AN INVESTIGATION OF OUTCOMES IN RELATION TO THIAMIN STATUS OF AMBULATORY PATIENTS WITH HEART FAILURE

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Science

Graduate Department of Nutritional Sciences

University of Toronto

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Masters of Science, 2012
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Abstract
Thiamin is a required coenzyme in energy producing reactions that subsequently fuel myocardial contraction. Therefore, thiamin deficiency (TD) might contribute to the reduction in myocardial function observed in patients with heart failure (HF) by limiting the available energy and subsequently aggravating cardiac performance. While the prevalence of TD as well as the impact of supplementation has been examined in patients with HF, none of these studies to date has examined the impact of TD on clinical outcomes. Therefore, this study investigated the associations between erythrocyte [TPP] levels and outcomes in ambulatory patients with HF. Time-to-event probabilities were found to be not significant for acute decompensated heart failure, mortality, all-cause hospitalizations, arrhythmias, myocardial infarctions and other adverse events. Further investigations into the longer term impact of TD on outcomes and the effects of thiamin supplementation as an adjunct therapy in delaying the disease progression are needed.
Acknowledgements

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My sincerest gratitude goes to Dr. JoAnne Arcand. Thank you for being an inspirational role model and mentor. I have sought to follow in your footsteps and today, I would not have been an MSc without your motivating dedication and perseverance to strive to reach for the sky.
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<thead>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>αKGDH</td>
<td>alpha-ketoglutarate dehydrogenase</td>
</tr>
<tr>
<td>ACC</td>
<td>American College of Cardiology</td>
</tr>
<tr>
<td>ADA</td>
<td>American Dietetic Association</td>
</tr>
<tr>
<td>ADHF</td>
<td>Acute Decompensated Heart Failure</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine monophosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine tri-phosphate</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BNP</td>
<td>Brain Natriuretic Peptide</td>
</tr>
<tr>
<td>HF</td>
<td>Heart Failure</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary Artery bypass graft</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CI</td>
<td>Cardiac index</td>
</tr>
<tr>
<td>cMRI</td>
<td>Cardiac Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>DC</td>
<td>Dietitians of Canada</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary reference intakes</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated Average Requirement</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiograms</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetraacetic acid</td>
</tr>
<tr>
<td>ETKA</td>
<td>Erythrocyte transketolase assay</td>
</tr>
<tr>
<td>sq-FFQ</td>
<td>Semi-quantitative food frequency questionnaire</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HMS</td>
<td>Hexose monophosphate shunt</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>ICD</td>
<td>Implantable Cardioverter Defibrillator</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricle</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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</tr>
<tr>
<td>MSH</td>
<td>Mount Sinai Hospital</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NE</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>N-terminal peptide fragment brain natriuretic peptide</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
</tr>
<tr>
<td>PCr</td>
<td>Phosphocreatine</td>
</tr>
<tr>
<td>PCI</td>
<td>Percutaneous coronary intervention</td>
</tr>
<tr>
<td>PCWP</td>
<td>Pulmonary capillary wedge pressure</td>
</tr>
<tr>
<td>PDHC</td>
<td>Pyruvate dehydrogenase complex</td>
</tr>
<tr>
<td>PND</td>
<td>Paroxysmal nocturnal dyspnea</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended dietary allowance</td>
</tr>
<tr>
<td>SGA</td>
<td>Subjective Global Assessment</td>
</tr>
<tr>
<td>SMH</td>
<td>St. Michael's Hospital</td>
</tr>
<tr>
<td>SVR</td>
<td>Systemic vascular resistance</td>
</tr>
<tr>
<td>TCA</td>
<td>Trichloroacetic acid</td>
</tr>
<tr>
<td>TD</td>
<td>Thiamin Deficiency</td>
</tr>
<tr>
<td>TDP</td>
<td>Thiamin diphosphate</td>
</tr>
<tr>
<td>TGH</td>
<td>Toronto General Hospital</td>
</tr>
<tr>
<td>TK</td>
<td>Transketolase</td>
</tr>
<tr>
<td>TMP</td>
<td>Thiamin monophosphate</td>
</tr>
<tr>
<td>TPP</td>
<td>Thiamin pyrophosphate</td>
</tr>
<tr>
<td>TPPE</td>
<td>Thiamin pyrophosphate effect</td>
</tr>
<tr>
<td>TTP</td>
<td>Thiamin triphosphate</td>
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</table>
1.0 - Introduction

Heart Failure (HF) is associated with significant morbidity and mortality and is considered as one of the most costly and burdensome cardiovascular diseases (The Heart and Stroke Foundation of Canada, 2011). Exacerbations of chronic HF result in episodes of acute decompensated heart failure (ADHF), which is a fatal cause of respiratory distress and is associated with reductions in cardiac output (Heart Failure Society of America, 2010). The incidence of HF is expected to rise as a result of the aging population (Heart Failure Society of America, 2010). Despite improvements in the clinical management of HF, mortality rates remain high (Tsuyuki et al., 2003; Yancy et al., 2004). As a consequence, recent research has focused on the investigation of alternative and novel factors such as altered energy metabolism and the role of micronutrients in the modulation of the progression of HF in an effort to improve outcomes (Keith et al., 2001; Soukoulis et al., 2009; Jeejeebhoy et al., 2002).

Thiamin, a water-soluble B vitamin, is a required coenzyme for carbohydrate metabolism and is involved in the production of adenosine triphosphate (ATP) (Gropper et al., 2005). Therefore, thiamin deficiency (TD) may contribute to the observed depletion of ATP reserves in patients with HF (Neubauer et al., 1997). Reductions in high energy phosphate stores may limit the energy available for myocyte contraction and thereby contribute to myocardial weakness and aggravation of cardiac performance in patients with HF (Neubauer et al., 1997). Recent trials have shown some potential improvement in cardiac function associated with thiamin supplementation, suggesting that thiamin might positively influence cardiac remodelling (Seligmann et al., 1991; Shimon et al., 1995).
There are several factors that may play a role in the development of TD in patients with HF. These factors include frequent hospitalizations, inadequate thiamin intake, malnutrition, disease severity, advanced age and use of diuretic medications (O’Keeffe et al., 2000; Kwok et al., 1992; Brady et al., 1995; Zenuk et al., 2003; Seligmann et al., 1991). Given the important role of thiamin as a cofactor in several energy producing reactions, it is possible that thiamin deficiency may negatively impact outcomes in patients with HF. To date, studies have been limited by their small sample sizes, variations in thiamin assay methods and their focus on hospitalized patients. Moreover, to our knowledge, there are no studies that have included an examination of the impact of thiamin status on clinical outcomes in compensated appropriately medicated patients with HF. Therefore, this study was designed to investigate the associations between thiamin status and outcomes as well as to determine the prevalence of TD in ambulatory patients with HF.
2.0 – Literature Review

2.1 - Heart Failure

2.1.1 Definition, Aetiology and Pathophysiology:

The development of Heart Failure (HF) is associated with significant morbidity and mortality resulting in approximately 400,000 hospitalizations per year in Canada (Heart and Stroke, 2011). The prevalence and incidence of HF is growing and the number of people diagnosed with HF is expected to double within the next 20 years (Tsuyuki et al., 2003). Despite advances in therapy, HF remains a common and serious condition with an average annual mortality rate of 10% (Ho et al., 1993). HF is also considered to be one of the most costly cardiovascular diseases as patients are usually on multiple medications and require frequent monitoring to prevent exacerbation of their symptoms (Liu et al., 2001). As the severity of HF increases, there is an associated increase in the number of hospitalizations placing an increased burden on the health care system as well as on the quality of life of the patient (O'Connell., 2000).

HF is a complex clinical syndrome which results from a cardiac disorder impairing the ventricle’s ability to fill with or eject blood (Zipes et al, 2005). Common aetiologies of HF include hypertension, valvular heart disease, coronary artery disease, idiopathic processes and viral myocarditis (Zipes et al., 2005). The diagnosis of HF is based on physical symptoms, attenuation of symptoms with medical therapy and objective tests such as electrocardiograms, echocardiograms and chest x-rays (Johnstone et al., 1994). Assessments of New York Heart Association (NYHA) classification and left ventricular ejection fraction (LVEF) are used for the quantification of the severity of HF (further discussed in sections 2.1.3 (A) and 2.1.3 (B)). The Framingham criterion can also be used to diagnose HF, though it is more commonly utilized in epidemiological studies rather than in the clinical setting. According to the Framingham
criterion, an individual should have at least two major or one major and two minor clinical factors in order to be diagnosed with HF (Table 1.1) (Ho et al. 1993). The cardinal manifestations of HF include dyspnea, fatigue and fluid retention leading to pulmonary congestion or peripheral edema (Hunt et al., 2009). Chronic HF can exacerbate into a condition known as **Acute Decompensated Heart Failure (ADHF)**. ADHF occurs in patients with pre-existing HF and is defined as an exacerbation of symptoms consisting of dyspnea, edema or fatigue and requiring urgent medical care (Heart Failure Society of America., 2010). After being diagnosed with HF, patients may generally follow one of three clinical pathways: 1) remain stable without ADHF and/or mortality; 2) experience sudden cardiac death; or 3) present with ADHF and subsequent hospitalization (Lepage., 2008).
**Table 1.1:** Framingham Criteria for Diagnosis of Heart Failure

- Diagnosis of HF requires that two major or one major and two minor criteria are present at the same time. Acceptability of minor criteria occurs only in cases if they cannot be attributed to another medical condition. (Adapted from Ho et al., 1993).

<table>
<thead>
<tr>
<th>Major Criteria</th>
<th>Minor Criteria</th>
</tr>
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<tbody>
<tr>
<td>Paroxysmal nocturnal dyspnea (PND) or orthopnea</td>
<td>Edema</td>
</tr>
<tr>
<td>Jugular venous distention</td>
<td>Nocturnal cough</td>
</tr>
<tr>
<td>Hepatojugular reflex</td>
<td>Shortness of breath on exertion</td>
</tr>
<tr>
<td>Cardiomegaly</td>
<td>Hepatomegaly</td>
</tr>
<tr>
<td>Acute pulmonary edema</td>
<td>Pleural effusion</td>
</tr>
<tr>
<td>Third sound (S₃) gallop</td>
<td>Tachycardia (rate≥120 beats/min)</td>
</tr>
<tr>
<td>Increased central venous pressure</td>
<td></td>
</tr>
<tr>
<td>Weight loss ≥ 4.5kg in 5 days after treatment</td>
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</tbody>
</table>
The pathophysiology of HF occurs gradually and progressively following left ventricle (LV) systolic dysfunction due to an injury or stress (e.g. myocardial infarction {MI}) on the myocardium that results in LV dilatation and hypertrophy (Cohn, 1996). Cardiac remodelling is the principal manifestation of HF (Cohn et al., 1996). Several abnormalities (hemodynamic, neurohumoral and molecular) have been found in the failing myocardium, which contributes to myocardial dysfunction, myocyte apoptosis and progressive HF (Sole et al., 2002). Injury to the myocardium can result in myocyte hypertrophy due to systolic and diastolic wall stress. This stress stimulates ventricular enlargement and leads to further decline in contractile function, contributing to progressive HF (Sole et al., 2002). Neurohumoral activation is a central mechanism of progressive HF. It involves activation of the sympathetic nervous system that, in turn, augments cardiac output by increasing heart rate and contractility (Zipes et al., 2005). Initially, increased sympathetic outflow promotes vasoconstriction to maintain blood pressure and venoconstriction to optimize venous return and thereby, allowing for an increase in cardiac output (Zipes et al., 2005). However, this response becomes deleterious as cardiac filling pressures are further elevated due to an increase in sympathetic outflow, leading to ventricular wall stress (Zipes et al., 2005). As a result, ventricular hypertrophy develops, which is thought to reduce ventricular wall stress and maintain cardiac output. In the short term, these responses are sustained by increased contractility (Guyton et al., 2006). As a consequence, patients with chronic HF may have elevated levels of neurohumoral factors including norepinephrine, angiotension II, vasopressin and cytokines which increase the hemodynamic stresses caused by sodium retention and exert apoptotic effects on myocardial cells (Hunt et al., 2009). Moreover, increases in proinflammatory cytokines have also been shown to contribute to alterations in
body composition and muscle wasting, resulting in a syndrome known as cardiac cachexia (Anker et al., 2004). Cardiac cachexia is a complication found in some patients with HF and further worsens the prognosis of these patients (Anker et al., 2004). This syndrome is further discussed in sections 2.1.6 and sections 2.3.1 (A). In some patients with time, the heart may decompensate due to loss of myocardial cells, increasing fibrosis and impairments in contractility, further leading to decreases in cardiac output (Guyton et al., 2006). Subsequently, the sympathetic nervous system is further stimulated and the heart is unable to respond adequately (Guyton et al., 2006). Chronic HF can switch to an acute episode of HF and there has been a lot of interest in determining the factors that may contribute to the movement from compensated to decompensated HF. Some of the precipitating factors include concomitant illnesses (e.g pneumonia), myocardial infarction (MI), anemia, and adherence to medications or dietary noncompliance (Rodgers., 2006).

Recent research suggests that there is altered energy metabolism and metabolic abnormalities found in HF, which contribute to both the loss of myocytes as well as to myocardial dysfunction (Weiss et al., 2005; Ingwall et al., 2004, Gosker et al., 2000, Neubauer et al., 2007, Sole et al., 2002). The process of cardiac energy metabolism includes the following: 1) anabolism of free fatty acids and glucose through beta-oxidation and glycolysis, 2) oxidative phosphorylation, which is the process of production of ATP and 3) the transfer and utilization of ATP to the myofibrils through creation of phosphocreatine (PCr) by creatine kinase (CK) (Neubauer et al., 2007). In the healthy human heart, about 60%-80% of ATP is generated through beta-oxidation of fatty acids and the remainder is produced from glycolysis and lactate oxidation (Stanley et al., 2002). In compensated HF, increases occur in glucose uptake and utilization whereas fatty acid
oxidation either remains the same or is decreased (Ingwall et al., 2009). However, this results in a state of diminished energy reserves where the heart has limited contractile reserves and is at an increased risk for acute mechanical failure (Ingwall et al., 2004). In this acute decompensated state, fatty acid oxidation decreases and any increases in glucose uptake or utilization are not sufficient to meet the demands for ATP supply and thus the concentration of required ATP falls (Ingwall et al. 2009). When there is a mismatch between the energy demand and energy supply, the concentration of phosphocreatinine [PCr] falls in order to keep the concentration of ATP [ATP] at a constant level (Ingwall et al., 2009; Neubauer et al., 1997). However, this may lead to an increase in the concentration of adenosine diphosphate [ADP], decrease average [ATP] or reduce ATP transfer capacity to the myofibrils, which consequently inhibits the function of many intracellular enzymes, reducing contractility (Weiss et al., 2005; Neubauer et al., 1997). The previously observed reductions in myocardial PCr:ATP ratios suggest that stores of high energy phosphate compounds are necessary for contractile function. Furthermore, a reduced PCr:ATP ratio has also been shown to be a significant predictor of cardiovascular mortality in patients with HF (Weiss et al., 2005; Neubauer et al., 2007). These ratios have also been shown to correlate with the NYHA functional class and indices of systolic and diastolic function including LVEF (Neubauer et al., 1997; Weiss et al., 2005).

2.1.2 Acute Decompensated Heart Failure (ADHF):

ADHF encompasses patients with HF of sufficient severity to require hospitalization and is defined as an exacerbation of dyspnea, edema and fatigue requiring urgent medical care (Heart Failure Society of America., 2010). ADHF is defined as progression of chronic HF that involves active inflammation, increases in neurohormonal activation and reductions in cardiac output. It
is a fatal cause of acute respiratory distress most commonly due to left ventricular systolic or diastolic dysfunction (Heart Failure Society of America., 2010). It is associated with significant mortality and morbidity (Heart Failure Society of America., 2010). Though patients at any stage of HF can become decompensated, ADHF episodes occur more frequently in patients with overt, symptomatic HF (McBride et al., 2003).

According to the AHA, the prevalence of ADHF is approximately 5 million episodes per year with an incidence rate of 500,000 new cases per year (Roger et al., 2012). The economic burden of ADHF is approximated at 29.6 billion dollars and is predicted to elevate due to the increase in aging population (Rodgers, 2006). The rate of hospitalizations due to ADHF is approximately one million per year (Rodgers, 2006). The readmission rate for patients admitted with ADHF is 47% within 90 days (Vinson et al., 1990). Hospitalization is a serious event in the life of a patient with HF as it is associated with in-hospital mortality of 15.8% (Tsuyuki et al., 2003).

Data on the clinical characteristics of patients with ADHF as well as outcomes associated with hospitalization for ADHF remains inconsistent and incomplete (Adams et al., 2005).

The common causes of ADHF are medication noncompliance (24%), failure to seek care (19%), dietary noncompliance (24%), inappropriate pharmaceutical prescribing (16%) and others (17%) (Rodgers, 2006). In diagnosing ADHF, patients may present with symptoms of fluid overload which include weight gain, dyspnea, peripheral edema, jugular venous distension, pulmonary capillary wedge pressure of >18mm Hg and elevated arterial pressure of > 10mm Hg. Some patients may also present with low cardiac output and worsening symptoms which includes fatigue, nausea, early satiety, weight loss and increased serum creatinine (Rodgers, 2006).
The pathophysiology of ADHF is related to high filling pressures causing pulmonary congestion. This occurs due to chronic elevation of neurohumoral factors present in patients with HF (McBride et al., 2003). Moreover, neurohumoral activation is linked to worsening symptoms of HF as it stimulates proinflammatory cytokines and myocyte apoptosis by elevating many neurohormones and immunomodulators (McBride et al., 2003). In patients with ADHF, ventricular tachyarrhythmias become enhanced due to hyperactive sympathetic nervous system activity, increased intracellular calcium concentrations and anaerobic metabolism. The activity of angiotension II is also augmented and causes increased myocardial wall stress and sodium retention, thus, worsening the degree of cardiac decompensation (McBride et al., 2003).

The goals of therapy for ADHF is to improve symptoms of congestion, identify aetiology and minimize adverse effects. This is currently being achieved by diuretics, inotropes, vasodilators and natriuretic peptides (McBride et al., 2003). Diuretics are mainly used to reduce congestion but there is paucity of data to support their impact on outcomes (Rodgers, 2006). Most often, patients presenting with ADHF are resistant to diuretics which limit their efficacy (Rodgers, 2006). Also, the chronic use of diuretic therapy in ADHF has been associated with mortality as indicated by the data from the ADHERE trial showing that patients hospitalized for ADHF and receiving intravenous (IV) diuretics had an increased risk of mortality in comparison with patients who were not given IV diuretics (OR 1.3 (95% CI, 1.2-1.5)) (Peacock et al., 2009; Emerman, 2003). Other observational studies in ADHF patients support the findings of the ADHERE trial in which increased mortality and length of stay in the hospital was observed in patients given diuretics (Neuberg et al., 2002; Domanski et al., 2003). Because of the above-mentioned concerns regarding the use of diuretics in the management of ADHF, newer therapies
are being actively pursued and one such therapy is peripheral ultrafiltration (Allen et al., 2007). Peripheral ultrafiltration is an alternative to loop diuretics for the treatment of volume overload. It utilizes the mechanism of veno-venous filtration to remove excess fluid (Allen et al., 2007). The ultrafiltration versus intravenous diuretics for patients hospitalized for ADHF (UNLOAD) trial indicated that in 200 patients with ADHF, peripheral ultrafiltration reduced the rate of readmission to hospital at 90 days (Costanzo et al., 2007). Though ultrafiltration may seem like a new promising treatment option for patients with ADHF, data on the benefit to risk ratio as well as the overall cost effectiveness of this technique has yet to be determined. Another pharmacotherapy approach commonly used to treat patients with ADHF is inotropes. Inotropes are given in order to improve hemodynamics by increasing cardiac output. However, several studies have shown adverse effects of these drugs such as increased mortality, arrhythmias, tachyphylaxis and neurohumoral activation (Rodgers, 2006; Cuffe et al., 2002). One such trial showed no significant difference between an inotrope (milrinone) and a placebo on total days spent in hospital as well as on mortality rates (Cuffe et al., 2002). Nevertheless, there was a trend for the mortality rate to be higher in the inotrope group than in the placebo group (Cuffe et al., 2002). Given the burden of hospitalizations, the high rate of readmissions after discharge and concern regarding the safety and efficacy of therapies currently being used in managing ADHF, there is a need for active management and control of stimulating factors in ADHF (McBride et al., 2003).
2.1.3 Phenotyping HF

2.1.3 (A) Left Ventricular Ejection Fraction (LVEF):

Left ventricular ejection fraction (LVEF) is a significant prognostic indicator of the severity of HF and is defined as the fraction of end-diastolic volume that is pumped from the left ventricle into the aorta (Guyton et al., 2006). LVEF is depressed in patients with cardiac hypertrophy and reduced contractile function (Chatterjee et al., 2008). LVEF is classified by a LV Grade, where; an EF of >60% is considered normal. Grade I (EF>50%) includes patients with mild LV dysfunction. The severity of LV dysfunction is in direct relationship to the increase in LV grades from Grade II (EF 30-50%) to Grade III (EF 20-30%) and Grade IV (EF<20%). Although, LVEF is found to be a significant predictor of mortality attributable to cardiovascular diseases (Neubauer et al., 1997), recently, it has not been shown to correlate with disease severity (Rose., 2005). This is linked to the lack of ability of EF to reflect the strength of myocardial contractility (Rose., 2005). Moreover, patients in the upper numerical value for EF may have overlapping phenotypes that makes it difficult to measure EF in relation to severity. Therefore, subsequently, other indicators of cardiac function including biomarkers have been found in relation to disease severity.

2.1.3 (B) New York Heart Association (NYHA):

The New York Heart Association (NYHA) functional classification system quantifies the degree of HF by the presentation of physical symptoms during the performance of activities of daily living (Hunt et al., 2009). This framework places individuals in four different classes which correspond to their degree of functional limitation during physical activity. Table 1.2 summarizes the characteristics of patients assigned to each class (McBride et al., 2003).
This framework is limited due to its subjective nature with significant inter-observer variability (Hunt et al., 2009). As a result, American Heart Association (AHA) has proposed a newer complementary method for the classification of HF patients (McBride et al., 2003). This complementary method is known as the AHA Prognostic Classification (Stages A-D) system and is discussed below (McBride et al., 2003).
Table 1.2: New York Heart Association (NYHA) Functional Classification System for Heart Failure (HF) (Adapted from McBride et al., 2003).

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>Patients have no functional limitation and no symptoms of HF.</td>
</tr>
<tr>
<td>Class II</td>
<td>Patients have symptoms with moderate exertion.</td>
</tr>
<tr>
<td>Class III</td>
<td>Patients have symptoms with minimal exertion.</td>
</tr>
<tr>
<td>Class IV</td>
<td>Patients have symptoms at rest.</td>
</tr>
</tbody>
</table>
2.1.3 (C) American Heart Association (AHA) Prognostic Classification (Stages A-D):

The development and progression of HF is categorized into four stages (Stage A-D), which includes patients who are at a risk of developing HF as well as those with overt and refractory HF (Hunt et al., 2009). Stage A encompasses patients who are at a risk of developing HF but do not demonstrate hypertrophy, such as those with diabetes, obesity or hypertension. Stage B includes patients with structural heart disease and low ejection fraction but have asymptomatic valvular disease. Stage C includes patients who have developed overt, symptomatic HF. At this stage, patients with pre-existing HF can be diagnosed with ADHF if they present with symptoms related to worsening HF. Stage D designates patients with refractory HF and occurs when a patient has symptoms at rest despite optimal medical therapy (Hunt et al., 2009) (Table 1.3). This framework allows for therapeutic interventions based on disease severity in order to reduce population morbidity and mortality (McBride et al., 2003). It also recognizes established risk factors and pathophysiologic abnormalities as they occur in the development of HF (Hunt et al., 2009).
Table 1.3: American Heart Association (AHA) Prognostic Classification. HF = Heart failure; LV= Left ventricle (Adapted from McBride et al., 2003).

<table>
<thead>
<tr>
<th>Stages</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>STAGE A</td>
<td>Patients with risk factors for the development of overt HF or structural heart disease</td>
</tr>
<tr>
<td>STAGE B</td>
<td>Presence of structural heart disease (e.g. LV hypertrophy) without symptoms of HF</td>
</tr>
<tr>
<td>STAGE C</td>
<td>Patients with structural heart disease and current or prior symptoms of HF amenable to therapy</td>
</tr>
<tr>
<td>STAGE D</td>
<td>Refractory HF to conventional treatment requiring devices, transplantation or palliative care</td>
</tr>
</tbody>
</table>
2.1.4 Diagnostic Tools and Biomarkers:

One way of achieving optimal care and regular follow-ups for patients with HF is through the specialized clinics using a multi-disciplinary approach (Hunt et al., 2005). Patients may present with symptoms of decreased exercise tolerance, fluid retention or symptoms/no symptoms of another cardiac/noncardiac disorder (Hunt et al., 2009). Data collection on patient demographics, medical history including risk factors, clinical symptoms, physical examination, and laboratory biomarker testing are the first steps in evaluating individuals suspected of having HF (Hunt et al., 2005). Routine diagnostic tests performed on patients with HF include electrocardiograms (ECG) and chest x-rays (Hunt et al., 2009; Johnstone et al., 1994). The size, dimension and volume of the ventricle, measurement of wall thickness, scar tissue, myocardial viability and functional capacity can be determined by standardized diagnostic imaging techniques and formal tests of exercise tolerance (Johnstone et al., 1994; Hunt et al., 2009). These techniques include echocardiography, radionucleic angiography, cardiac catheterization, 6-minute walk test and cardiac magnetic resonance imaging technique (cMRI) (Hunt et al., 2009).

Assessing the aetiology of HF can allow for the application of treatments that may help reverse or delay the progression of the condition. Laboratory testing allows for identifying potential causes that can exacerbate or lead to HF (Hunt et al., 2009). This testing includes a complete blood profile including blood count, haemoglobin, electrolytes, lipids and some circulating biomarkers. Measurements of circulating biomarkers may provide insight into the pathophysiology of HF and are now applied in routine clinical practice (Felker et al., 2006). These include but are not limited to norepinephrine, natriuretic peptides and troponin.
Brain Natriuretic Peptide (BNP) is a hormone found in ventricular myocardium and it is secreted in response to increased myocardial wall stress (Felker et al., 2006). BNP secretion during volume overload occurs in order to promote natriuresis and diuresis which helps to lower blood pressure, cause vasodilation and decrease myocardial fibrosis (Frigerio et al., 2003). Elevated plasma levels of natriuretic peptides, including brain natriuretic peptide (BNP) and N-terminal peptide fragment (NT-proBNP) are associated with reduced LVEF (Hunt et al., 2009). The prognostic value of measuring BNP is indicated by a pooled analysis of four studies that used continuous levels of BNP to predict all-cause mortality (Frigerio et al., 2003). These studies showed that for each 100pg/ml increase in BNP, the relative risk of death increased by 35% (Frigerio et al., 2003). Some other studies used BNP as a dichotomous variable. One such study indicated a doubling of mortality among patients with BNP levels greater than 97pg/ml (Anand et al., 2003). BNP levels have also been shown to predict mortality or readmission rates for hospitalization in patients with HF. Logeart & colleagues (2004) reported that the risk of death or readmission after decompensated HF increased in a stepwise fashion across increasing predischarge BNP levels ($p<0.0001$) However, the use of BNP to monitor the response to HF therapy has not been widely advocated since natriuretic peptides are sensitive to other biological factors including kidney function, disease stage and genetic polymorphisms (Weinfeld et al., 1999 and Felker et al., 2006).

Abnormal sympathetic nerve activation has been shown to augment the risk of HF and plasma norepinephrine, a marker of systemic sympathetic nerve activity, has been reported to be a useful prognostic biomarker (Tsutamoto, 2008). Many studies have demonstrated a relationship between elevated plasma levels of norepinephrine and risk of increased mortality in end-stage
HF (Tsutamoto, 2008; Cohn, 1984; Hunt et al., 2009). Chronic exposure of myocardial cells to increased levels of circulating norepinephrine has been shown to induce cardiac hypertrophy and ventricular wall stress, thereby aggravating HF (Knowlton et al., 1993).

Another biomarker that has received recent interest in detecting myocardial injury in patients with HF is troponin. Troponins are proteins involved in the regulation of cardiac and skeletal muscle contraction (Kociol et al., 2010). Though, there are many isoforms of troponin, the isoform cardiac troponin I is the only one expressed during myocardial injury (Kociol et al., 2010). Multiple mechanisms contribute to the release of troponin and these include increased wall stress, oxidative stress, inflammatory cytokines, neurohormonal activation, myocyte necrosis due to ischemia and apoptosis (Kociol et al., 2010). Because of the specificity of cardiac troponin I to myocardial injury, it has been suggested that elevated troponins in patients with HF may reflect ongoing injury and progression of HF (Latini et al., 2007). Missov et al. (1997) conducted one of the first studies illustrating increased levels of circulating troponin I in patients with stable HF. Since then, troponin levels have been widely accepted to act as a marker of HF severity (Kociol et al., 2010). Cardiac troponin I has been shown to have prognostic value in predicting ongoing myocardial necrosis and thereby, predicting worsening HF. This suggests a role for troponin-based laboratory testing to monitor high-risk patients (Perna et al., 2004).

2.1.5 Management of Heart Failure:

Goals of therapy for chronic HF are to alleviate symptoms, improve quality of life and delay disease progression (Jessup et al., 2003). Medical intervention forms the basis of HF management. It focuses on improving cardiac contractility while reducing LV remodelling, vasoconstriction and congestion (Johnstone et al., 1994; Barker et al., 2009). Patients with
advanced symptomatic HF may benefit from implantable devices (i.e. pacemakers, implantable defibrillators), surgical interventions such as revascularization (i.e. coronary artery bypass grafting) or valve replacement (Arnold et al., 2007; Bristow et al., 2004; Moss et al., 2002; Chatterjee et al. 2008).

Pharmacological therapy includes beta blockers to inhibit the effects of sympathetic nervous system activation, ACE inhibitors & angiotensin II receptor antagonists to reduce vasoconstriction while restoring fluid/sodium balance, cardiac glycosides to control heart rate and nitrates to reduce peripheral vascular resistance (Johnstone et al., 1994; Barker et al., 2009). Diuretics are prescribed to symptomatic patients with ongoing sodium and water retention in order to lower blood pressure and increase cardiac output (Johnstone et al., 1994; Brater., 1998). Diuretics are categorized as thiazides, carbonic anhydrase inhibitors, aldosterone-antagonists and loop diuretics (Rose, 1991). The categorization of diuretics into these types relates to their mechanisms of action for inducing diuresis.

Despite advances in therapeutic approaches to HF management, HF continues to be an ongoing challenge for patients. Also, there is a concern that the current pharmacological and device therapy may have achieved the maximum benefit (Keith et al., 2001). Moreover, pharmacological agents including diuretic therapy have been shown to be associated with adverse neurohumoral activation and hemodynamic effects (Chatterjee et al., 2008).

Nonpharmacological interventions are being considered as adjunctive therapies to improve outcomes in patients with HF. Lifestyle modification for HF management and prevention include monitoring sodium/fluid intake, body weight, smoking, and cholesterol levels (Hunt et al., 2009). Physical activity may also be encouraged to improve life quality and functional
capacity (McKelvie et al., 1995; Piepoli et al., 2004). Dietary sodium reduction and lipid management together with a heart healthy diet have formed the mainstay of nutritional management (Arnold et al., 2006). However, other nutritional modifications as an adjunct therapy have been fairly unexplored in the management of patients with HF. Education of patients regarding lifestyle modifications, adherence to medications, reinforcing healthy dietary choices and regular visits to the clinic all play a vital role in the management of HF (Arnold et al., 2006).

2.1.6 Nutritional Management of Heart Failure:

Most of the research efforts to manage HF have either focused on pharmacological therapies and devices and/or the wasting syndrome of cardiac cachexia while little attention has been paid to non-pharmacological approaches, particularly nutrition (de Lorgeril et al., 2005). Nutritional interventions are an area of ongoing investigation in models for HF management due to their potential to positively modulate cardiac energetics and homeostasis (Sole et al., 2002 and Briet et al., 2008). While the therapeutic role of nutrition has primarily focused upon sodium restriction and lipid modification, there is evidence suggesting that the hypertrophied cardiomyocyte is depleted of several nutrients, which are key to cellular cardiac energetics (Sole et al., 2002). These nutrients include thiamin, potassium, carnitine, taurine and coenzyme Q10 and their deficiencies have been shown to aggravate myocyte dysfunction (Sole et al., 2002 and Jeejeebhoy et al., 2002).
2.1.6 (A) Cardiac Cachexia:

Cardiac cachexia is a clinical syndrome defined as an unintentional, non-edematous weight loss of $>6\%$ of body mass over 6 months (Anker et al., 2004). Cardiac cachexia is also a recognized disorder of altered energy-protein metabolism in HF (Anker et al., 1999). The prevalence of cardiac cachexia has been estimated to be 12%-15% in patients with HF of NYHA class II to IV (Anker et al., 2009). The development of cachexia is associated with increases in resting energy expenditure and catabolism (protein loss) (Anker et al., 1999). The mechanism for hypercatabolism and hypermetabolism (increased resting energy expenditure) in cachetic patients with HF is thought to be the result of elevated activity of the sympathetic nervous system, neuroendocrine abnormalities and an increase in catabolic factors such as norepinephrine and inflammatory cytokines (Anker et al., 1999). Resting energy expenditure is also thought to increase due to elevations in breathing, fever, increased resting oxygen consumption and increased cardiac function (Freeman et al., 1994, Riley et al., 1991 and Berry et al., 2000).

Cachetic patients with HF undergo body composition alterations including muscle atrophy, fatigue, cardiac wasting, loss of lean body mass, reduced fat tissue mass and bone mineral density (Anker et al., 2004; Anker et al., 1998; Anker et al., 1999). Although cachexia is a well recognized syndrome in patients with HF, the mechanism by which a patient becomes cachetic is not well defined (Anker et al., 2004) and nutritional interventions in the prevention and treatment of this syndrome requires further investigation (Freeman et al., 1994).
2.1.6 (B) Protein-Energy Malnutrition:

The presence of protein-energy malnutrition has been recognized in patients with HF and has been investigated in observational studies (Freeman et al., 1994; Carr et al., 1989; Mancini et al., 1992). Surveys done in hospitalized patients suggest that 50-68% of patients with HF are significantly malnourished (Freeman et al., 1994, Schewengel et al., 1994). Factors such as excessive drug therapies, restrictive diets or hypermetabolism may compromise food and nutrient intake, subsequently contributing to an overall poor nutritional status (Schewengel et al., 1994; Poehlman et al., 1994). Direct relationships have been illustrated between the clinical severity of HF and an increased resting metabolic rate (Anker et al., 2004; Obisesan et al., 1996). Poehlman et al. (1994) observed patients with HF to have an 18% higher resting energy expenditure in comparison with that of healthy controls. Aquilani et al., (2003) demonstrated negative calorie and nitrogen balance in patients with HF, suggesting that patients with HF do not consume enough energy and protein to sustain daily physical activity. However, some studies show no associations between dietary energy intake and nutritional status while others demonstrated that the underlying disease processes also contributed to dietary intake and subsequently, to the nutritional status of patients with HF (Lourenco et al., 2009; Gariballa et al., 2007). Since most of the studies on protein-energy status in HF are conflicting and methods are limited in detecting malnutrition, it becomes difficult to determine actual energy requirements of this population.

2.1.6 (C) Macronutrient Modifications:

There is a paucity of literature on the role of macronutrient modification in HF. One epidemiological study has suggested an important role for essential fatty acids in the setting of
HF (Marchioli et al., 2002). This study found that the intake of very long chain omega-3 polyunsaturated fatty acids (PUFAs) was associated with a 37% lower risk of development of HF in the highest quintile of intake (Mozaffarian et al., 2005). The GISSI-Prevenzione Trial similarly suggested a positive effect of omega-3 fatty acid supplementation on sudden cardiac death in patients with LV dysfunction (Marchioli et al., 2002). However, there is conflicting evidence in regards to the cardioprotective effects of omega-3 fatty acids in population groups at risk of cardiovascular disease (de Lorgeril et al., 2005). Most randomized prevention trials do not support that an increased intake of fatty fish can prevent against risk and death from HF (Nordoy, 2001; Poppitt et al., 2009; Tanaka et al., 2008). In addition, although the use of omega-3 supplements are becoming popular, the PUFAs are particularly sensitive to oxidation, suggesting the need for additional recommendations of antioxidants along with these concentrated supplements (Nordoy, 2001). Future studies need to document the interactions of omega-3 PUFAs with drug therapy along with reporting the safety and efficacy of dietary and supplemental intake of omega-3 PUFAs in relation to HF. Furthermore, additional research is required in order to define the optimal dosage of these essential fatty acids that is beneficial for patients with HF as well as their mechanisms of cardioprotection (Nordoy, 2001).

2.1.6 (D) Micronutrient Modifications:

It has been suggested that patients with HF may be more susceptible to the effects of micronutrient deficiency as a result of increased oxidative stress, impaired skeletal muscle function and impaired myocardial contraction (Witte., 2005). For example, carnitine, coenzyme Q10 and taurine play key roles in substrate transfer to the mitochondrial matrix and thereby, ensure adequate ATP stores. These nutrients are shown to have cardioprotective effects in
preserving mitochondrial energy metabolism, improving contractility and reducing oxidative stress (Sole et al., 2002; Briet et al., 2008).

Investigations assessing alterations in ATP production because of impairments in cardiac function suggest that a lower rate of ATP production may contribute to a poor energy state in the myocardium. Hence, the risk of development of HF may increase, in part because of limited energy available for myocyte contraction (Neubauer et al., 1997; Ingwall et al., 1990). Considering that B-vitamins play key roles in the production of cellular energy, it is logical to hypothesize that having a deficiency of one or more B-vitamins might contribute to the observed depletion of energy reserves to fuel the myocardium, and subsequently contribute to myocardial dysfunction. Thiamin, riboflavin and pyridoxine all play a role in the production of red blood cells and are essential cofactors for the production of cellular energy. In addition, B-vitamins are water-soluble therefore; the levels may be adversely affected in patients with HF as they are subject to urinary excretion in patients taking loop diuretics, have limited tissue storage and are dependent on intake. Thiamin deficiency (TD), in particular, has been linked with dysfunction of the cardiovascular system, commonly known as wet beriberi. An increased risk of TD may be related to factors such as increased urinary thiamin excretion in patients taking diuretics, elderly age, malnourishment, disease severity and compromised dietary intake (Briet et al., 2008; Jeejeebhoy et al., 2002). These factors are further discussed in section 2.3.1. Some preliminary evidence exists, showing a beneficial effect of thiamin supplementation on myocardial contractility and LV function in patients with HF (Shimon et al., 1995; Seligmann et al., 1991). This suggests a potential role for thiamin as an adjunct therapy in modulating cardiac function.
Several metabolic abnormalities are present in the myocardium, which are linked with both myocardial dysfunction as well as with the progression of HF (Sole et al., 2002; Jeejeebhoy et al., 2002; Briet et al., 2008). These mechanisms include abnormalities in aerobic oxidation, loss of energy reserves and oxidative stress which affect cardiac pump efficiency (Sole et al., 2002; Briet et al., 2008). Moreover, patients with HF may have nutritional deficiencies as a result of factors such as chronic use of medications including diuretics, poor dietary intake, increased nutrient needs or excessive nutrient losses (Briet et al., 2008; Sole et al., 2002; Keith et al., 2009). Evidence supporting a therapeutic benefit of nutrient modification in patients with HF remains an area to be explored. Due to the heterogeneity of patients with HF, individual responses to nutritional interventions will differ. Dietary inadequacies of several vitamins and minerals have been shown in some patients with HF (Catapano et al., 2008; Gorelik et al., 2003; Lemon et al., 2010). A recent study indicated that in outpatients with HF on stable medication regimens, more than 50% had inadequate dietary intakes of thiamin, calcium, magnesium, folate, vitamin E, vitamin D and zinc (Arcand et al., 2009). Considering that myocardial energy production is aerobic and dependent on a continual and adequate flow of nutrients, it is possible that nutrient deficiencies may contribute to worsening symptoms of HF and may subsequently offer some therapeutic benefit in the setting of HF (Sole et al., 2002). One of these nutrients of interest is thiamin. Thiamin is a required cofactor in energy producing reactions that subsequently fuel myocardial contraction (Gropper et al., 2005). Thiamin is required in the tricarboxylic acid (TCA) cycle for oxidative decarboxylation of both pyruvate and alpha-ketoglutarate (Gropper et al., 2005). Impediments in these reactions, as a consequence of TD, can result in changes in pH, prevent adequate synthesis of ATP, and increase pyruvate, lactate
and alpha-ketoglutarate in the blood, subsequently slowing the TCA cycle (Bettendorff., 1995). This results in impairments in oxidative metabolism of both fats and carbohydrates and limits ATP production (Neubauer et al., 1997; Weiss et al., 2005). Impaired ATP production may contribute to the depletion of energy stores previously observed in the progression of HF (Weiss et al., 2005). Considering the significant role of thiamin as a coenzyme in ATP production, it is logical to assume that TD might reduce the rate of ATP production and thereby, contribute to cardiac dysfunction and impairments in contractile function (Ingwall et al., 1990, Neubauer et al., 1997; Pichard et al., 1988; Weiss et al., 2005).
2.2-Thiamin (Vitamin B1)

2.2.1 Chemistry:

Thiamin or vitamin B₁ is a water-soluble vitamin (Gropper et al., 2005). Its structural formula consists of a pyrimidine ring and a thiazole moiety linked by a methylene bridge (Figure 2.1) (Gropper et al., 2005).
Figure 2.1: Structure of Thiamin. Adapted from Gropper et al., 2005.
2.2.2 Dietary Sources:

Thiamin is widely distributed in foods, including meat, legumes, cereals, breads and whole, fortified enriched grain products (Shils et al., 2006). Wheat germ and yeast also contain significant amounts of thiamin (Table 2.1) (Gropper et al., 2005). However, dairy products and seafood are poor sources of thiamin (Shils et al., 2006). In supplements, thiamin is found mainly as thiamin hydrochloride or thiamin mononitrate salt (Gropper et al., 2005).
Table 2.1: Thiamin content of common foods (modified from Shils and Shike, 2006).

<table>
<thead>
<tr>
<th>Food Type</th>
<th>Thiamin Content (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>0.4-0.5</td>
</tr>
<tr>
<td>Rice</td>
<td></td>
</tr>
<tr>
<td>Whole rice</td>
<td>0.5</td>
</tr>
<tr>
<td>Polished rice</td>
<td>0.03</td>
</tr>
<tr>
<td>Rice bran</td>
<td>2.3</td>
</tr>
<tr>
<td>Vegetables</td>
<td></td>
</tr>
<tr>
<td>Peas</td>
<td>0.36</td>
</tr>
<tr>
<td>Other Legumes</td>
<td>0.4-0.6</td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>0.04</td>
</tr>
<tr>
<td>Meats</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>0.3</td>
</tr>
<tr>
<td>Lamb</td>
<td>0.2</td>
</tr>
<tr>
<td>Poultry/Pork</td>
<td>≤0.1</td>
</tr>
</tbody>
</table>
2.2.3 Digestion, Absorption, Transport, Metabolism, Storage and Excretion:

Thiamin is present as four different compounds in human tissue: free thiamin, thiamin monophosphate (TMP), thiamin pyrophosphate (TPP) or thiamin diphosphate (TDP) and thiamin triphosphate (TTP) (Thiamine.monograph.,2003; Gropper et al., 2005). In animals, >95% of thiamin occurs in a phosphorylated form, primarily as thiamin diphosphate (TDP) (Gropper et al., 2005; Shils et al., 2006).

Absorption of thiamin from foods is thought to be high except in cases where antithiamin factors may be present in the diet known as thiaminases (Gropper et al., 2005). For example, thiaminases found in raw fish may destroy this vitamin. However, these thiaminases are thermolabile therefore cooking seafood can render thiaminase inactive (Gropper et al., 2005). Other thiaminases include polyhydroxyphenols such as tannic and caffeic acids. These are thermostable and are found in coffee, tea, betel nuts and certain fruits and vegetables including black currants, blueberries, and red cabbage (Shils et al., 2006; Gropper et al., 2005). The polyhydroxyphenols inactivate thiamin by an oxyreductive process. However, if vitamin C or citric acids are present, thiamin destruction may be prevented (Gropper et al., 2005).

Once ingested, thiamin is hydrolyzed to free thiamin, which is readily absorbed into the intestinal cells. Most of the absorption of thiamin occurs in the jejunum but some can also occur in the ileum (Gropper et al., 2005). Lesser amounts of thiamin are absorbed in the duodenum (Gropper et al., 2005). Depending on the amount of the vitamin present in the intestine, the absorption of thiamin can be either active or passive. At low physiological concentrations of less than 1umol/L, thiamin absorption is active and sodium-dependent (Laforenza et al., 1998).
Absorption is predominantly by passive diffusion when intakes of thiamin are high (Laforenza et al., 1998).

Thiamin in the blood is typically either in its free form, bound to albumin or found as TMP. About 90% of thiamin in the blood is present within the red blood cells (erythrocytes) and not found in the plasma (Tanphaichitr, 2001). In red blood cells, most thiamin is found as TDP. Transport of thiamin into the blood cells is thought to occur by facilitated diffusion whereas transport into other tissues requires energy (Tanphaichitr, 2001).

Most of the free thiamin is taken up by the liver and phosphorylated into TDP after absorption. Conversion of thiamin to TDP requires adenosine triphosphate (ATP) and the enzyme, thiamin pyrophosphokinase (Figure 2.2). TTP is synthesized by the action of a TDP-ATP phosphoryl transferase that phosphorylates TDP. TMP is derived from the catabolism of the terminal phosphate on TDP and is believed to be inactive (Gropper et al., 2005).
Figure 2.2: Conversion of thiamin (Adapted from Gropper et al., 2005).
Total thiamin concentrations in healthy adults are estimated to be between 25-30mg (Filer et al., 1996, Sauberlich et al., 1974). Eighty percent (80%) of the total thiamin concentration is found as TDP, 10% as TTP and the rest of it as TMP and free thiamin (Shils et al., 2006). Skeletal muscles are thought to contain about half of the body’s thiamin and small concentrations are stored in the liver, kidney, heart and brain (Gropper et al., 2005). The half-life of thiamin is between 9-18 days and as a result, body stores can deplete within two to three weeks (Ariaey-Nejad et al., 1970).

Thiamin and its metabolites, when in excess of storage capacity and tissue needs, is excreted in the urine (Goodhart et al., 1988). Degradation of thiamin begins with the cleavage of the molecule into its pyrimidine and thiazole moieties to form free thiamin and a variety of other thiamin catabolites in order to be excreted in the urine (Gropper et al., 2005).

**2.2.4 Functions and Mechanism of Action:**

Thiamin as TDP plays an important role in carbohydrate metabolism in three main enzyme complexes: energy transformation of the pyruvate dehydrogenase (PDH) complex, the alpha-ketoglutarate dehydrogenase (KGDH) complex, and the branched-chain alpha-keto acid dehydrogenase complex (Figure 2.3).
**Figure 2.3**: Thiamin dependent enzymes.

TPP = Thiamin Pyrophosphate (thiamin diphosphate)

TCA = Tricarboxylic Acid Cycle

PPP = Pentose Phosphate Pathway
Thiamin plays the following essential roles in the body:

1) Acts as a coenzyme for energy transformation

2) Acts as a coenzyme for synthesis of pentoses and nicotinamide adenine dinucleotide phosphate (NADPH)

3) Works as a noncoenzyme in membrane and nerve conduction

4) Acts as an antioxidant to combat oxidative stress

### 2.2.4 (A) Function of TDP as a coenzyme:

TDP acts as a coenzyme necessary for the oxidative decarboxylation of pyruvate and alpha-ketoglutarate in the TCA cycle as well as a coenzyme for oxidation of the three branched-chain amino acids isoleucine, leucine and valine (Gropper et al., 2005). These reactions are important for generating energy (ATP). This reaction requires a multienzyme complex known as the PDH complex which is made up of three enzymes (Tanphaichitr et al., 2001; Gropper et al., 2005). Another enzyme complex that thiamin plays a key role in is the alpha-KGDH complex which serves to decarboxylate alpha-ketoglutarate and forms succinyl CoA. Inhibition of these decarboxylation reactions will prevent the synthesis of ATP and of the acetyl CoA needed for the synthesis of fatty acids, cholesterol and other important compounds (Gropper et al., 2005). The inhibition of these reactions prevents synthesis of ATP, increases anaerobic glycolysis and also results in accumulation of pyruvate, lactate and alpha-ketoglutarate in the blood (Gropper et al., 2005). This can lead to changes in the pH and cause depletion in ATP stores, resulting in reduced contractility and subsequently affecting LVEF (Bettendorf et al., 1994; Neubauer et al., 1997).

Decarboxylation of the branched-chain alpha-keto acids, which occurs due to the transamination of valine, isoleucine and leucine, is an oxidative process that also requires TDP (Gropper et al.,
2005). Failure to oxidize the alpha-keto acids results in the accumulation of both the branched-chain amino acids and their alpha-keto acids in blood and other body fluids which is a characteristic feature of the maple syrup urine disease (Gropper et al., 2005). If left untreated, this disease can cause severe brain damage and consequently, result in mortality. People with maple syrup urine disease must limit their intake of protein-containing foods in order to limit high levels of valine, isoleucine and leucine in the body (Gropper et al., 2005).

2.2.4 (B) Synthesis of Pentoses and NADPH:

Thiamin as TDP functions as a prosthetic group of transketolase, a key cytosolic enzyme in the hexose monophosphate shunt (HMS) (Gropper et al., 2005; Filer et al., 1996). The HMS serves as an alternate pathway to glycolysis as a means of generation of NADPH which is needed for fatty acid synthesis and interconversion of sugars, particularly ribose 5-phosphate (Gropper et al., 2005). Transketolase catalyzes a series of reversible reactions that convert excess 5-ribose phosphate and xylulose 5-phosphate to the glycolytic intermediates, fructose 6-phosphate and glyceraldehydes 3-phosphate (Goodhart et al., 1988; Stipanuk et al., 2000). These intermediates are subsequently used for energy production as they are channelled back into the glycolytic pathway and other pathways of glucose metabolism (Stipanuk et al., 2000).

2.2.4 (C) Noncoenzyme roles: Membrane and Nerve Conduction:

In nerve membranes, TTP is thought to activate chloride ion transport (Haas., 1988; Bettendorff et al., 1993; Bettendorf et al., 1994). Thiamin is also thought to be involved in nerve impulse transmission via regulation of sodium channel (Haas., 1988; Bettendorff et al., 1993; Bettendorf et al., 1994). The data on the role thiamin plays in membrane and nerve conduction remains limited.
2.2.4 (D) Thiamin as an Antioxidant:

Epidemiologic evidence has shown that fruits, grains, teas, vegetables, alcoholic beverages which are rich in antioxidants have a cardioprotective effect (La Vecchia et al., 1998; Rimm et al., 1996; Wiseman et al., 1997; Halliwell, 2000). This is in line with the hypothesis that oxidative stress contributes to the pathology of atherosclerosis and vascular dysfunction (Halliwell, 2000; Rosenfeld, 1998). Also, free radicals are involved in ischemia-reperfusion injury and thereby, increase the risk of the loss of viability of myocardial cells, consequently affecting cardiac function (Halliwell, 2000). Although it has been difficult to elucidate the singular effect of a nutrient as an antioxidant, several studies have suggested that thiamin may act as an antioxidant (Stepuro et al., 2005; Lukienko et al., 2000).

There are two different mechanisms that have been hypothesized to explain the antioxidant effect of thiamin. The first pathway proposes the loss of hydrogen and one electron to form thiamin disulfide and nitric oxide as a result of the opening of the thiazole ring (Stepuro et al., 2005). An alternative pathway suggests the separation of two hydrogens and two electrons from NH$_2$ group of the pyrimidine ring as thiamin oxidizes to thiochrome and thiamin disulfide (Lukienko et al, 2000). The way thiamin has been hypothesized to function as an antioxidant is due to the transfer of H (thiazole ring) and 2H (pyrimidine ring) to oxidative substances (Lukienko et al., 2000; Stepuro et al., 2005). One study assessing the antioxidant activity of thiamin found an inhibitory effect of thiamin on lipid peroxidation in rat liver microsomes and on free radical oxidation of unsaturated fatty acids in vitro (Lukienko et al; 2000). Further support for the role of thiamin as an antioxidant comes from a study looking at copper-induced toxicity in Wilson’s disease (Sheline et al., 2004). In Wilson’s disease, copper facilitates the
formation of free radical scavengers that inhibit pyruvate dehydrogenase complex and alpha-ketoglutarate dehydrogenase complex. Thiamin lessened this enzymatic inhibition in vitro (Sheline et al., 2004). Moreover, this group of scientists also found that oral thiamin supplementation restored the activities of liver PDH and KGDH, which had been reduced in the mouse model of Wilson’s disease (Sheline et al., 2004). Most research in vitro has illustrated that thiamin interacts with free radicals and hydroperoxides, undergoes oxidation and therefore, is capable of producing antioxidant effects (Lukieno et al, 2000).

Recent data has indicated benfotiamin, a synthetic S-acyl derivative of thiamin and a lipophilic thiamin diphosphate prodrug, to have direct antioxidant activity (Schmid et al., 2008). One study showed benfotiamine to prevent oxidative stress and oxidative DNA damage induced by angiotensin II in vitro (Schmid et al., 2008). Incubation with benfotiamin also increased TK expression and activity in vitro (Schmid et al., 2008). Another recent study reported a protective effect of benfotiamin on haemoglobin and plasma membrane of erythrocytes against oxidation induced by sodium nitrite in vitro (Marouf et al., 2010). Research has also shown the benefits of benfotiamin in preventing complications of neuropathy, nephropathy and retinopathy found in diabetes mellitus by inhibiting advanced glycation end products (AGEs), which are inversely correlated with vascular complications (Stirban et al., 2007; Stracke et al., 2001). However, there is paucity of literature studying the antioxidant effects of benfotiamin as well as the mechanism of action and clinical value of using benfotiamin in treatment of diabetic complications (Ceylan-Isik et al., 2006; Stirban et al., 2007). Benfotiamin may have mild side effects including skin hypersensitivity and nausea. Effects resulting from long term use of this supplement have not been reported (Ceylan-Isik et al., 2006; Stirban et al., 2007).
2.2.5 Intake Requirements:

The Dietary Reference Intakes (DRI) developed for thiamin is the estimated average requirement (EAR) and recommended dietary allowance (RDA). The basis for the RDA of thiamin relies on the results of metabolic studies examining urinary excretion of thiamin, changes in erythrocyte transketolase activity and thiamin intake data (Gropper et al., 2005). The 1998 Dietary Reference Intakes RDA for thiamin for adult men is 1.2mg/day and for adult women is 1.1mg/day. The EAR for thiamin for adult men and women are 1.0 and 0.9 mg/day, respectively (Gropper et al., 2005; Institute of Medicine, 1998). While RDA is used as a goal for dietary intake for healthy individuals, EAR is applied in the assessment of the prevalence of inadequate intakes within a group (Institute of Medicine, 1998). Thiamin needs differ between men and women as they are based on differences in body size and energy needs. Thiamin intakes during pregnancy and lactation increase to 1.4 and 1.5 mg/day (Gropper et al., 2005). No tolerable upper level for thiamin has been established.

2.2.6 Toxicity:

Oral intake of large amounts (500 mg daily for 1 month) of thiamin has not been linked to any toxic effects as thiamin is readily excreted by the kidneys (Goodhart et al., 1988; Guyton et al., 2006 and Gropper et al., 2005). Excessive thiamin (multiple 100mg boluses) administrated parenterally has been associated with headache, convulsions, cardiac arrhythmia and anaphylactic shock (Institute of Medicine, Food and Nutrition Board., 1998).

2.2.7 Thiamin Deficiency (TD) Disorders:

Thiamin deficiency disorders in humans include Beriberi and Wernicke’s encephalopathy (Shils et al., 2006). One of the first symptoms of TD is appetite loss which results in weight loss. As
deficiency worsens, neurological symptoms such as apathy, confusion, irritability and decreased short-term memory appear and the cardiovascular system is also affected (Horwitt et al., 1949; Williams et al., 1942). Due to thiamin’s relatively short storage time as well as its dependence on intake, marginal TD can occur within 10 days and more severe TD can occur within 21 days if intake is stopped (Lynch et al., 2000).

Three types of beriberi have been identified. Dry beriberi, usually found in older adults, is thought to result from a long term low thiamin intake, especially if coupled with a high carbohydrate intake (Gropper et al., 2005). Dry beriberi is characterized by muscle weakness and wasting in the lower extremities. Wet beriberi is described as a more extensive cardiovascular system deterioration including HF and respiratory problems (Gropper et al., 2005). Clinical signs of wet beriberi include edema, tachycardia, cardiomegaly, and muscle weakness (Shils et al., 2006). Acute beriberi is a fatal form of wet beriberi and is also known as Shoshin Beriberi (Shils et al., 2006). It is found mostly in infants who present with hypotension, tachycardia and circulatory collapse (Loma-Osorio et al., 2011). Beriberi is uncommon in developed countries (Loma-Osoria et al., 2011; Shils et al., 2006). However, TD associated with alcoholism is common in developed countries and is known as Wernicke’s encephalopathy or Wernicke-Korsakoff syndrome (Loma-Osoria et al., 2011).

Alcohol-dependent individuals are at an increased risk for TD as a result of a combination of factors including decreased intake of thiamin from food consumption or due to an increased requirement of the vitamin as well as decreases in thiamin absorption (Gropper et al., 2005). Wernicke’s encephalopathy is characterized by ophthalmoplegia (paralysis of the ocular muscles), nystagmus (involuntary eyeball movement), ataxia, loss of recent memory and
confusion (Gropper et al., 2005). It has been suggested that during Wernicke’s encephalopathy, TD results in a decreased activity of the alpha-KGDH enzyme (Butterworth et al., 1993), leading to irreversible neurological damage, including lactic acidosis and impaired brain metabolism (Butterworth et al., 1993).

Treatment for beriberi and Wernicke’s encephalopathy consists of therapeutic doses of thiamin (~100 mg) (Bridges et al., 1999; Loma-Osorio et al., 2011; Sheline et al., 2004). Response to thiamin administration in TD patients is usually a rapid clinical improvement in symptoms (Lynch et al., 2000).

People with diabetes may also have increased thiamin requirements as a result of impaired carbohydrate metabolism, catabolism, malabsorption and increased urine thiamin excretion (Havivi et al., 1991; Saito et al., 1987). Moreover, some studies have shown that people with diabetes have a low dietary thiamin intake, which may be a result of a restricted intake of foods rich in thiamin such as grain products (Saito et al., 1987; Havivi et al., 1991). This topic is further discussed in section 2.3.1 (F) in relation to HF.

Acute TD is also found to be prevalent (49%) in people who undergo gastric bypass surgery (specifically Roux-Y-Gastric Bypass surgery) due to obesity (Matrana, 2009; Lakhani et al., 2008). Acute TD occurs as nonspecific symptoms including fatigue, nausea, headache, and muscle aches and pains (Matrana, 2009). Bariatric beriberi is associated with small intestinal bacterial overgrowth and antibiotic therapy is often required to treat the complications (Koch et al., 2010). Individuals who have undergone gastric bypass surgery and present with symptoms of TD are given oral thiamin supplement or IV doses of thiamin to treat TD (Matrana, 2009).
Monitoring thiamin levels in individuals post-gastric bypass surgery have been suggested as part of routine clinical practice (Herve et al., 1995; Koch et al., 2010).

TD is common in developing countries where the diet mainly consists of non-enriched grains such as wheat and white rice (Stipanuk et al., 2000). However, TD is also found in developed countries due to other associated risk factors such as age, diseases and lifestyle. Elderly people are at a risk for TD. Iber and colleagues (1982) estimated that 5% of US adults > 60 years of age have impaired thiamin status (Iber et al., 1982). People with diseases that impair absorption of vitamins (e.g. cancers, HIV-AIDS infection, renal failure, inflammatory bowel disease) are also at a greater risk for developing deficiency. Chronic alcoholism, hyperemesis gravidarum and consumption of foods that contain thiaminases or antithiamin factors have been shown to cause TD (Willett, 1998).

2.2.8 Assessment of Nutriture:

Adequacy of thiamin nutriture may be assessed by measuring the stimulation of erythrocyte transketolase (TK) by TPP in haemolysed whole blood or by measuring thiamin in the blood or urine (High Performance Liquid Chromatography) (Finglas et al., 1993; Warnock et al., 1978; Sauberlich et al., 1974).

2.2.8 (A) Erythrocyte Transketolase Activity (ETKA) and Thiamin Pyrophosphate Effect (TPPE):

The measurement of erythrocyte TK activity and thiamin pyrophosphate effect (TPPE) is the most common method of assessing thiamin nutriture in the general population and both are dependent on the TK enzyme activity (Gropper et al., 2005; Institute of Medicine; 1998). In the HMS pathway, the TK enzyme, with the help of TPP, catalyzes the following reaction:
Xylulose-5-Phosphate + Ribose-5-Phosphate $\rightarrow$ Sedoheptulose-7-Phosphate + Glyceraldehyde-3-Phosphate

The rate of production of hexose is used as an indicator for TK activity, which is an indirect measure of TPP levels. ETKA is measured before the addition of TPP and the increase in ETKA after addition of TPP is the thiamin pyrophosphate effect (Akbarian et al., 1968). A large change in enzyme activity implies a relative deficiency of thiamin (Willett, 1998; Sauberlich et al., 1974). An increase in TK activity of >25% is indicative of TD, while an increase in activity of 15% to 25% suggests marginal status and an increase of <15% indicates adequate thiamin status (Gropper et al., 2005).

Several criticisms have rendered this method as inadequate for the measurement of thiamin status. One major limitation is its dependence on the TK enzyme which can be affected by several factors including decreased levels of TK enzyme, altered binding affinity of TK for TPP, loss of TK activity, and low TK activity (Kjosen et al., 1977). Other limitations include the variation in the interpretation of ETKA because of poor inter-assay precision, difficulty in standardization and instability of TK enzyme during storage (Talwar et al., 2000).

### 2.2.8 (B) High-Performance Liquid Chromatography (HPLC):

High-Performance Liquid Chromatography (HPLC), in contrast to ETKA, is considered to be a more sensitive indicator of thiamin status as it directly measures the erythrocyte TPP (Lynch et al., 2000). Measurement of erythrocyte thiamin is considered direct because 1) 80% of thiamin is stored in erythrocytes 2) erythrocytes TPP levels fall before changes in transketolase activity occurs (Warnock et al., 1978), and 3) erythrocyte TPP is stable in frozen erythrocytes and less
susceptible to factors that alter enzymatic activity (Baines et al., 1988). HPLC is also considered to be advantageous in measuring TPP with high precision (5% to 11%) and high recovery rate (87% to 102%) (Baines et al., 1985; Mancinelli et al., 2003; Tallaksen et al., 1991; Talwar et al., 2000; Warnock et al., 1978).

Many different HPLC methods have been reported for the determination of thiamin in food, pharmaceutical preparations, and in biological fluids and tissues (Lynch et al., 2000). The basis for these HPLC methods involve the oxidation of thiamin compounds to thiochrome, which is detected either by fluorescent or ultraviolet detection (Lynch et al., 2000). Fluorescent detection is more sensitive than ultraviolet detection but requires a pre- or post-column conversion of thiamin to thiochrome. Pre-column conversion is considered to be more convenient due to sharper peaks and better resolutions although post-column derivatizations uses less caustic mobile phases which allows for extended column stability and lifetime (Lynch et al., 2000).

**2.2.8 (C) Urinary Thiamin:**

Another way to assess adequacy of thiamin nutriture is by measurement of thiamin in urine by HPLC. Urinary thiamin excretion decreases with decreased thiamin status and excretion is also correlated with thiamin intake (Bayliss et al., 1984). This is illustrated by Oldham et al (1962) who demonstrated a positive correlation between thiamin intake and 24-hour urine excretion in young adults on selected diets.

Urinary thiamin excretion of >40ug is suggestive of TD (Wood et al., 1980). A study by Wood et al (1980) demonstrates the usefulness of urinary thiamin levels in determining dietary thiamin intake. In this study, nineteen healthy volunteers were randomized to receive either a control diet (500 ug thiamin + 5mg thiamin hydrochloride) or a thiamin deficient diet (500 ug
thiamin+placebo). At the end of 5 weeks, the thiamin deficient group had a significant decrease in urinary thiamin excretion whereas, in controls, urinary thiamin excretion rose at the beginning of the weeks and after remained constant (Wood et al., 1980).

However, urinary thiamin levels are not considered a good index of thiamin nutriture as these levels are generally considered to be indicative of recent dietary intake and not an accurate reflection of thiamin status of the individuals (Ziporin et al., 1965 and Willett, 1998). Moreover, urinary thiamin has also been shown to vary with renal function, variable volume of urine excreted and inadequate bladder emptying (Powers et al., 1993; Wood et al., 1977).
2.3 - Heart Failure and Thiamin

Despite advances in therapeutic innovations in cardiology, the disability and mortality associated with HF continues to pose a significant burden on the health care system as well as on the patient (O’Connell et al., 2000). Moreover, there is a concern that the commonly used device and drug therapies in improving heart function may provide little additional benefits to the current treatment regimens (Keith et al., 2001).

Several metabolic abnormalities have been found in the failing myocardium, which may contribute to myocardial dysfunction and progressive HF (Sole, 2002, Neubauer et al., 2007, Ingwall et al., 2004). A study by Neubauer et al., (1997) indicated an impaired energetic state in HF as shown by a low [PCr]:[ATP] ratio. Another group demonstrated an increased risk for the development of systolic and diastolic HF with decreasing energy reserves for ATP synthesis (Ingwall et al., 1990). These abnormalities are deleterious as they limit the contractile reserves of [ATP] for efficient heart pumping, subsequently resulting in worsening HF (Neubauer et al., 1997). Moreover, these processes may adversely affect skeletal muscle metabolism, thereby impacting the functional capacity of patients with HF (Clark et al., 1995).

The pathogenesis of these abnormalities and alterations in cardiac energy metabolism may be linked to hypoxia, oxidative stress and micronutrient deficiencies (Keith et al., 2001, Witte et al., 2001; Sole et al., 2002). Several micronutrients including thiamin, carnitine, selenium, taurine, coenzyme Q10 and vitamin E are found to be decreased or deficient in the state of HF (Keith et al., 2001; Seligmann et al., 1991; Witte et al., 2001; Sole et al., 2002). Some of these nutrients have been linked with individual and additive cardioprotective effects that include improvements in indices of systolic and diastolic function (Jeejeebhoy et al., 2002, Keith et al.,
2001, Shimon et al., 1995). However, considering the discrepant literature on the views of the role nutrients may play in heart function, further research is required to better understand which micronutrients are of importance in managing HF as well as distinguish between mono- or poly-nutrient therapy.

As discussed in section 2.2.4 (A), thiamin is an important coenzyme in energy producing reactions that subsequently fuel myocardial contractions (Keith et al., 2001; Jeejeebhoy et al., 2002). Thiamin is of particular interest in the management of HF for many reasons: 1) HF patients are at a risk of inadequate nutrient intake therefore potentially reduced thiamin intake 2) use of drugs such as loop diuretics can cause hyperexcretion of thiamin and 3) Severe TD may alter cellular energy metabolism, contributing to an overall reduction in energy stores and consequently, potentially resulting in cardiac muscle weakness and reduction in cardiac output (Jeejeebhoy et al., 1988). Therefore, it is possible that TD aggravates the cardiac performance in patients with HF.

**2.3.1 Factors associated with TD in HF:**

Several factors contributing to TD in HF include: Increased metabolic rate, renal failure, malnutrition, elderly age, frequent hospitalizations, use of diuretics, decreased appetite, disease severity and inadequate thiamin Intake (Figure 3.1).
Figure 3.1: Factors contributing to Thiamin Deficiency (TD).
2.3.1 (A) Cardiac Cachexia, Anorexia and Malnourishment:

Cardiac cachexia (as discussed in section 2.1.6 (A)) results in depletion of metabolically active lean body mass, with subsequent decline in function, performance and immune system (Anker, 1999 and section 2.1.6). It is characterized by an imbalance of catabolic and anabolic body systems, which may lead to development of body wasting (Anker et al., 2004; Pittman, 1964). Cachexia is also considered to be a strong independent predictor of mortality (Anker et al., 1997). The frequency of malnutrition in patients with HF increases with the severity of HF (Freeman et al, 1994; Schewengal et al, 1994). For example, one study showed that malnourished adults who had lost 40% of their total body weight had a 35% reduction in their heart weight (Hill., 1992). This can aggravate myocardial dysfunction and increase the risk of mortality. Three mechanisms have been proposed that may contribute to the development of cachexia and these include: 1) dietary deficiency, 2) malabsorption and metabolic dysfunction and 3) loss of nutrients via urinary or digestive system (Anker et al., 2004).

Current nutritional support for the treatment of malnutrition and cachexia in HF population consists of high energy/protein diets using oral supplements or total parenteral nutrition (ADA/DC, 2000; Anker et al., 2004). However, the use of such nutrition regimens has not been particularly successful in improving cardiac function (Paccagnella et al., 1994; Broqvist et al., 1994; Heymsfield et al., 1989). Since cachetic patients with HF experience a higher metabolic rate and suffer from a mismatch between dietary intake of nutrients and nutritional requirements, these patients are at a higher risk of having nutrient deficiencies such as thiamin deficiency (TD). Therefore, it becomes necessary to better understand the extent to which
micronutrient deficiencies might modulate functional capacity and cardiac progression of patients with HF.

2.3.1 (B) Advanced Age:

Thiamin deficiency (TD) is found to be prevalent in elderly individuals with or without HF (Wilkinson et al., 2000 & O’Keefe et al., 2000). The prevalence of TD in elderly individuals without HF is 10-15% and primarily occurs in those of lower socio-economic status or diseased individuals (Bailey et al., 1997; Baum et al., 1984, Wilkinson et al., 1997). TD was found to be 13%-91% in elderly patients with HF (Seligmann et al., 1991; Kwok et la., 1992; Pfitzenmeyer et al., 1994). Biochemical evidence of TD has been reported to range from 13%-48% in elderly individuals who are hospitalized (Kwok et al., 1992; Seligmann et al., 1991; Peppersack et al., 1999 & O’Keefe et al., 2000). All studies comparing thiamin status between young adults and elderly individuals have shown that TD worsens with aging (Pfitzenmeyer et al., 1994 & Wilkinson et al., 2000). Wilkinson et al. (2000) examined whether changes in erythrocyte TPP concentrations in elderly patients could be attributed to age or to other co-morbidities. The authors reported that the lower TPP concentrations in their elderly population were related more to age than to co-existent illnesses (Wilkinson et al., 2000). Though TD is rare in developed countries, it is possible that TD is underdiagnosed as clinical features of this syndrome might not always be clear (O’Keefe et al., 2000). Moreover, subclinical TD may contribute to nonspecific symptoms such as impaired mobility, anorexia and fatigue which are common in elderly individuals (O’Keefe et al., 2000) and thus, difficult to diagnose in relation to TD.
2.3.1 (C) Frequent Hospitalizations:

Frequent hospitalizations of patients with HF have shown to increase the risk of TD. The reported prevalence of TD is 25-40% in hospitalized patients admitted with various conditions including liver disease, gastrointestinal disease, and critical illness (Cruickshank et al., 1987; Lemoine et al., 1980; Truswell et al., 1972). The reported prevalence of TD in hospitalized patients with HF ranges between 23-83% (Hanninen et al., 2006; Kwok et al., 1992; Pfitzenmeyer et al., 1994; Yue et al., 1997). A local study indicated that one-third of hospitalized patients with HF had biochemical evidence of TD (Hanninen et al., 2006). The high prevalence of TD in institutionalized subjects may be related to an increased disease severity, presence of comorbidities, drug intoxication, appetite loss or suboptimal food quality (Lemoine et al., 1980; Pepersack et al., 1999 and Vir et al., 1977).

2.3.1 (D) Disease Severity:

Disease severity has been shown to be a contributor to TD. Several studies have observed associations between worsened NYHA classification or increased LV dysfunction and TD (Brady et al., 1995, Pfitzenmeyer et al., 1994). Patients with severe HF have also been reported to have a higher metabolic rate which may, in turn, influence the requirements for thiamin (Gosker et al., 2000; Stanley et al., 2002). Furthermore, worsened HF requires higher dosages of loop diuretics, which may contribute to excessive thiamin losses in the urine.

2.3.1 (E) Diuretic Therapy:

Several studies have found an impact of diuretic therapy on the development of TD in patients with HF (Lubetsky et al., 1999; Yui et al., 1980; Seligmann et al., 1991; Shimon et al., 1995 & Zenuk et al., 2003). The mechanisms by which diuretics may contribute to TD are: 1) diuretics...
increase urinary flow and thus, the urinary excretion of thiamin also increases (Yui et al., 1980) and 2) diuretics inhibit thiamin uptake by cardiac cells which leads to a reduction in [TPP] production in patients with HF (Yui et al., 1980). Both animal and human studies have reported increased thiamin losses associated with increased diuretic doses. Yui et al (1980) found that the intra-peritoneal administration of furosemide (a loop diuretic) in rats for four weeks resulted in a significantly lower thiamin status and increased urinary thiamin excretion compared to rats that were not on furosemide (Yui et al., 1980). Another group showed that diuretics other than furosemide such as mannitol, acetazolamide and amiloride may also contribute to TD by increasing urinary excretion of thiamin (Lubetsky et al., 1999). Similarly in humans, one study in 25 patients with HF found a 98% prevalence of TD associated with furosemide use at doses of 80mg/d or greater (Zenuk et al., 2003). This group also found that 57% of patients taking a lower dose of diuretic therapy also developed TD (Zenuk et al., 2003). Suter et al (2000) showed a similar association between diuretic therapy and TD in 149 older adult patients hospitalized with HF. Multivariate analysis indicated that the only significant predictor of the change in thiamin status was the use of diuretics (P<0.001) (Suter et al., 2000). In another study conducted in healthy volunteers, it was found that furosemide could lead to TD in a dose-dependent manner (Rieck et al., 1999). Given the potential adverse effects of diuretic-induced TD, it has been suggested to monitor patients for TD who are on chronic use of diuretic therapy as well as to explore thiamin replacement in patients with HF who are on high dosages of diuretics (Dunn et al., 2009).
2.3.1 (F) Co-morbidities:

Patients with HF often have co-morbidities such as diabetes and renal insufficiency which may also contribute to TD. The prevalence of TD is 18%-75% in individuals with diabetes treated with oral hypoglycaemic agents or insulin (Havivi et al., 1991; Saito et al., 1987). In the case of diabetic patients with HF, thiamin requirements may alter due to impaired renal handling of thiamin as well as changes in carbohydrate metabolism (Thornalley et al., 2007; Havivi et al., 1991; Saito et al., 1987). It has been hypothesized that renal mishandling of thiamin may occur due to the decreased re-uptake of thiamin in renal proximal tubules as a result of increased renal clearance and fractional excretion of thiamin (Page et al., 2011; Thornalley et al., 2007). However, the mechanisms by which diabetic patients may be at a risk for TD remains unclear (Page et al., 2011). It has also been observed that ambulatory patients with diabetes have an estimated thiamin intake that is less than the RDA (Saito et al., 1987). Recently, new data has emerged studying other reasons for TD in diabetic patients as well as the relation between TD and diabetic nephropathy (Rabbani et al., 2011; Page et al., 2011). The reason for an increased risk of TD in diabetes has also been postulated to occur due to reductions in the rate of thiamin transport across the intestine, leading to marked impairments in insulin synthesis and secretion (Page et al., 2011). TD has been postulated to impair insulin secretion due to decreased glucose oxidation (Rathanaswami et al., 1991). Diabetes has also been shown to be associated with tissue-specific TD characterized by a marked decrease in plasma thiamin concentration and a decreased activity of TK (Thornalley et al., 2007). Moreover, hyperglycaemia leads to the production of harmful byproducts which may contribute to the development of diabetic complications. In studies assessing risk of TD in patients with diabetes, TD has been shown to
have direct effect on endocrine function, thereby contributing to hyperglycaemia (Page et al., 2011). In two trials, high-dose thiamin therapy (300mg/day) was shown to prevent the development of microvascular complications \textit{in vivo} (Rabbani et al., 2011; Riaz et al., 2011). However, data is limited in view of the actual requirements of thiamin and whether thiamin therapy has any beneficial effects in individuals with diabetes.

In the case of patients suffering from renal insufficiency, it is hypothesized that their condition might be protective against the risk of TD. This protection is attributed to the reduced amount of urine and therefore, decreased urinary thiamin losses. On the other hand, it may also be hypothesized that patients with impaired renal handling might lose more nutrients as a result of increased resting energy expenditure, vomiting, nausea, chronic inflammation and gastrointestinal alterations (e.g. delayed gastric emptying, abnormalities in absorption) (Stenvinkel et al., 1999; Caimi et al., 2005). The aetiology of malnutrition in renal function is complex and there may be several factors including disease stage, co-morbidities, and relative levels of visceral proteins, energy stores and body fat that may regulate nutrient status of patients with impaired renal function (Stenvinkel et al., 1999). Therefore, it is difficult to elucidate the complex interplay of factors that may contribute to nutritional status of these patients. However, nutrient losses have been reported in pre-dialysis patients with severe chronic renal insufficiency and have been linked with factors such as inflammation, oxidative stress and malnutrition (Stenvinkel et al., 1999). Several studies have observed the development of encephalopathy in non-alcoholic patients with end-stage renal disease undergoing haemodialysis and peritoneal dialysis (Hung et al., 2001; Ihara et al., 1999; Ueda et al., 2006). It may be that patients on dialysis are at an increased risk for TD due to poor dietary intake and
increased losses of water-soluble vitamins (Ueda et al., 2006; Hung et al., 2001). Thus, TD might be a contributor to the encephalopathy observed in this population. Studies of thiamin replacement have shown improvements in the symptoms of encephalopathy observed in dialysis patients (Hung et al., 2001; Ihara et al., 1999). However, the literature remains largely limited in view of the TD in renal insufficient and diabetic patients.

Given the complex interplay of various factors that may contribute to TD in patients with HF, it becomes necessary to further investigate these factors simultaneously. This will help in identifying patients with HF at risk of TD.

2.3.2 Studies:

This section explores studies relevant to investigations of factors which may contribute to TD, the prevalence of TD found in patients with HF and implications of thiamin replenishment in modulating heart function.

2.3.2 (A) Animal Studies:

One of the first studies to determine a relationship between TD and diuretic use was conducted by Yui et al (1980) in male Wistar rats. After 4 weeks of furosemide administration, thiamin concentration and TK activity were significantly decreased and the thiamin pyrophosphate effect (TPPE) was significantly increased in the blood. Moreover, these authors also showed a significant increase in urinary thiamin excretion in rats that were administered furosemide. The authors concluded that long term furosemide use can result in TD (Yui et al., 1980). Previously, the authors had shown decreased blood thiamin levels, decreased erythrocyte TK activity and increased TPP effect in patients with HF on long-term diuretic use (Yui et al, 1978). However,
since the study (Yui et al., 1978) is communicated in a foreign language, we are unable to fully comment on the details of this trial and hence, it will not be discussed in subsequent literature. On the contrary, Lubetsky et al (1999) examined the mechanism of furosemide-induced urinary thiamin loss in rats and failed to find any evidence of diuretic-specific thiamin loss. Instead, these authors concluded that urinary thiamin loss occurs via a non-drug-specific mechanism and occurs as a result of the increase in urinary flow observed due to diuresis (Lubetsky et al., 1999). Although it has been suggested that diuretics contribute to thiamin excretion, this relationship remains controversial. Zangen et al (1998) further extended the findings of Yui et al. (1980) and Lubetsky et al. (1999) by examining the mechanism by which diuretics, specifically furosemide and digoxin, may cause TD in rat cardiac cells in culture. These authors reported that both furosemide and digoxin caused a significant decrease in thiamin pyrophosphate levels in cardiac cells. It was found that diuretics exhibited a dose-dependent decrease in thiamin uptake by cardiac cells. Moreover, co-administration of these drugs resulted in an additive effect on inhibition of thiamin uptake (Zangen et al., 1998). The data above indicate that chronic use of diuretics, drugs which are commonly prescribed in the treatment of HF, may contribute to TD.

2.3.2 (B) Prevalence Studies:

Several studies have evaluated the thiamin status of patients with HF and shown a prevalence of TD ranging from 3% to 91% (Seligmann et al., 1991; Kwok et al., 1992; Levy et al., 1992; Pfützenmeyer et al., 1994; Brady et al., 1995; Hanninen et al., 2006 & Abou-Hashem et al., 2009). The breadth of this range reflects variations in sample size, underlying nutritional status and disease state of subjects, use of medications and supplements and differences in the measurement techniques for the assessment of thiamin status (Wooley et al., 2008).
Seligmann et al., (1991) conducted a study to investigate the prevalence and significance of TD in 23 elderly hospitalized patients with chronic HF on long-term furosemide therapy (80-240 mg/d, for 3 months) who were compared to 16 age-matched hospitalized controls. Thiamin status was measured by the erythrocyte transketolase activity assay (ETKA) and urinary thiamin excretion. The prevalence of TD was reported to be 91% in patients with HF on furosemide therapy, which was significantly higher compared with the prevalence of TD of 12% in controls ($p<0.001$). The authors noted a high urinary thiamin excretion in the majority of the patients on furosemide therapy compared with that of their controls although the difference was not found to be significant. Similarly, Pfitzenmeyer et al., (1994) assessed the prevalence of TD in 35 elderly hospitalized patients with HF and compared them with 35 elderly inpatients with diagnoses other than HF. Thiamin status was measured using the thiamin pyrophosphate effect (TPPE) and high performance liquid chromatography (HPLC). The prevalence of TD was found to be 11.5% in patients with HF which was not significantly different in comparison with the 6% prevalence rate in non-HF patients. Moreover, the authors did not find differences in TD between patients on furosemide treatment and those not taking furosemide (Pfitzenmeyer et al., 1994).

Some studies have shown a low prevalence of TD in patients with HF. Levy et al., (1992) investigated the prevalence of TD in a group of younger HF outpatients (n=38, mean age 47 years) on diuretic therapy, predominantly furosemide (20-960mg/d). Using the ETKA method, the prevalence of TD was 3%. To explain the discrepancy from Seligmann et al., it was noted that advanced age may be a risk factor for TD in patients with HF. However, TD was also not found to be common in elderly population with HF by another group (Kwok et al., 1992). Kwok
et al., (1992) compared the thiamin status of 37 elderly hospitalized HF patients with that of 35 elderly patients with diagnoses other than HF and with that of 41 healthy elderly people. The prevalence of TD, as measured by TPPE, was significantly lower (13%) in HF than the non-HF group (29%) but not significantly different from that of the elderly healthy population. This study was limited by lack of data on the type, dose and duration of the diuretic administered to the patients. Furthermore, the non-HF group from this study was comprised of individuals with a wide range of comorbidities, which in and of themselves may have been associated with TD (Leslie et al., 1996).

The studies conducted thus far to assess the prevalence of TD in patients with HF are limited by their lack of rigorous data on the dietary intake of subjects. In response, Brady et al., (1995) conducted a study to assess the prevalence of TD using ETKA and sought to evaluate dietary thiamin adequacy with a semiquantitative food frequency questionnaire (sq-FFQ) in patients with HF on furosemide therapy. The prevalence of TD was found to be 21% in patients with HF (n=38). Of the TD patients, the majority (88%) had a dietary thiamin intake that met the criteria for inadequate intake (based on the RDA). However, the study was limited due to its heterogeneous (inpatients and outpatients) population and small sample size. Moreover, most prevalence studies to date have used indirect methods of assessing thiamin status. Yue et al., (1997) evaluated the erythrocyte [TPP] levels using HPLC in 81 hospitalized patients with HF on furosemide therapy and compared these with 18 control subjects. The authors did not find a significant difference in the erythrocyte TPP concentration between the HF patients and controls. The findings of this study was explained by hypothesizing that this population had adequate thiamin intake due to high content of thiamin found in Swedish diet.
Thus far, trials aimed at defining the prevalence of TD in patients with HF have been hampered by their small sample sizes. In 2006, Hanninen et al. conducted a large cross-sectional study to assess the prevalence of TD using the HPLC method in 100 hospitalized patients with HF on optimal long-term furosemide therapy (60mg/d for 14 months) and compared these with erythrocyte [TPP] from 50 matched control subjects. One-third of the hospitalized patients with HF were found to have biochemical evidence of TD compared with 12% in control subjects ($P<0.007$). Abou-Hashem et al., (2010), recently compared the thiamin status using HPLC of 63 elderly patients with HF on furosemide therapy with 63 age-matched controls. The prevalence of TD was 27% in the patients with HF compared with 14% in controls, although this was not significant ($p=0.08$). However, these authors found a significant correlation between TPP levels and LVEF ($r=0.63, p<0.001$).

2.3.2 (C) Randomized Controlled Trials:

From the above mentioned studies, the relationship between TD and worsening symptoms remains unclear as does the efficacy of supplementation. In addition, TD may also be a feature of the late stages of ADHF, which remains to be elucidated. In view of these arguments, several studies of thiamin supplementation and replenishment in TD individuals with HF have been conducted. Freye et al., (1982), conducted the first clinical study assessing the potential clinical use of thiamin supplementation in HF. These authors observed a significant increase in systolic arterial pressure and central venous pressure after treatment with incremental doses of thiamin (thiamin administered intravenously until a total dose of 50mg/kg was given) in six patients with pulmonary or myocardial impairment. It was suggested that thiamin may serve as a mild
peripheral vasodilator in the failing heart, thereby improving cardiac performance (Freye et al., 1982).

Seligmann et al (1991), assessed the effect of thiamin replacement on left ventricular function in six patients with HF who received IV thiamin twice a day for 7 days followed by oral thiamin supplementation of 200mg/d. The authors reported a significant decrease in TPPE measurements together with improvements in functional capacity of one NYHA class in all six patients. The LVEF, measured by echocardiography, increased in four out of the five patients while remaining unchanged in one. Similarly, Pfitzenmeyer et al (1994) randomized 35 hospitalized patients with HF to receive either 200mg/d of IV or intramuscular thiamin daily for one week and compared them with a group not receiving thiamin. The authors demonstrated a significant improvement in thiamin status in individuals on thiamin supplementation in comparison with individuals without thiamin supplementation ($P<0.026$).

Shimon et al (1995) completed a follow-up study to assess the effect of thiamin repletion on thiamin status, functional capacity and LVEF in 30 patients with HF who were on furosemide therapy (atleast 80mg/d) for three months. Patients were randomized to receive either IV thiamin (200mg/d) or a placebo for one week ($n=15$ in each arm). Following this, all patients received an oral thiamin supplement (200mg/d) for six weeks. The group on IV thiamin showed increases in thiamin level, measured by TPPE, as well as a significant improvement in LVEF ($p<0.05$) after one week. After six weeks, patients receiving oral thiamin supplements showed a significant improvement in LVEF ($p<0.001$), mean NYHA class ($p<0.01$) and plasma thiamin levels ($p<0.01$). The authors concluded that thiamin repletion improved left ventricular function and biochemical evidence of TD in patients with HF on chronic furosemide therapy. This may
be an important finding as an increase in ejection fraction has been associated with a favourable effect on survival in patients with HF (Leslie et al., 1996).

Limited data exists on the role of thiamin supplementation in an acute setting. Smithline et al (2007) conducted a small trial in which patients with ADHF (n=50) were randomized to receive either a placebo or IV thiamin (100mg) within 30 minutes of arrival at the emergency department. The authors did not find any differences in their primary outcome measures which included change in dyspnea score from arrival to enrolment (4-hour change) and duration of hospitalization. However, several limitations are evident in this trial which include a small sample size, lack of measurement of initial thiamin levels, and baseline differences of dyspnea score between thiamin and placebo groups, making interpretation of this study challenging. Considering this is the only study to date assessing the importance of thiamin in an acute decompensated setting; it becomes necessary to further research the role thiamin may play in managing ADHF.

Collectively, the studies conducted thus far are limited by their sample size, the heterogeneity of inclusion criteria, lack of randomization, lack of information on dietary intake and differing methods for assessment thiamin status. Although the results of most studies are discrepant in regards to the prevalence of TD, none have ruled out a potential role of thiamin supplements on positively influencing cardiac function.

Given the potential adverse effects of TD on myocardial function and the potential of thiamin replenishment to improve cardiac function, it is important to further elucidate the mechanisms by which thiamin may play a role in potentially enhancing cardiac performance. Furthermore, the majority of the studies thus far have been conducted in patients with chronic HF whereas
data on the role thiamin may play in ADHF is limited. Our group has previously studied the prevalence of TD in ambulatory patients with HF. This thesis will extend the previous work exploring the prevalence of TD and the outcomes of patients with HF in response to their erythrocyte [TPP] levels to further understand the implication of TD on cardiac function.
3.0 - Rationale

Thiamin acts as a coenzyme in many energy producing reactions, and therefore, TD may contribute to the observed depletion of ATP reserves in patients with HF. Reductions in ATP production may contribute to a lower cardiac output and myocardial weakness by limiting the energy available for myocyte contraction. Therefore, the risk of TD might adversely influence disease progression as well as outcomes in mortality and morbidity of patients with HF. Correction of TD through supplementation has been shown to have potential to improve cardiac function in patients with HF and thereby, have the potential to positively influence cardiac remodelling.

Risk factors such as malnutrition, increased metabolic rate, frequent hospitalizations, use of diuretic medications and elderly age have been shown to contribute to TD in patients with HF. Studies have shown a wide range in the prevalence of TD in patients with HF. This wide variation can be related to the inherent differences in the individual studies such as underlying nutrition status of patients, medication usage, disease severity and technique for assessment of thiamin status. To date, studies have been limited by small sample sizes, use of indirect measurement of thiamin status and lack of dietary assessments. Moreover, majority of the studies have focused on the thiamin status of hospitalized patients, whereas ambulatory patients with HF have received little attention.

Patients with chronic HF may experience adverse outcomes as their disease progresses. These outcomes include development of ADHF, hospitalizations, mortality, myocardial infarctions and/or arrhythmias and other adverse events (such as renal dysfunction, gastrointestinal problems). Although current drug and device therapeutics have improved morbidity and mortality in patients with HF, additional interventions are urgently required to delay progression
of HF and improve outcomes. While the risk of being thiamin deficient is linked with the development of arrhythmias, encephalopathy in renal dysfunction and decreased functional improvements post-MI; to our knowledge, no studies have examined the impact of TD on outcomes in patients with chronic HF.

Although chronic HF is progressive and often fatal, patients can become stabilized through possible improvements in myocardial dysfunction and remodelling. Therefore, patients with ADHF can often revert back to the state of chronic HF. There has been interest in various factors, including dietary noncompliance, which may contribute to the development of ADHF. However, data is largely lacking on the information on dietary adequacy and nutrient losses in patients with ADHF. To date, we were unable to find studies of prevalence of TD specific to this condition and there is only one study (Smithline et al., 2007) looking at the implications of thiamin supplementation in patients with ADHF. However, this study has several limitations including no prior knowledge of thiamin status, small sample size and significant confounding issues (Smithline et al., 2007).

To our knowledge there is no evidence to date that demonstrates beneficial or adverse outcomes associated with thiamin status in compensated, appropriately medicated HF outpatients.

Therefore, this study aims:

1) To determine whether retrospectively collected thiamin status is associated with adverse cardiovascular outcomes, including ADHF, in a population of stable, ambulatory compensated patients with HF and;

2) To investigate the prevalence of TD in ambulatory patients with HF
3.1 Study Design:

The patients included in this project are part of a larger study that is divided into three trials. We have previously conducted a cross-sectional study to investigate the prevalence of TD in a large group of ambulatory patients with compensated HF on stable medications. Subsequently, we followed a cohort to evaluate the incidence of adverse events and to link these incident rates with levels of erythrocyte [TPP] in ambulatory patients with compensated HF. Some of these patients are also part of an ongoing randomized controlled trial assessing the effect of thiamin supplementation in improving heart function.

3.2 Hypothesis:

3.2 (A) Primary Hypothesis:

1) Incidence of ADHF will be inversely related to erythrocyte [TPP] levels.

2) The prevalence of TD will be estimated at 20% in our population of outpatients with HF.

3.2 (B) Secondary Hypothesis:

1) The rate of total adverse events including all-cause hospitalization, mortality, arrhythmias and myocardial infarctions will be inversely related to erythrocyte [TPP] levels.

2) Age, thiamin intake, HF severity and use of diuretic medications will be associated with prevalence of TD.
3.3 Objectives:

3.3 (A) Primary Objective:

1) To determine cumulative ADHF events associated with low, mid and high erythrocyte [TPP] levels.

2) To determine the prevalence of TD using HPLC in ambulatory patients with HF.

3.3 (B) Secondary Objectives:

1) To determine cumulative events for total adverse events including all-cause hospitalizations, mortality, myocardial infarction and arrhythmias in association with low, mid and high erythrocyte [TPP] levels.

2) To investigate the correlation between TD and clinical and demographic variables of HF including HF severity, nutritional status, thiamin intake and diuretics.
4.0 - Methodology

4.1 Study Participants
Participants included in this project were recruited for participation in a study investigating the prevalence of TD in ambulatory patients with HF. During the first phase of the study, I collaborated with another graduate student (Parastoo Azizi-Namini) to recruit and collect data on the first 100 patients participating in the prevalence study. Following this, I extended the sample size of the study to 150 patients. Subsequently, I submitted a research ethics approval and carried out the study protocol to investigate incidence of adverse events in relation to thiamin status of these ambulatory patients with HF.

4.1 (A) Prevalence Study:
Participants were approached and recruited in the cardiology outpatient clinics at St. Michael’s Hospital (SMH), Mount Sinai Hospital (MSH) and Toronto General Hospital (TGH).

Inclusion Criteria:

1) Primary diagnoses of ischemic, dilated, idiopathic or valvular HF

2) Evidence of impaired LV function (LVEF<45%) as indicated from an echocardiogram, MUGA scan or cardiac catheterization, which is performed within the last five years.

3) NYHA class I-IV symptoms

Exclusion Criteria:

1) Are unable or unwilling to provide informed consent

2) Have any concurrent condition which would result in TD, namely gastrointestinal disorders (E.g Crohn’s disease, ulcerative colitis), known magnesium deficiency, liver or kidney disease, thyrotoxicosis, known vitamin B12 or folate deficiency, prolonged
diarrhoeal disease, dialysis, fever or infection, recent myocardial infarction, coronary revascularization (percutaneous coronary intervention or coronary artery bypass surgery (Within 3 months) ).

3) Are rapidly deteriorating, or not on stable medication regimen (> 2months)

4) On experimental medications

5) Consume excessive alcohol (>3 drinks per day), have a documented history of alcoholism or have documented alcoholic cardiomyopathy

6) Are pregnant

Eligible and interested participants were given an information sheet about the study by the study coordinator. Subsequently, an appointment was booked for each interested patient to participate in the study. Participants were provided with a $5 Tim Hortons® gift card (registered trademarks owned by TDL Marks Corporation, Canada) and were compensated for their transportation.

4.1 (B) Outcome Study:

Patients in this analysis included some that were previously enrolled in a study that assessed the prevalence of TD in ambulatory patients with HF. A total of 150 patients were approached to participate in the study. Data is available for 138 of these patients. The cohort included eligible ambulatory patients with HF who were consecutively enrolled between September 2009 and March 2011 from multidisciplinary heart failure programs at three tertiary care hospitals (St. Michael’s Hospital, Mount Sinai Hospital and University Health Network). The outcome study included patients who had measured erythrocyte [TPP] levels and who provided informed consent over the telephone.
Both studies were approved by the Research Ethics Board at St. Michael’s Hospital, Mount Sinai Hospital and University Health Network (Toronto, Canada).

4.2 Collection of Information

4.2 (A) Prevalence Study:

Following informed consent, participants attended a screening visit which consisted of collecting anthropometric data, demographic variables, and information on causes, symptoms and duration of HF as well as measures of cardiac function including LVEF and NYHA class. A detailed medical history including the name of each medication and its dosage was also recorded. Information on over the counter medications and supplements was collected. Each patient’s nutritional status was assessed using the Body Mass Index (BMI), Subjective Global Assessment (SGA) technique and a semi-quantitative food frequency questionnaire (sq-FFQ). Each participant’s provided a fasting blood sample for the determination of the levels of erythrocyte TPP by using HPLC according to the procedure outlined by Mancinelli et al (2003).

4.2 (A1) Study Protocol:

On the study day, participants had their height and weight measured, filled out the appropriate questionnaires including one on dietary assessment and gave a fasting blood sample.

4.2 (A1.1) Nutritional Assessment:

1) BMI:

According to the Health Canada guidelines, BMI is used as a proxy for human body fat based on an individual’s height and weight. There are four categories of BMI ranges in the Canadian weight classification system (Table 4.1):
Table 4.1: Body Mass Index (BMI) and its associated categories:

<table>
<thead>
<tr>
<th>BMI Range</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;18.5\text{kg/m}^2)</td>
<td>Underweight</td>
</tr>
<tr>
<td>19-24.9 kg/m(^2)</td>
<td>Normal</td>
</tr>
<tr>
<td>25-29.9 kg/m(^2)</td>
<td>Overweight</td>
</tr>
<tr>
<td>(\geq30\text{kg/m}^2)</td>
<td>Obese</td>
</tr>
</tbody>
</table>
In order to calculate the BMI, we measured the weight of our patients using an electronic scale (Scale-Tronix, Wheaton, IL). Height was obtained from patient’s clinical charts. BMI (kg/m$^2$) was obtained by dividing the body weight (kg) by the square of the height (m).

2) **SGA:**

SGA is used as an assessment for risk of malnutrition based on a history and a physical examination of the patient (Detsky et al., 1987). In the questionnaire, we assessed the amount and rate of weight loss, changes in the habitual dietary pattern, presence of any persisting adverse gastrointestinal symptoms in the last 2 weeks and changes in functional capacity. By physical examination, we assessed the patient’s subcutaneous fat loss and presence of muscle wasting as well as edema and ascites. The SGA evaluation was performed by the study coordinator who was trained by a Registered Dietitian. Based on the results of history and physical examination, patients were placed in one of the three SGA categories for nutritional status. These categories are A= well-nourished, B=mild to moderately malnourished and C=severely malnourished.

3) **sq-FFQ:**

sq-FFQ was used to estimate recent thiamin and macronutrient intake in the subjects within the past month. The one month time frame was suitable since thiamin stores in the body can deplete within two to three weeks of a thiamin deficient diet (Ariaey-Nejad et al., 1970). The Block Brief 2000 FFQ was used, modified from its full-length version to a reduced version (Block, 2000; Block et al., 1990), and previously validated (Hanninen et al., 2006) to provide assessment of nutrient intake in a population of patients with HF.
To help facilitate estimation of portion sizes, a sheet containing photos of different portion sizes (originally part of the Block 2000 FFQ) was provided to the patients. The dietary information collected was evaluated by the ESHA Food Processor SQL nutrient analysis software with the Canadian Nutrient File as an add-in (ESHA Research Inc, version 10.8.0, Salem, Oregon and 2010 Canadian Nutrient File). All data entered into the software was double-checked by a trained FFQ analyst.

4.2 (A1.2) Evaluation of NT-pro BNP and Erythrocyte [TPP] Levels:

1) Collection of Blood Sample:

Participants were asked to fast overnight but were allowed to take their medications with water. St. Michael’s Hospital laboratory phlebotomists collected a fasting blood sample from study participants. Four 4.0ml vacutainer tubes with anticoagulant agent (ethylenediamine-tetraacetic acid (EDTA)) were collected to assess erythrocyte [TPP] levels.

One 5.0 ml SST vacutainer was used for the evaluation of plasma NT-proBNP of patients. Plasma NT-proBNP levels were determined by the SMH laboratory phlebotomists using the electrochemiluminescence immunoassay (ECLIA, Rocha Diagnostics). The normal reference ranges (age-dependent) were: age < 50: NT-proBNP < 125ng/L, age 50-75: NT-proBNP < 250ng/L, age >75: NT-proBNP < 500ng/L.

2) Erythrocyte [TPP] Extraction:

Collected blood samples were kept on ice until they were transferred to the research laboratory. Within two hours of collection, samples were centrifuged (1500 g, 30 minutes, 4°C) to separate the plasma from erythrocytes. Erythrocyte [TPP] was extracted
and analysed according to the procedure outlined by Mancinelli et al (2003). Briefly, the erythrocytes were separated from the buffy coat and washed four times with four volumes of saline. The sample was centrifuged after each wash (1000g, 10 minutes, 10°C. The fourth wash was centrifuged at 1400 g, 20 minutes and 10°C. This allowed us to obtain well-packed red blood cells. 20 ul of trichloroacetic acid (TCA) (Sigma-Aldrich Co., Oakville, ON) was added to the 1:1 mixed cell suspension of red blood cells and saline and incubated for 1 hour in the dark at room temperature. Samples were centrifuged again at 1900 g, 20 min and 20°C to release the supernatant which was transferred and washed with 5 volumes of water saturated diethyl ether to remove TCA. The extract was filtered through 1ml syringe, using MINISART RC4 filters 0.45um (VWR, Toronto, ON) and stored at -80°C until analysis.

3) Erythrocyte [TPP] Analysis by HPLC:

Erythrocyte [TPP] was determined using HPLC according to the procedure used by Mancinelli et al. (2003). The chromatography was performed with the column Ultra Amino 5µm, 250 x 4.6 mm with an Ultra Amino 5µm, 10 x 2.1 mm Guard Cartridges (Restek, Bellefonte, PA). The mobile phase was 65% 85nM potassium phosphate buffer (pH 7.5) and 35% acetonitrile. The HPLC was performed by precolumn, alkaline oxidation of TPP to thiochrome. A fluorescence detector was set at an excitation wavelength of 375 nm and an emission wavelength of 430 nm. Based on the results of the study by Mancinelli et al(2003) on the plasma TPP levels in healthy individuals, the normal levels of erythrocyte TPP is at 222.2 + 56.3 nmol/l and an erythrocyte TPP ≤ 180 nmol/l is considered thiamin deficient. This method has a high recovery rate and a low
elution time of 20 minutes. The injection volume was 50ul. The intra-day HPLC precision was evaluated by processing duplicates of each patient blood sample separately. The inter-day HPLC precision was verified by running same patient samples on two different days. To evaluate extraction recovery, washed erythrocytes were spiked with 10ul of 10uM of TPP standard before the addition of TCA and deprotenization. The difference in the erythrocyte [TPP] between the regular and spiked samples was measured to obtain the percent recovery.

4.2 (B) Outcome Study

4.2 (B1) Study Protocol:

Participants from the prevalence study were called and explained the following: 1) The purpose of the call, 2) The purpose of the outcome study and 3) Whether they would agree to participate in the outcome study or not? Participants that agreed were then further asked questions regarding their outcomes since the time of their screening visit. Changes in medications including diuretics and use of supplements including thiamin supplements were also assessed. The form used during the telephone interview to note down responses is appended (10.1).

Patients participating in the screening study were contacted by telephone by a study coordinator to follow-up on subsequent events that they may have encountered since their initial study visit. Consent to inquire about their events was obtained on the telephone. Participant events were compared to the events recorded in the patient’s hospital chart and adjudicated by a cardiologist blinded to the erythrocyte [TPP] levels. Events were assessed from the time of enrolment to the completion date of data collection (telephone interview). The study collection forms are appended and include information on ADHF, death, transplant, hospitalizations or emergency
visits, arrhythmias, MI/angina, other adverse events, medication and dose changes, supplement intake and dietary counselling (Appendix 10.0).

4.2 (B2) Outcome variables:

The primary outcome for this study is the rate of ADHF in tertiles of erythrocyte [TPP] levels. Secondary outcomes include all-cause hospitalizations, emergency visits, arrhythmias, MI/angina, other adverse events and death or transplantation. Outcomes are defined below.

1) **ADHF:**

This category includes patients who were hospitalized with a primary diagnosis of ADHF and patients who visited the emergency department for the management of ADHF. Admittance to a hospital as well as the purpose of visiting the emergency department were confirmed by discharge summaries and reviewed by the cardiologist blinded to erythrocyte [TPP] levels. The presence of ADHF was defined according to the occurrence of symptoms of worsening HF and the use of IV diuretics based on the diagnoses of the attending physician (see Appendix 10.5). The date of presentation of ADHF was recorded (defined as the date the attending physician diagnosed ADHF).

2) **All-Cause Hospitalization:**

Includes patients admitted to the hospital for any cause, including cardiovascular causes (e.g. documented MI, unstable angina, CABG, CHF, PCI, arrhythmia and vascular causes (stroke)). Date of admission (date on which patient was hospitalized for known causes) and discharge (date on which patient was discharged from hospital or if patient died in the hospital, date of discharge is recorded as date of death) was recorded (Appendix 10.6).
3) **Emergency Visits:**

This category includes patients presenting to the emergency department for any cause (management of ADHF symptoms, noncardiovascular and cardiovascular causes). The date that the patient presented to the emergency department for relief of symptoms was recorded (Appendix 10.6).

4) **Mortality:**

Mortality outcome includes patients who died during our followup period. The event of mortality was communicated by a family member and subsequently, we confirmed the cause of mortality by reviewing the patient charts. Medical Records/Source Documents as listed in the study case report form (Appendix 10.3) were collected and appended to the study chart for confirmation of death/transplant. Date of death was recorded. Death was defined as cardiac, noncardiac or unknown. Confirmed cause of mortality was adjudicated by a cardiologist. Cardiovascular death included causes such as sudden cardiac death, myocardial infarction (MI), unstable angina or other CAD; vascular death (e.g stroke, pulmonary/arterial embolism); HF or arrhythmia. Noncardiovascular death included respiratory failure, pneumonia, cancer, trauma, suicide or any other already defined cause (e.g liver disease or renal failure) (Cannon et al., 2001; Radford et al., 2005, Arnold et al., 2006) (Appendix 10.3).

5) **Myocardial Infarction:**

This outcome included individuals who had a documented MI based on the criteria defined by American College of Cardiology and Canadian Cardiovascular Society guidelines (Cannon et al., 2001, Radford et al., 2005; Arnold et al., 2006). This diagnosis of MI was based on the observation of: a gradual rise and fall of troponin-T/ troponin-I or a more rapid rise and fall of
creatine-kinase/muscle-brain type (CK-MB)) which are biochemical markers of myocardial necrosis with at least one of the following: a) ischemic symptoms b) development of pathological Q waves on the ECG c) ECG changes indicative of ischemia (ST-segment elevation or depression) and d) coronary artery intervention or pathological finding of an acute MI. Angina was defined as recurrent ischemic pain suggestive of cardiac origins (Cannon et al., 2001; Radford et al., 2005; Arnold et al., 2006) (Appendix 10.2).

6) Arrhythmias:
This category included patients who experienced arrhythmias as defined by the Canadian Cardiovascular Society (CCS) and American College of Cardiology (ACC) guidelines (Cannon et al., 2001, Radford et al., 2005; Arnold et al., 2006). According to the CCS and ACC, atrial arrhythmia is documented by atrial fibrillation/flutter or supraventricular tachycardia requiring treatment (such as cardioversion or drug therapy). Ventricular arrhythmia is defined as ventricular tachycardia or ventricular fibrillation requiring cardioversion and/or intravenous antiarrhythmics (Cannon et al., 2010) (Appendix 10.2).

7) Diet Counselling:
Patients were assessed for diet counselling defined as advice/counselling given by a certified registered dietitian (RD) that emphasized fruit, vegetables, low-fat dairy products, moderate sodium restriction and increased consumption of omega-3 fatty acids. Patients were also asked whether they had attended a rehabilitation center and seen a dietitian at the center for dietary assessment (Appendix 10.1).
4.3 Statistical Analysis:

All statistical analysis was performed with IBM SPSS Statistics version 20.0.

4.3.1 Prevalence Study:

The determination of sample size was based on the estimated prevalence of TD of 20% based on the study by Brady et al. (1995). The sample size was calculated using the following formula (Naing et al., 2006) which is designed for prevalence studies:

\[
\text{Sample size (n)} = \frac{Z^2 \cdot P \cdot (1-P)}{d^2}
\]

where;

\[Z = Z \text{ statistic for level of confidence}\]

\[P = \text{expected prevalence}\]

\[D = \text{precision}\]

Considering the level of confidence of 95% and precision of 7%, the required sample size was 126 subjects. Therefore, our sample size of 138 patients was sufficient for the prevalence study.

Parametric analysis of the continuous data was performed using the independent samples t-test and presented as mean (SD). One-way ANOVA was used to test for differences in three or more groups, including LV function and diuretic types. Categorical data was analyzed with chi-squared test or Fisher’s Exact Test when the number in any cell was less than five. Erythrocyte [TPP] levels were determined to be normally distributed, therefore parametric statistics were used.

Non-parametric analysis of continuous data was performed using the Mann-Whitney U test and presented as median (range). Non-parametric analysis was used for dietary level of thiamin intake, total thiamin intake and NT-proBNP which was found to be not normally distributed.
Spearman’s rho coefficient was used to determine the association between erythrocyte [TPP] and dietary thiamin intake, total thiamin intake and NT-proBNP.

P-values were two-tailed and a p value < 0.05 was considered significant.

4.3.2 Outcome Study:

Patients were equally divided into lower, middle and upper tertiles based on erythrocyte [TPP] levels. This approach was planned prospectively and since no identifiable standard cutpoints are readily available for erythrocyte [TPP] levels, statistical methods were used to define the cutpoints (Mandrekar., 2012).

Continuous variables are described by using descriptive statistics including means, medians and standard deviations (SDs). Frequencies were used for categorical variables. Mann-Kendall tau-b was used for bivariate comparisons of continuous variables. The choice of Kendall tau-b was based on its ability to compute the direction of the trend for equally spaced data (Field., 2009). Pearson’s chi-square test was used to determine the differences among tertiles as they relate to categorical variables and the p-value of linear by linear association was chosen to signify the direction of trend.

The follow-up period is computed in person-days and calculated as time to event or if no event occurred, time to follow-up (date of contact). The cumulative event probability for time-to-event outcomes was calculated by Kaplan-Meier method. To test the hypothesis that total adverse events are inversely related to erythrocyte [TPP] levels, the tertiles were compared with each other. P-values were two tailed and a p-value of < 0.05 was considered significant.
5.0 - Results

5.1 Study Subjects:

Participants included in this analysis were enrolled in a study assessing the prevalence of thiamin deficiency in ambulatory patients with HF. Comprehensive patient demographics, relationship of erythrocyte [TPP] to cardiac factors and dietary intake from this cohort will be described below. Participants enrolled in this study were recruited between September 2009 and February 2011 from St. Michael’s Hospital (SMH), Mount Sinai Hospital (MSH) and Toronto General Hospital (TGH) heart function clinics. A total of 150 patients took part in the prevalence trial and were approached to participate in the study assessing the outcomes in relationship to erythrocyte [TPP] levels. Data is available from 138 patients with HF in the final analysis as 6 patients declined participation, 4 patients were lost to followup and 2 patients’ erythrocyte [TPP] levels were not successfully analyzed. The data for the first 100 patients has been reported in Parastoo Azizi-Namini, MSc Thesis, University of Toronto. During a telephone interview, information on changes in health condition, medications, visits to the emergency department, hospitalizations and changes in supplement uses were recorded (Appendix 10.2).

5.2 Subject Characteristics:

Patient characteristics are summarized in Table 5.1. The majority of our participants were male (76%) and Caucasian (79%) with the mean age of 63 years. The estimated body mass index (BMI) was found to be within the following BMI ranges of 19 kg/m² to 30 kg/m². Only one patient was found to have a BMI ≤ 18.5 kg/m² (n=1, 1%).
Table 5.1: Summary of Patient Characteristics (n =138):

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HF Patients (n=138)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Male) ¹</td>
<td>105 (76)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>109 (79)</td>
</tr>
<tr>
<td>African-American</td>
<td>11 (8)</td>
</tr>
<tr>
<td>Asian</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Other</td>
<td>14 (10)</td>
</tr>
<tr>
<td>Age (Years) ²</td>
<td>63 ± 12</td>
</tr>
<tr>
<td>BMI ³ (kg/m²)</td>
<td></td>
</tr>
<tr>
<td>≤ 18.5</td>
<td>1 (1)</td>
</tr>
<tr>
<td>19 ≤ BMI ≤ 24.9</td>
<td>41 (30)</td>
</tr>
<tr>
<td>25 ≤ BMI ≤ 29.9</td>
<td>55 (40)</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>41 (30)</td>
</tr>
</tbody>
</table>

¹ n (%)  
² Mean (SD)  
³ Body Mass Index (BMI)
5.3 Cardiac Disease Characteristics:

HF etiology, disease severity and risk of malnutrition are summarized in Table 5.2. HF etiology included cardiomyopathy (93%) and ischemic heart disease (54%). Co-morbidities of patients with HF included hypertension (49%), family history of heart disease (70%), hypercholesterolemia (71%), respiratory disease (20%), stroke or transient ischemic attack (TIA) (17%) and diabetes (28%). Of our patients, 44% were past smokers and 10% were found to be current smokers. Most of our participants had an implantable cardioverter defibrillator (ICD) or a pacemaker (80%).

The majority of patients with HF were found to have mild HF symptoms (NYHA Class I-II, 70%) (Table 5.3). In regards to LV function, the majority of the patients were found to have Grade III LV function (65%) whereas 22% had Grade II LV function and 14% had Grade IV LV function (Table 5.3). Our participants were found to be well-nourished (SGA Grade A: 98%) (Table 5.3).
Table 5.2: Cardiac Disease Characteristics and HF Risk Factors of patients (n=138):

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HF Patients (n=138)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Etiology of HF¹</strong></td>
<td></td>
</tr>
<tr>
<td>Valvular Disease</td>
<td>23 (17)</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>128 (93)</td>
</tr>
<tr>
<td>Ischemic Heart Disease</td>
<td>75 (54)</td>
</tr>
<tr>
<td>Previous Cardiac Surgery</td>
<td>66 (48)</td>
</tr>
<tr>
<td>ICD²/Pacemaker</td>
<td>110 (80)</td>
</tr>
<tr>
<td><strong>HF Risk Factors¹</strong></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>75 (54)</td>
</tr>
<tr>
<td>Current</td>
<td>61 (44)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>14 (10)</td>
</tr>
<tr>
<td>Family History of heart Disease</td>
<td>67 (49)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>97 (70)</td>
</tr>
<tr>
<td>Respiratory Disease</td>
<td>71 (51)</td>
</tr>
<tr>
<td>Asthma</td>
<td>27 (20)</td>
</tr>
<tr>
<td>COPD³</td>
<td>21 (77)</td>
</tr>
<tr>
<td>Stroke/TIA⁴</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>23 (17)</td>
</tr>
<tr>
<td></td>
<td>40 (28)</td>
</tr>
</tbody>
</table>

¹ n (%)
² Implantable cardioverter defibrillator
³ Chronic Obstructive Pulmonary Disease
⁴ Transient Ischemic Attack
Table 5.3: Cardiac Disease Severity (n=138).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HF Patients (n=138)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left Ventricular Dysfunction</strong>&lt;sup&gt;1,2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Grade II</td>
<td>30 (22)</td>
</tr>
<tr>
<td>Grade III</td>
<td>89 (65)</td>
</tr>
<tr>
<td>Grade IV</td>
<td>19 (14)</td>
</tr>
<tr>
<td><strong>Ejection Fraction</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
<td>29 ± 8</td>
</tr>
<tr>
<td><strong>NYHA Classification</strong>&lt;sup&gt;1,4&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Class I-II</td>
<td>96 (70)</td>
</tr>
<tr>
<td>Class III-IV</td>
<td>42 (30)</td>
</tr>
<tr>
<td><strong>NT-ProBNP levels (ng/L)</strong>&lt;sup&gt;5&lt;/sup&gt;</td>
<td>750 (324, 1612)</td>
</tr>
<tr>
<td><strong>SGA Grade</strong>&lt;sup&gt;1,6&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>135 (98)</td>
</tr>
<tr>
<td>B/C</td>
<td>3 (2)</td>
</tr>
</tbody>
</table>

<sup>1</sup> n (%)

<sup>2</sup> **Left Ventricular Dysfunction**: Grade II=Ejection Fraction (EF) 30%-50%, Grade III=EF 20%-30%, Grade IV=EF<20%.

<sup>3</sup> Mean ± SD

<sup>4</sup> **New York Heart Association (NYHA)**: Class I-II: Patients with mild or no limitation in ordinary physical activity; Class III-IV: Patients with severe limitation in ordinary physical activity.

<sup>5</sup> Median (25<sup>th</sup>, 75<sup>th</sup>)

<sup>6</sup> **Subjective Global Assessment (SGA)**: Class A=well-nourished, Class B/C=mild to severely malnourished
5.4 Medication and Supplementation History:

Medication usage as well as the dietary intake of participants is summarized in Table 5.4 and Table 5.5. Fifty-two percent of participants were taking furosemide with a median daily dose of 40 mg/d (range of doses from diuretic users=20-320 mg/day). Forty-one patients with HF were on spironolactone with a median daily dose of 25mg/day (5-100 mg/day) and 25% of them were taking two diuretics.

Other medications common among our participants included: cholesterol-lowering medications (74%), angiotensin II receptor blockers (20%), ACE inhibitors (74%), beta-blockers (87%), calcium channel blockers (9%), anticoagulants (41%), aspirin (51%) and digoxin (23%).

Thirty-four patients were taking thiamin-containing supplements in the form of multivitamins (Vitamin B₁ mean dose = 25.95 mg). Six patients were taking only B-complex vitamins (Vitamin B₁ mean dose =100 mg). Some of the other vitamins or herbal supplements used by study participants included vitamin D, vitamin C, potassium, folic acid, niacin, calcium, omega-3, vitamin B12, co-enzyme Q10, vitamin E, chondroitin, glucosamine and cod liver oil.

Participants had a median caloric intake of 1385 kcal/day, median carbohydrate intake of 178g/day, median thiamin intake of 1.16 mg/day and mean thiamin intake from diet and supplements of 1.52mg/day (Table 5.5).
Table 5.4: Medication history and supplemental usage of patients with HF (n=138).

<table>
<thead>
<tr>
<th>Medication and Supplements</th>
<th>HF Patients (n=138)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diuretic Medications</strong></td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>72 (52)</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>41 (30)</td>
</tr>
<tr>
<td>Metalozone</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Two Diuretics</td>
<td>34 (25)</td>
</tr>
<tr>
<td><strong>Cholesterol-lowering medications</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>102 (74)</td>
</tr>
<tr>
<td>Angiotensin II Receptor Blockers</td>
<td>27 (20)</td>
</tr>
<tr>
<td>ACE Inhibitors</td>
<td>102 (74)</td>
</tr>
<tr>
<td>Beta Blockers</td>
<td>120 (87)</td>
</tr>
<tr>
<td>Calcium Channel Blockers</td>
<td>12 (9)</td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>57 (41)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>70 (51)</td>
</tr>
<tr>
<td>Digoxin</td>
<td>32 (23)</td>
</tr>
<tr>
<td><strong>Thiamin Containing Supplements</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 (29)</td>
</tr>
<tr>
<td><strong>Other Supplements</strong></td>
<td>68 (49)</td>
</tr>
</tbody>
</table>

1 n (%)

3 *Thiamin containing supplements*: This category encompasses HF patients taking supplements that contain vitamin B1 (e.g. multivitamin or B-complex)

4 *Other supplements*: This category encompasses HF patients who are taking any other vitamins, minerals or herbal supplements (with or without B1).
### Table 5.5: Dietary Intake of patients with HF:

<table>
<thead>
<tr>
<th>Dietary Intake</th>
<th>HF Patients (n=138)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories/day (kcal/day)</td>
<td>1385 (950, 1667)</td>
</tr>
<tr>
<td>Carbohydrates/day (g/day)</td>
<td>178 (121, 213)</td>
</tr>
<tr>
<td>Thiamin/day (mg/day)²</td>
<td>1.2 (0.8, 1.6)</td>
</tr>
<tr>
<td>Total Thiamin/day (mg/day)³</td>
<td>1.5 (0.9, 2.8)</td>
</tr>
</tbody>
</table>

¹ Median (25th, 75th)
² Thiamin/day(mg/day): Includes dietary thiamin intake as reported by a semi-quantitative food frequency questionnaire.
³ Total Thiamin/day(mg/day): Includes thiamin from diet and supplements combined as reported.
5.5 Erythrocyte TPP and its relationship with other factors:

5.5A Prevalence of TD:

The prevalence of TD is presented in Figure 5.0. Eight percent of patients (n=138) were found to be thiamin deficient (n=11) based on the TD cutoff <180nmol/L (Section 4.2 (A1.2)). When patients taking B1-containing supplements were excluded, (n=11) 11% were found to be thiamin deficient. Figure 5.1 shows the distribution of erythrocyte [TPP] levels among the total sample size of 138. The mean (SD) [TPP] was 360.72 (168.33) nmol/L. Figure 5.2 shows the distribution of erythrocyte [TPP] levels in B1 supplement users only (n=40). The mean (SD) of erythrocyte [TPP] for B1-supplement users was 398.84 (194.84) nmol/L. There was no significant difference in the erythrocyte [TPP] levels between those who were not taking B1 supplements (n=98, mean=345.16, SD=154.65) and those who were taking B1 supplements (n=40, mean=398.84, SD=194.84) (p=0.2) (Figure 5.3). The prevalence of TD was found to be significantly different between those who were not taking B1 supplements (11%) compared with those who were taking B1 supplements (0%) (p=0.02).
Figure 5.0: Prevalence of TD in patients with HF (n=138)
The graph shows the count (n) of patients in each patient group. The prevalence of TD was 8% in all patients with thiamin deficiency and 11% in patients not taking B1-containing supplements.
**Figure 5.1:** Distribution of erythrocyte [TPP] in all subjects.

Distribution of erythrocyte [TPP] in all patients with HF. The mean (SD) TPP concentration was 360.72 (168.33) nmol/L. The horizontal line indicates the TD cut-off at 180nmol/L.
Figure 5.2: Distribution of erythrocyte TPP in thiamin-containing supplement users.
Distribution of erythrocyte [TPP] in all thiamin-containing supplement users (n=40). The mean (SD) TPP concentration was 398.84 (198.84) nmol/L. The horizontal line indicates the TD cutoff at 180 nmol/L.
Figure 5.3: Mean Erythrocyte [TPP] (nmol/L) and thiamin containing supplement users.

Erythrocyte [TPP] in thiamin-containing supplement users (n=40) and those not taking thiamin-containing supplements (n=98). Figure shows mean (SD) for each group (p=0.2).
5.5B Relationship between TD and Estimated Thiamin Intake:

No relationship was found between TD and the estimated intake of dietary thiamin (n=138, r= 0.03, p=0.7) (Figure 5.4). Similarly, no relationship was found between TD and estimated total thiamin intake (diet+supplements) (n=138, r=0.09, p=0.3) (Figure 5.5). No significant difference was found in dietary thiamin intake (n, median, range) (11, 1.15, {0.24-4.13}) between TD and non-TD groups (Mann-Whitney U=664, p=0.8). As well, no relationship was found in total thiamin intake (n, median, range) (127, 1.52, {0.24-105.65}) between the TD and non-TD groups (Mann-Whitney U= 500, p=0.1).

The estimated dietary thiamin intake and total thiamin intake (diet+supplements) were compared to the EAR cut-off for adults (male: 1.0mg/day, female: 0.9mg/day) (Figures 5.6 & 5.7). Both the dietary (median intake 1.2mg/d) and total thiamin intake (median intake 1.2mg/day) in TD patients was higher than the EAR cutoff. Sixty-three percent of the subjects had a dietary thiamin intake that was ≥ the EAR cutoff and 72% of the subjects had total thiamin intake from diet and B1-containing supplements ≥ the EAR cutoff. No significant difference was found in erythrocyte [TPP] levels between those with a dietary thiamin intake of ≥ EAR (n=87, mean=363.80, SD=179.70) and subjects with a dietary thiamin intake of < EAR (n=51, mean=355.46, SD=148.52) (p=0.6). No difference was found in the erythrocyte [TPP] levels between those with a total thiamin intake ≥ EAR (n=100, mean=361.05, SD=171) and subjects with a total thiamin intake < EAR (n=38, mean=359.85, SD=163.32) (p=0.8).

The estimated median (range) of the daily caloric intake based on the sq-FFQ results was 1318 (283-3958) kcal/day. The normal resting energy expenditure for each participant was
estimated using the Harris-Benedict (Harris JS et al., 1919) equation. The median and range for the basal metabolic rate was found to be 1617 (903-4563) kcal/day.

No correlation was found between the reported caloric intake (kcal/day) and the mean erythrocyte [TPP] levels (n=138, r=0.05, p=0.5) (Figure 5.8). A significant positive correlation was found between dietary thiamin intake and caloric intake (n=138, r=0.86, p<0.001) (Figure 5.9). However, no relationship was found between total thiamin intake (mg/day) and caloric intake (kcal/day) (n=138, r=0.07, p=0.43). A significant positive correlation was found between dietary thiamin intake and carbohydrate intake (n=138, r=0.81, p<0.001) (Figure 5.10). No correlation was found between total thiamin intake and carbohydrate intake (Data not shown).
Figure 5.4: Distribution of Erythrocyte [TPP] and dietary thiamin intake. Relationship between Erythrocyte [TPP] and estimated dietary thiamin intake (r=0.03, p=0.7, n=138).
Figure 5.5: Distribution of Erythrocyte [TPP] and estimated total thiamin intake.
Relationship between Erythrocyte [TPP] and estimated total thiamin intake (n=138, r=0.09, p=0.3).
Figure 5.6: Mean Erythrocyte [TPP] and dietary thiamin intake compared to EAR. Erythrocyte [TPP] in patients with dietary thiamin intake of <EAR (n=51) compared to those with dietary thiamin intake of ≥EAR (n=87). Figure shows mean (SD) for each variable (p=0.6).
Figure 5.7: Mean Erythrocyte [TPP] and total thiamin intake compared to EAR.
Erythrocyte [TPP] in patients with total thiamin intake of <EAR (n=38) compared to those with dietary thiamin intake of ≥EAR (n=100). Figure shows mean (SD) for each variable (p=0.8).
Figure 5.8: Mean Erythrocyte [TPP] and reported caloric intake. Relationship between Erythrocyte [TPP] and reported caloric intake (kcal/day) (r=0.05, p=0.5, n=138).
Figure 5.9: Dietary thiamin intake and reported caloric intake.
Relationship between dietary thiamin intake and reported caloric intake (kcal/day) (n=138, r=0.86, p<0.001).
Figure 5.10: Dietary thiamin intake and reported carbohydrate intake.
Relationship between dietary thiamin intake and reported carbohydrate intake (kcal/day) (n=138, r=0.81, p=<0.001).
5.5 (C) Relationship between TD and Disease Severity:

No difference was found in the prevalence of TD between NYHA class I-II subjects and NYHA class III-IV subjects (Table 5.6). 82% of patients at risk of TD patients were in the NYHA class I-II. In addition, no association was found between erythrocyte [TPP] levels and ejection fraction (%) \((p=0.4)\) (Figure 5.11). As well, no relationship was found between erythrocyte [TPP] levels and ejection fraction (%) obtained from diagnostic tests that were done within one year of the measurement of thiamin status \((n=74, p=0.7)\) (Figure 5.12). Similarly, no relationship was found in the mean erythrocyte [TPP] levels between NYHA class I-II and NYHA class III-IV \((p=0.2)\) (Figure 5.13). Plasma NT-proBNP was available for 113 patients. No relationship was found between erythrocyte [TPP] and plasma NT-pro BNP levels \((p=0.5)\) (Figure 5.14).
Table 5.6: Cardiovascular Disease characteristics (n=138).

<table>
<thead>
<tr>
<th></th>
<th>Erythrocyte TPP ≤180nmol/L</th>
<th>Erythrocyte TPP &gt;180nmol/L</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NYHA class</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>9 (82%)</td>
<td>87 (69%)</td>
<td>0.5</td>
</tr>
<tr>
<td>III-IV</td>
<td>2 (18%)</td>
<td>40 (32%)</td>
<td></td>
</tr>
<tr>
<td><strong>LV Grade</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>2 (18%)</td>
<td>28 (22%)</td>
<td>0.6</td>
</tr>
<tr>
<td>III</td>
<td>7 (64%)</td>
<td>82 (65%)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>2 (18%)</td>
<td>17 (13%)</td>
<td></td>
</tr>
</tbody>
</table>

1 n (%) 
2 **Left Ventricular Dysfunction**: Grade II=ejection fraction (EF) 30%-50%(n=30) , Grade III= EF 20%-30% (n=89) , Grade IV= EF<20% (n=19).
3 **New York Heart Association (NYHA)**: Class I-II: Patients with mild or no limitation in ordinary physical activity. Class III-IV: Patients with severe limitation in ordinary physical activity.
Figure 5.11: Distribution of erythrocyte [TPP] levels and ejection fraction (%).
Relationship between erythrocyte [TPP] levels and ejection fraction (%) (n=138, r=-0.07, p=0.4).
Figure 5.12: Erythrocyte [TPP] levels and ejection fraction obtained within a year of thiamin status measurement.
The relationship between erythrocyte [TPP] levels and ejection fraction (%) obtained from diagnostic tests done within a year of the thiamin status measurement (n=75, r=0.04, p=0.7).
Figure 5.13: Mean erythrocyte [TPP] and NYHA Class.
Mean Erythrocyte [TPP] in subjects with NYHA functioncal Class I-II (n=73) and Class III-IV (n=42) (p=0.2).
Figure 5.14: Erythrocyte [TPP] levels and plasma NT-proBNP (ng/L).
The relationship between erythrocyte [TPP] levels and plasma NT-proBNP (ng/L) concentrations (n=113, r=0.06, p=0.5).
5.5 (D) Relationship between TD and Diuretic Intake:

No significant differences in erythrocyte [TPP] levels were observed among patients who were taking furosemide only, spironolactone only or two diuretics (furosemide+spironolactone or furosemide+metalozone) ($p=0.4$) (Table 5.7). The erythrocyte [TPP] was not different between diuretic users in comparison with those who were not taking any type of diuretics ($p=0.4$, n=138) (Figure 5.15). TD was not related to either the use or the dose of furosemide ($p=0.4$; $p=1.0$) (Figure 5.16).
Table 5.7: Relationship between TD and diuretic use (n=138).

<table>
<thead>
<tr>
<th></th>
<th>Erythrocyte TPP $\leq 180$nmol/L</th>
<th>Erythrocyte TPP $\geq 180$nmol/L</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Furosemide</strong>†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4 (36%)</td>
<td>68 (54%)</td>
<td>0.4</td>
</tr>
<tr>
<td>No</td>
<td>7 (64%)</td>
<td>59 (47%)</td>
<td></td>
</tr>
<tr>
<td><strong>Furosemide Dose</strong> (mg/day)†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\leq 80$</td>
<td>4 (100%)</td>
<td>60 (87%)</td>
<td>1.0</td>
</tr>
<tr>
<td>&gt;80</td>
<td>0 (0%)</td>
<td>9 (13%)</td>
<td></td>
</tr>
<tr>
<td><strong>Spironolactone</strong>†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (27%)</td>
<td>38 (30%)</td>
<td>1.0</td>
</tr>
<tr>
<td>No</td>
<td>8 (73%)</td>
<td>89 (70%)</td>
<td></td>
</tr>
<tr>
<td><strong>Multiple Diuretics</strong>†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/1 Diuretic</td>
<td>9 (82%)</td>
<td>95 (75%)</td>
<td>1.0</td>
</tr>
<tr>
<td>&gt;1 Diuretic</td>
<td>2 (18%)</td>
<td>32 (25%)</td>
<td></td>
</tr>
</tbody>
</table>

† n (%)
Figure 5.15: Mean erythrocyte [TPP] and diuretic use.
Mean Erythrocyte [TPP] in subjects taking no diuretics (n=57), furosemide only (n=39), spironolactone only (n=8) and two diuretics (furosemide+spironolactone) or (furosemide+metolazone) (n=34) (p=0.4).
Figure 5.16: Mean erythrocyte [TPP] and furosemide dose.
Mean Erythrocyte [TPP] in subjects taking furosemide at a dose of ≤80mg/day (n=64) versus those taking furosemide only at a dose of >80mg/day (n=9) (p=0.4).
5.6 Subject Characteristics and Outcomes in relation to their erythrocyte [TPP] levels:

Characteristics of the patients in each erythrocyte [TPP] tertile are reported in Table 5.8. Medication history and dietary intake is reported in Table 5.9 and Table 5.10. Distribution of clinical characteristics, comorbidities, medication usage and dietary intake were similar between tertiles. Mean erythrocyte [TPP] levels were 215 ± 52 (range: 79-284nmol/L), 330 ± 28 nmol/L (range: 287-383) and 536 ± 169 nmol/L (range: 384-1035) in the low, middle and upper tertiles, respectively.

The total amount of person-time contributed to this study by 138 patients was 60,681 days.

5.6.1 Acute Decompensated Heart Failure (ADHF):

Cumulative ADHF events for the lower, middle and upper tertiles were 44%, 39% and 17%, respectively. Although, the percentage of ADHF events was found not to be significantly different among groups, it was observed that the lowest tertile had a higher number of ADHF events in comparison with the middle or upper tertiles ($p=0.12$) (Table 5.9; Figure 5.17). The cumulative event probability did not differ for days to ADHF between tertiles (log rank $p=0.44$) (Figure 5.18). The incidence rate for ADHF in this cohort of patients with HF is 0.11 cases per year.
Table 5.8 Summary of Patient Characteristics based on tertiles:

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>&lt;284nmol/L TPP (n=46)</th>
<th>285-382nmol/L TPP (n=46)</th>
<th>≥383nmol/L TPP (n=46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)(^1)</td>
<td>36 (34%)</td>
<td>34 (32%)</td>
<td>35 (33%)</td>
<td>0.8</td>
</tr>
<tr>
<td>Age (y)(^2)</td>
<td>64 ± 10</td>
<td>63 ± 13</td>
<td>64 ± 12</td>
<td>0.8</td>
</tr>
<tr>
<td>BMI (kg/m)(^2)</td>
<td>27 ± 5</td>
<td>28 ± 5</td>
<td>28 ± 5</td>
<td>0.5</td>
</tr>
<tr>
<td>NYHA Class I-II (%)(^1)</td>
<td>36 (78%)</td>
<td>33 (72%)</td>
<td>27 (59%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NYHA Class III-IV (%)(^1)</td>
<td>10 (22%)</td>
<td>13 (28%)</td>
<td>19 (46%)</td>
<td></td>
</tr>
<tr>
<td>Ejection Fraction(^2)</td>
<td>30 ± 8</td>
<td>27 ± 8</td>
<td>29 ± 9</td>
<td>0.1</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)(^2)</td>
<td>117 ± 18</td>
<td>119 ± 20</td>
<td>122 ± 22</td>
<td>0.2</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)(^2)</td>
<td>70 ± 12</td>
<td>71 ± 13</td>
<td>69 ± 13</td>
<td>0.7</td>
</tr>
<tr>
<td>Heart Rate (beats/min)(^2)</td>
<td>69 ± 13</td>
<td>67 ± 16</td>
<td>69 ± 13</td>
<td>1.00</td>
</tr>
<tr>
<td>Cardiomyopathy(^1)</td>
<td>43 (34%)</td>
<td>42 (33%)</td>
<td>43 (34%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Ischemic Heart Disease(^1)</td>
<td>27 (36%)</td>
<td>25 (33%)</td>
<td>23 (31%)</td>
<td>0.4</td>
</tr>
<tr>
<td>ICD(^1)</td>
<td>35 (32%)</td>
<td>40 (36%)</td>
<td>35 (32%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Hypertension(^1)</td>
<td>23 (34%)</td>
<td>24 (36%)</td>
<td>20 (30%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Current Smoker(^3)</td>
<td>7 (50%)</td>
<td>3 (21%)</td>
<td>4 (29%)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

\(^1\) n (%)
\(^2\) Mean (SD)
Table 5.9 Medication history based on tertiles:

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>&lt;284nmol/L TPP (n=46)</th>
<th>285-382nmol/L TPP (n=46)</th>
<th>&gt;383nmol/L TPP (n=46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furosemide Use¹</td>
<td>20 (28%)</td>
<td>26 (36%)</td>
<td>26 (36%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Furosemide Dose (mg/d)²</td>
<td>66 ± 49</td>
<td>70 ± 72</td>
<td>54 ± 35</td>
<td>0.3</td>
</tr>
<tr>
<td>Spironolactone use¹</td>
<td>11 (27%)</td>
<td>17 (42%)</td>
<td>13 (32%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Beta Blocker¹</td>
<td>41 (34%)</td>
<td>41 (34%)</td>
<td>38 (32%)</td>
<td>0.4</td>
</tr>
<tr>
<td>ACEI²</td>
<td>32 (31%)</td>
<td>39 (38%)</td>
<td>31 (30%)</td>
<td>0.8</td>
</tr>
<tr>
<td>ARBs³</td>
<td>8 (30%)</td>
<td>8 (30%)</td>
<td>11 (41%)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

¹ n (%)
² Mean (SD)
Table 5.10 Dietary Intake based on tertiles:

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>≤284nmol/L TPP (n=46)</th>
<th>285-382nmol/L TPP (n=46)</th>
<th>≥383nmol/L TPP (n=46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Intake(^{\text{3}})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calories/day (kcal)</td>
<td>1296 (891, 1660)</td>
<td>1405 (867, 1996)</td>
<td>1250 (970, 1582)</td>
<td>0.5</td>
</tr>
<tr>
<td>Carbohydrates/day (g)</td>
<td>170 (126, 206)</td>
<td>174 (116, 258)</td>
<td>159 (119, 195)</td>
<td>0.4</td>
</tr>
<tr>
<td>Thiamin/ day (mg)</td>
<td>1.2 (0.8, 1.6)</td>
<td>1.3 (0.7, 1.9)</td>
<td>1.1 (0.8, 1.6)</td>
<td>0.8</td>
</tr>
<tr>
<td>Thiamin/d (mg) (diet+suppl)</td>
<td>1.4 (0.9, 2.7)</td>
<td>1.9 (0.8, 3.7)</td>
<td>1.5 (0.9, 2.5)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

\(^{\text{3}}\)Median (25\(^{\text{th}}\), 75\(^{\text{th}}\))
Figure 5.17: Number of ADHF events in each tertile.
The graph shows the total number of ADHF events in lower (≤284nmol/L TPP), middle (285-382nmol/L TPP) and upper (≥383nmol/L TPP) tertiles (p=0.1).
Figure 5.18: Kaplan-Meier plots for erythrocyte [TPP] levels and Acute Decompensated Heart Failure (ADHF) in lower (≤284nmol/L TPP), middle (285-382nmol/L TPP) and upper (≥383nmol/L TPP) tertiles (p=0.4).
5.6.2 All-Cause Hospitalization:

At a person-time of 60,681 days, event rate for all-cause hospitalizations were 41%, 34% and 25% ($p=0.2$) for patients in the lower, middle and upper tertiles, respectively (Table 5.9). The cumulative event probability did not significantly differ among tertiles (log rank $P=0.8$; Figure 5.19).

5.6.3 Mortality:

The event rate for mortality was similar (33%; $p=1$) across all tertiles (Table 5.9). The cumulative event probability did not differ among tertiles (log-rank $P=0.9$; Figure 5.20). All patients died of cardiovascular causes. Three patients out of six died from a combination of kidney dysfunction and HF. None of the study participants underwent cardiac transplantation.

5.6.4 Emergency Visits:

The event rate for emergency visits for cardiovascular causes were 11%, 13% and 10% ($p=0.8$) in the lower, middle and upper tertiles, respectively (Table 5.9) and the cumulative event probability did not differ significantly among tertiles (log-rank $P=0.7$; Figure 5.21).

5.6.5 Arrhythmias:

Overall cumulative event arrhythmias were rare and were not significantly different among tertiles (log-rank $P=0.2$; Figure 5.22). The event rate for arrhythmias was 10%, 50% and 40% in the lower, middle and upper tertiles, respectively ($p = 0.2$; Table 5.9).
5.6.6 Myocardial Infarction (MI)/Angina:

Similar to arrhythmias, cumulative events for MI/Angina were rare and were not significantly different among tertiles (log-rank $P=0.3$, Figure 5.23). The event rate for MI/Angina was found to be 60% and 40% in the lower and middle tertiles and 0% in the upper tertile ($p=0.1$) (Table 5.9).

5.6.7 Other Adverse Events:

No significant difference was observed among tertiles in regards to cumulative events for other adverse events (log-rank $P=0.8$; Figure 5.24). The event rate for other adverse events was similar across tertiles (37% (lower), 29% (middle), 34% (upper); $p=0.8$) (Table 5.9). Other adverse events included gastrointestinal diseases, kidney dysfunction, cancer or others.

5.6.8 Composite Endpoint:

The composite endpoint was defined as the time to first event or follow-up. No significant difference was observed in the composite endpoints among the three groups (log-rank $p=0.9$; Figure 5.25). The event rate for the composite endpoint was also similar across the lower (37%), middle (37%) and upper (32%) tertiles ($p=0.5$) (Table 5.9).
Figure 5.19: Kaplan-Meier plots for erythrocyte [TPP] levels and All-Cause Hospitalizations in lower ($\leq 284$nmol/L TPP), middle (285-382nmol/L TPP) and upper ($\geq 383$nmol/L TPP) tertiles ($p=0.8$).
Figure 5.20: Kaplan-Meier plots for erythrocyte [TPP] levels and Mortality in lower (≤284nmol/L TPP), middle (285-382nmol/L TPP) and upper (≥383nmol/L TPP) tertiles (p=0.9).
Figure 5.21: Kaplan-Meier plots for erythrocyte [TPP] levels and Emergency Visits in lower (≤282nmol/L TPP), middle (283-382nmol/L TPP) and upper (≥383nmol/L TPP) tertiles (p=0.7).
Figure 5.22: Kaplan-Meier plots for erythrocyte [TPP] levels and Arrhythmias in lower (≤284nmol/L TPP), middle (285-382nmol/L TPP) and upper (≥383nmol/L TPP) tertiles (p=0.2).
Figure 5.23: Kaplan-Meier plots for erythrocyte [TPP] levels and Angina/Myocardial Infarction (MI) in lower ($\leq 284\text{nmol/L}$ TPP), middle (285-382nmol/L TPP) and upper ($\geq 383\text{nmol/L}$ TPP) tertiles ($p=0.3$).
Figure 5.24: Kaplan-Meier plots for erythrocyte [TPP] levels and other adverse events; which includes gastrointestinal issues, kidney disease, cancer and others (in lower (≤284nmol/L TPP), middle (285-382nmol/L TPP) and upper (≥383nmol/L TPP) tertiles) (p=0.8).
Figure 5.25: Kaplan-Meier Curves for erythrocyte [TPP] and Composite Endpoint. Composite Endpoint is defined as first event or follow-up ($p=0.9$).
5.6.9 Observations on medical therapy and dietary supplements:

During the telephone interview, patients were asked about medication changes and use of dietary supplements. These results are summarized in Table 5.9. Twenty-one patients had an increase in dose and/or duration of the prescribed diuretic since the time of their initial study visit. A total of 58 (42%) patients had other medication changes that included Beta-Blockers, ARBs, ACE-inhibitors, cholesterol-lowering medications and others. There was no significant difference in the number of changes of medications among the tertiles. Two patients previously not taking thiamin-containing supplements had started during the follow-up period. Equal numbers of patients in the middle and upper tertile group had received dietary counselling from a registered dietitian while only 2 people were found to have used the services of a dietitian in the lower erythrocyte [TPP] level tertile.
Table 5.9 Event Rate Table based on tertiles:

<table>
<thead>
<tr>
<th>Outcome</th>
<th>&lt;284nmol/L TPP (n=46)</th>
<th>285-382nmol/L TPP (n=46)</th>
<th>&gt;383nmol/L TPP (n=46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHF 1,2</td>
<td>8 (44%)</td>
<td>7 (39%)</td>
<td>3 (17%)</td>
<td>0.1</td>
</tr>
<tr>
<td>Emergency Visits 1</td>
<td>11 (32%)</td>
<td>13 (38%)</td>
<td>10 (29%)</td>
<td>0.8</td>
</tr>
<tr>
<td>All-Cause Hospitalization 1</td>
<td>13 (41%)</td>
<td>11 (34%)</td>
<td>8 (25%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Mortality 1</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Arrhythmia 1</td>
<td>1 (10%)</td>
<td>5 (50%)</td>
<td>4 (40%)</td>
<td>0.2</td>
</tr>
<tr>
<td>MI/Angina 1,3</td>
<td>3 (60%)</td>
<td>2 (40%)</td>
<td>0 (0%)</td>
<td>0.1</td>
</tr>
<tr>
<td>Other Adverse Events 1</td>
<td>13 (37%)</td>
<td>10 (29%)</td>
<td>12 (34%)</td>
<td>0.8</td>
</tr>
<tr>
<td>Composite Endpoint 1</td>
<td>22 (37%)</td>
<td>19 (32%)</td>
<td>19 (32%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Diuretic Change 1</td>
<td>10 (35%)</td>
<td>12 (41%)</td>
<td>7 (24%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Intensification of Diuretic 1</td>
<td>6 (29%)</td>
<td>8 (38%)</td>
<td>7 (33%)</td>
<td>0.8</td>
</tr>
<tr>
<td>Medication Change 1</td>
<td>19 (33%)</td>
<td>21 (36%)</td>
<td>18 (31%)</td>
<td>0.8</td>
</tr>
<tr>
<td>Multivitamin 1</td>
<td>8 (24%)</td>
<td>14 (41%)</td>
<td>12 (35%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Thiamin-Containing MVIT 1</td>
<td>12 (29%)</td>
<td>17 (41%)</td>
<td>13 (31%)</td>
<td>0.8</td>
</tr>
<tr>
<td>Other Supplements 1</td>
<td>23 (17%)</td>
<td>14 (10%)</td>
<td>17 (12%)</td>
<td>0.1</td>
</tr>
<tr>
<td>Dietitian 1</td>
<td>2 (14%)</td>
<td>6 (43%)</td>
<td>6 (43%)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

1 n (%)  
2 ADHF=Acute Decompensated Heart Failure  
3 MI=Myocardial Infarction
6.0 - Discussion

6.1 Prevalence of TD:

We found the prevalence of TD in the population of ambulatory patients with HF to be 8% (n=138) (Figure 5.0 & Figure 5.1). When we excluded patients taking B1-containing supplements, we found the prevalence of TD to be 11% (Figure 5.2). This is in accord with the study by Hanninen et al. (2006) where the use of thiamin-containing supplements was found to be significantly associated with not having TD in hospitalized patients with HF. This suggests that the risk of TD is decreased with the consumption of thiamin-containing supplements, even at low doses (1.5mg). We were unable to find any other studies which recorded detailed information on supplement use, including those of thiamin-containing supplements. Several studies have shown widespread nutritional inadequacies in patients with HF (Arcand et al., 2009; Price et al., 2007; Gorelik et al., 2003). This suggests that nutrient recommendations for patients with HF might be different than nutrient recommendations for healthy individuals and that the use of supplements may be warranted. Further research aimed at determining the efficacy of supplements in treating nutritional inadequacies should be considered.

Our results (8%; prevalence of TD) are consistent with those of Levy et al. (1992), who found a prevalence of 3% (n=38) in ambulatory patients with HF. The slightly higher prevalence in our study is probably due to the differences in the mean age and dose of furosemide in our population. The age of our population was older (mean age= 63 years old) compared with Levy et al. (1992), who had a younger group (mean age= 47 years) of patients with HF. The mean dose of furosemide in our patients was 63 mg/day versus a higher dose of furosemide (mean dose=184 mg/day) in the Levy et al. (1992) study. This suggests the possibility that factors such as age or diuretic dose/type might play a role in predicting the prevalence of TD in ambulatory
patients with HF. However, other factors such as dietary intake, cardiovascular etiologies and risk factors for heart disease might also influence the prevalence of TD. In addition, the prevalence of TD in our ambulatory patients with HF was found to be comparatively similar to the prevalence found in individuals without HF. The reported range of the prevalence of TD is 10-15% in individuals without HF (Bailey et al., 1997; Baum et al., 1984; Wilkinson et al., 1997). Moreover, it has been suggested that TD is more apparent in individuals of lower socio-economic status or individuals with other diseases (Bailey et al., 1997; Baum et al., 1984; Wilkinson et al., 1997). Therefore, it is possible that our population of ambulatory patients with HF (age range = 28-87) are similar in thiamin status in comparison with the general population without HF (age range = 60-90 years).

On the other hand, our findings were not consistent with the results of some of the other studies investigating the prevalence of TD in hospitalized patients with HF. These include study by Seligmann et al (1991), who found a prevalence of TD to be 91% in a group of hospitalized patients with HF (n=23) as well as a study of hospitalized elderly patients with HF (n=35) where the prevalence of TD was 37% (Pfitzenmeyer et al., 1994). Hanninen et al., (2006) also found evidence of TD in one-third of hospitalized patients with HF (n=100). On the other hand, Kwok et al. (1992) found a relatively low prevalence of TD of 13% in hospitalized patients with HF (n=37). Kwok et al. (1992) failed to report the type, dose and duration of diuretic prescribed to the patients. It remains possible that the low prevalence of TD found in the study by Kwok et al. (1992) is related to either diuretic therapy or disease severity. The higher relative prevalence of TD in hospitalized patients with HF may be related to chronic undernutrition but the duration at which thiamin is assessed during hospitalization may also influence the thiamin status of these
individuals. If patients had been consuming thiamin sufficient diets and their thiamin status was assessed at the point of admission, it is likely that they would show a lower prevalence of TD compared with patients who may have been consuming a diet consisting of low thiamin prior to their admission.

In comparing the above studies with our results, the population studied by Kwok et al. (1992) was different than ours, although the prevalence of TD was similar (8% vs 13%). Where Kwok et al. studied hospitalized patients with different co-morbidities (including infections, malignancies, diabetic coma), our population was comprised of ambulatory patients with HF only. Moreover, Kwok et al. (1992) had a small sample size (n=35) and used the TPPE method to assess TD compared with our large sample size (n=138) and the use of the more direct method of HPLC for assessing TD. Therefore, it is difficult to compare our study with Kwok et al. (1992) because of the discussed inconsistencies between the two studies. Other studies reported a relatively higher prevalence of TD in hospitalized patients with HF compared with our prevalence and this is discussed further in section 6.1 (C) and 6.1 (D).

A couple of studies included a mixed group of patients (ambulatory and hospitalized) and found a prevalence of TD to be 21% (n=27 ambulatory patients with HF/38) (Brady et al., 1995) and 27% (n=63) (Abou-Hashem et al., 2009). Brady et al. (1995) and Abou-Hashem et al. (2009) examined similar proportions of outpatients and inpatients and included information on the type of diuretic therapy prescribed to patients (furosemide only). However, Brady et al. (1995) had a small sample size (n=38) and a younger age group (age=55 years) of patients compared with Abou-Hashem et al., study (n=63, age = 67 years). Both these studies reported a higher prevalence of TD in comparison with the prevalence we found. This suggests the possibility that
the proportion of the population with HF that is hospitalized versus ambulatory might influence the prevalence of TD.

Altogether, these studies suggest a higher prevalence of TD in hospitalized patients with HF (Seligmann et al., 1991; Pfitzenmeyer et al., 1994; Hanninen et al., 2006) compared with a lower prevalence of TD found in ambulatory patients (Levy et al., 1992). Moreover, the prevalence of TD in hospitalized patients with HF was also found to be higher compared with the prevalence of TD in a mixed group of ambulatory and hospitalized patients (Brady et al., 1995; Abou-Hashem et al., 2009). Thus, it is likely that hospitalized patients with HF are at increased risk for TD.

6.1 (A) Speculations on reasoning behind decreased prevalence of TD in Ambulatory Patients:

There are several possible explanations for the higher prevalence of TD in hospitalized patients with HF in comparison with the relatively lower prevalence of TD observed in ambulatory patients with HF. These may include a low intake of dietary thiamin, increased disease severity and an intense drug regimen. There have been reports of an increased prevalence of TD in hospitalized patients in association with these above-mentioned factors (Thomas et al., 1988; Peipersack et al., 1999; O’Rourke et al., 1990, Lemoine et al., 1980; Chin et al., 1997).

Hospitalized patients may have more severe HF as indicated by systolic and diastolic indices (Abou-Hashem et al., 2009; Hanninen et al., 2006; Brady et al., 2006). Hanninen et al. reported that 75% of the hospitalized patients with HF were in NYHA class III-IV compared with only 30% of the ambulatory patients with HF in our study. Our patients were found to be well-nourished with mainly NYHA class I-II symptoms, and therefore, this stable cohort was found
to be more similar to a control population. Thus, it is hypothesized that disease severity might influence the risk of TD. This may be due to altered metabolism as a result of a combination of other factors including increased resting energy expenditure, increased diuretic requirements and worsened nutritional status as observed in hospitalized patients with HF.

In comparison with ambulatory patients with HF, hospitalized patients may have worsened nutritional status. This may be because hospitalized patients might be unwell for some time before they are admitted to the hospital, and thereby have a chronic reduced intake leading up to their admission. Moreover, reduced intake might further be compounded by aggressive medical therapy once they enter the hospital, consequently resulting in nutrient deficits. Lemoine et al., (1980) found that 57% of the patients hospitalized for various conditions had thiamin intakes lower than the RDA. Another study reported that hospitalized patients stated problems associated with mastication and/or illnesses that made it difficult for them to finish their meal completely (Thomas et al., 1988). The majority of our patients had adequate thiamin intake as well as were found to be relatively well-nourished (SGA class A [98%]).

Although, the use of diuretics in contributing to the increased risk of TD is not empiric, it has been suggested that hospitalized patients might be on an increased dose and/or duration of diuretics that might adversely influence their thiamin status. Studies that have reported a relationship between the use of diuretics and the prevalence of TD had patients that were on >40mg/day of diuretic dose (Seligmann et al., 1991; Zenuk et al., 2003). Our patients were relatively well-nourished with no or minimal limitations in their physical activity levels (NYHA class I-II) and were on a relatively low dose of diuretic (median furosemide dose=40mg/day).
Therefore, it is not surprising to find a relatively low rate of TD in our population of ambulatory patients with HF.

Overall, the literature is not comprehensive in regards to factors that may influence TD in hospitalized patients with HF in comparison with those found in ambulatory patients with HF. Future studies assessing patient’s state of health and dietary intake before and during hospitalization will be necessary to better understand the relationship between TD and HF. As well, deficiency of thiamin does not happen acutely and since suboptimal consumption of thiamin can influence body stores of erythrocyte [TPP] levels, it is important to consider the diet prior to assessing thiamin status for better understanding the relationship between the prevalence of TD in chronic HF.

6.2 Relationship between TD and Thiamin Intake:

The patient’s reported their dietary intake through a semi-quantitative food frequency questionnaire (sq-FFQ). We did not find any relationship between TD and either estimated dietary thiamin intake or total thiamin intake (diet + supplements). Moreover, no difference was found in the dietary thiamin intake or the total thiamin intake between the TD and non-TD group of ambulatory patients with HF. This is similar to the findings of Hanninen et al. (2006), who also did not find any relationship between erythrocyte [TPP] and estimated total or dietary thiamin intake. Several other studies have also reported no associations between blood thiamin levels and dietary thiamin intake (Bovet et al., 1998; Nichols et al., 1994). Nichols et al. (1994) assessed the dietary intake of thiamin using a 3-day food record and did not find any relationship between intake and TPPE in healthy individuals. Another study found a weak but statistically significant relationship between erythrocyte [TPP] and dietary thiamin intake, which
was measured using a 7-day weighed food record in healthy individuals (Bailey et al., 1997). There is a possibility that erythrocyte [TPP] levels are affected by factors other than thiamin intake. These factors may include those that alter thiamin absorption and utilization (Nichols et al., 1994). Some of these factors could be elevated resting metabolic rate (Obesesan et al., 1996; Poehlman et al., 1994; Riley et al., 1991), increased catabolism (Bourdel-Marchasson et al., 2001), intake of diuretics which may induce urinary thiamin excretion (Seligmann et al., 1991; Zenuk et al., 2003), presence of cardiac cachexia (Anker et al., 2004) and increased cardiac work (Berry et al., 2000). These factors can alter thiamin requirements for patients with HF and therefore, further research is required to better understand the nutrient recommendations of thiamin in relation to the factors that may increase risk of nutrient deficiencies in patients with HF.

We found that the median estimated thiamin intake from both diet alone and from thiamin-containing supplements was above the EAR in both the non-deficient and TD patients with HF and was not significantly different between the two groups. This suggests that thiamin intake in our patients was likely adequate. In addition, we did not find any association between mean erythrocyte [TPP] levels of subjects whose dietary thiamin intake or total thiamin intake were ≥ EAR or < EAR (Figure 5.6 & Figure 5.7). Similar to our study, Hanninen et al. (2006), using the sq-FFQ, reported the mean intake of thiamin from diet alone and thiamin-containing supplements to be adequate compared with the EAR in a group of hospitalized patients with HF. On the contrary, Brady et al. (1995) estimated thiamin intake in ambulatory and hospitalized patients with HF using a modified sq-FFQ and found that the mean dietary thiamin intakes were < RDA. They also observed that 60% of TD patients with HF had dietary thiamin intakes that
did not meet two-thirds of the RDA for thiamin, indicating inadequacy of thiamin intake in these patients. One of the reasons why Brady et al., (1995) may have found inadequate thiamin intakes is because they used the RDA as a reference, which is not recommended in cases of assessing the adequacy of nutrients of patient groups.

Using the estimated intake of total thiamin (diet + supplements), 28% of our patients reported having total thiamin intakes (median [25th, 75th]) (0.7 [0.6, 0.8]) of less than the EAR and 37% of our patients reported having dietary thiamin intakes (0.7 [0.6, 0.8]) of less than the EAR. This could be due to the underreporting of nutrient intake in the sq-FFQ. The sq-FFQ was used in this study as it has been validated in a HF population and is the same sq-FFQ used previously to collect data on the dietary intake of hospitalized patients with HF (Hanninen et al., 2006). However, the FFQ uses a retrospective method of collecting dietary intake and underreporting is a recurrent problem found with this method of dietary assessment (Willett, 1998). It is unlikely that the database we used to quantify thiamin in the foods plays a role in the underestimation of thiamin since according to the international nutrient databank directory, 53% of the items have thiamin calculated in the ESHA database and 93% of the items have thiamin calculated in the Canadian Nutrient File (Nutrient Databank Directory., 2010). As well, the database also takes into consideration the thiamin content of foods in relation to B-vitamin food fortification (Health Canada., 2012; Nutrient Databank Directory., 2010). Moreover, all FFQs were double-checked by a trained investigator, reducing the chances of an error. Underreporting of dietary intake may occur due to factors such as age-associated defects in memory that can hinder the effectiveness of using FFQ in an elderly diseased population (Sawaya et al., 1996; Willett, 1998). One way to solve the issue of memory-associated reporting in nutrient intakes is to use
the weighed food records. Using the 3 to 7 day food record is considered a gold standard for the assessment of dietary intake (Willett, 1998) but it was not feasible for this type of observational study where only one visit was involved. However, food records are not without limitations. These limitations include underreporting of nutrient intake and alteration in the eating behaviour as intake is collected during the consumption period (Willett, 1998). Moreover, food records also require literacy on the respondent’s part as well as it places a significant burden on the respondent to weigh and record food for several days (Willett, 1998). This can also lead to a poor response rate if subjects are not highly motivated (Willett, 1998). In addition, 24-hour recall method was also not considered for the prevalence study as it is dependent on the patient’s short-term memory and a single day of intake is not likely to be representative of usual intake (Willett, 1998).

We did not find any correlation between mean erythrocyte [TPP] levels and reported caloric intake (kcal/day) (Figure 5.8). However, while dietary thiamin intake was associated with caloric intake and carbohydrate intake (Figure 5.9 & Figure 5.10), total thiamin intake (diet+supplements) was found to be not associated with either caloric or carbohydrate intake. This is likely as an intake of supplements, specifically thiamin-containing supplements, is not linked to how much food is consumed throughout the day. We also made the observation that the reported number of calories consumed per day was relatively low compared with the amount reported of calories/day using a 3-day food record in another local study with similar patient characteristics (Arcand et al., 2009). Whereas our ambulatory patients with HF reported consuming $1385 \pm 623$ kcal/day of calories, patients in Arcand et al’s study consumed $1756 \pm 576$ kcal/day of calories (Arcand et al., 2009). This suggests the possibility that the level of
caloric intake may be underreported on the sq-FFQ. Equally, it is likely that carbohydrate intake as well as dietary thiamin intake is also underreported in our study and thus, is not an accurate representation of the mean erythrocyte [TPP] levels in our patients. Other factors such as alteration in thiamin absorption, use of several kinds of medications and relative disease severity may also play a role in determining the adequacy of thiamin intakes and its relationship to erythrocyte [TPP] levels. However, our observation of relatively low inadequacy of total thiamin intake (28% of patients with total thiamin less than the EAR) and caloric intake may also suggest that some patients with chronic HF might have an overall inadequate diet, contributing to the poor nutritional status of these patients. Impaired nutrient intake could be due to nausea, dietary restrictions, dyspnea and early satiety (Lennie et al., 2006). Moreover, patients with HF may not feel well enough to do grocery shopping or cook due to limited mobility which may further impair their diet (Arcand et al., 2009). Therefore, it is difficult to conclude from our data whether patients with HF have an overall inadequate diet or if patients underreported their nutrient intake on the FFQ. However, several other studies have reported inadequacy of various dietary nutrients in patients with HF (Arcand et al., 2009; Gorelik et al., 2003; Catapano et al., 2008). In view of these arguments, a registered dietitian, to counsel patients with HF on dietary management, should be considered as part of the multidisciplinary team.

Our patients were found to be well-nourished (98% as assessed using SGA) when compared with previous studies where the risk of malnutrition has been reported to be 50% or higher (Hanninen et al., 2006; Freeman et al., 1994). Furthermore, patients in SGA class B/C (2%), which is suggestive of mild to severe malnutrition, were not thiamin deficient. It is likely that
TD is not related to malnutrition risk in ambulatory patients with HF. The reason the majority of our patients were well-nourished could be due to having fewer limitations in ordinary physical activity (as indicated by NYHA class I-II, 70%) in comparison with hospitalized patients. This is also in support our findings of a low prevalence of TD. This supports the importance of introducing better treatments to minimize the burden of hospitalizations by educating patients on the benefits of physical activity as well as consumption of an adequate diet to support their self-sufficiency and independency in carrying out their daily activities.

6.3 Relationship between TD and HF Severity:

Although no difference was found in the relationship between TD and indices of cardiac function (NYHA and LVEF) (Figures 5.11, Figure 5.12 & Figure 5.13), it was noted that numerically, patients in NYHA class III-IV had a higher mean erythrocyte [TPP] level compared with patients in NYHA class I-II (Figure 5.13). We found our results of no associations between TD and NYHA class to be comparable to the results of Hanninen et al. (2006), who also did not find any relationship between NYHA and TD. The NYHA classification system has been criticized for its subjective nature of assessment and is generally considered to not be a sensitive classification to identify smaller changes that might be clinically significant (Packer et al., 1987; Hunt et al., 2005). The lack of relationship between NYHA and TD in our study may therefore reflect the subjectivity of the NYHA classification system (Packer et al., 1987). On the contrary, several studies (Brady et al., 1995; Abou-Hashem et al., 2009) including Hanninen et al., (2006) found an association between thiamin status and LV function as measured by LVEF, suggesting that patients with more severe HF are at a higher risk of TD. Our study did not find any relationship between erythrocyte [TPP] levels and LVEF.
LVEF is considered a significant prognostic indicator of HF severity (Guyton et al., 2006) and therefore has been found to be a better index of assessing cardiac function (Hunt et al., 2005). The reason we may not have found a significant relationship between TD and LVEF could be due to our data’s dependence on the latest available echocardiogram, MUGA or catheterization report, which were within the last five years of individual study visits. Although patients who are deteriorating, have changes in symptoms or been provided with change in therapy are recommended to have their LV function assessed more frequently (~ 6 months), there are no specific guidelines as to the precise time interval for longer-term follow-up of compensated patients on stable medications (Arnold et al., 2006).

As mentioned in section 2.1.4, NT-pro BNP levels can be used as a marker of LV dysfunction in patients with HF. We did not find a significant relationship between plasma NT-proBNP concentrations and erythrocyte [TPP] levels (Figure 5.14). This supports our finding of the lack of relationship between HF severity (as assessed by LVEF and NYHA) and erythrocyte [TPP] levels.

### 6.4 Relationship between TD and Diuretic Therapy:

Few studies commonly cite a relationship between risk of TD and diuretic use (Seligmann et al., 1991; Zenuk et al., 2003) while other studies (Hanninen et al., 2006; Pfitzenmeyer et al., 1994) do not. Thereby the link between diuretic therapy and TD remains controversial.

Our study did not find any association between TD and diuretic users (furosemide only, spironolactone only or multiple diuretics). Our results are consistent with some previous studies in which no relationship was found between TD and diuretic use in patients with HF (Pfitzenmeyer et al., 1994; Brady et al., 1995; Yue et al., 1997; Hanninen et al., 2006).
Moreover, the dose of diuretics prescribed to our patients was relatively low (median dose =40mg/day), which may have influenced the risk of TD. Other studies have reported a relationship between the use of furosemide and prevalence of TD in patients with HF (Zenuk et al., 2003; Seligmann et al., 1991) whereas ours did not. The mechanisms by which furosemide has been proposed to increase the risk of TD include either the increase in urinary flow through diuresis as shown using rat models (Lubetsky et al., 1999; Yui et al., 1980) or inhibition of thiamin uptake by cardiac cells in vitro (Zangen et al., 1998). Therefore, these studies have limited applicability to humans. One of the strongest supports for the relation between the risk of TD and diuretic use comes from reports of furosemide induced urinary losses of thiamin in healthy volunteers (Rieck et al., 1999). The authors showed that furosemide induced a dose-dependent increase in urine flow that correlated with thiamin excretion rate (Rieck et al., 1999). Approximately one-third to one-half of patients with HF have mild to moderate renal dysfunction (Shlipak, 2003), therefore, these patients may not have effects of furosemide induced urinary thiamin losses similar to those of healthy volunteers (Rieck et al., 1999). As well, some patients show resistance to furosemide (Perez-Ayuso et al., 1983), which can complicate the interpretation of the risk of TD in relation to the loop diuretic.

Interestingly, we did find a numerical trend in the mean erythrocyte [TPP] levels being relatively lower in patients who were taking spironolactone alone compared with patients taking furosemide only or multiple diuretics (Figure 5.15). Similar to our finding, Hanninen et al (2006) also observed that spironolactone use prior to hospitalization was significantly associated with TD (p<0.05). Spironolactone may play a role in contributing to TD in patients with HF, however, this is a hypothesis-generating finding as we are unable to draw a definite conclusion
at this point due to a small number of patients (n=8) taking spironolactone only. It is observed that the use of spironolactone is prescribed to patients for the management of more severe symptoms of HF (Liu et al., 2001). In view of these suggestions, future research investigating the relationship between spironolactone and TD should be conducted.

One of the reasons for the controversial debate regarding diuretic use and the risk of TD is related to the mechanism and site of action of individual diuretics. Loop diuretics such as furosemide have been proposed to act on the thick ascending Loop of Henle while potassium-retaining diuretics such as spironolactone act by hindering the reabsorption of Na\(^+\) at the distal tubules and collecting duct (Davison et al., 1998; Rose et al., 1991). The site for thiamin reabsorption is noted to be at the proximal tubules (Gastaldi et al., 2000; Laforenza et al., 1998) and in some cases, have been considered to be sodium independent (Gastaldi et al., 2000). Thus, it is possible that the actions of both loop diuretics and potassium-retaining diuretics do not influence the reabsorption of thiamin considering their respective sites of action. Regardless, more research is needed to better understand the mechanisms of various diuretics including those such as carbonic anhydrase inhibitors that may act at the proximal tubule and thereby influence thiamin reabsorption. As well, research understanding the influence of dose and duration of diuretic use on thiamin status should be considered.
6.5 Outcome Study:

To our knowledge, this is the first study investigating the relationship between erythrocyte [TPP] levels and clinical outcomes in stable compensated ambulatory patients with HF. However, our study failed to find any significant relationships between outcomes and circulating erythrocyte TPP levels.

6.6 Acute Decompensated Heart Failure (ADHF):

Our primary outcome was ADHF, for which we found a total of 18 cases at 60,681 total person-days. The highest percentage of ADHF events (44%) was in the lowest tertile of erythrocyte [TPP] levels (≥284nmol/L) although this relationship was not significant compared with the middle (39%) and the upper tertiles (17%). One of the reasons why we may not have seen a significant relationship is due to a relatively short follow-up time. A longer follow-up time period is recommended to increase the event rate in order to determine conclusively whether or not there is any relationship between erythrocyte [TPP] levels and ADHF events. Our event rate for ADHF was found to be low in comparison with another local study assessing outcomes in relation to sodium adherence (Arcand et al., 2009). Moreover, it is possible that this particular cohort of patients might be at a lower risk of developing an acute episode of HF in comparison with other populations. It has also been postulated that patients enrolled in heart function clinics at academic setting hospitals are receiving better treatment in comparison with treatment strategies that are used at community hospitals (Thomas et al., 2010; Howlett et al., 2009). One of the recent studies investigating the differences in treatments and their efficacy at academic and non-academic hospitals (Thomas et al., 2010) suggests that non-academic hospitals assess LV function less frequently as well as employ suboptimal use of effective drug therapies.
Another study suggests that implementation of a multidisciplinary HF clinic (from where our patients came from) is associated with a significant reduction both in hospitalization as well as mortality in patients with HF (Howlett et al., 2009). Moreover, most of our patients had a pacemaker or an ICD, which helps to stabilize heart function, and thereby, decrease the risk of acute episodes of HF. Another reason our patients may be at a lower risk of developing an acute episode of HF may be related to their overall health status. These patients were found to be well-nourished, have an adequate intake of thiamin (as measured by SGA and dietary assessment), be at low risk of TD as well as being able to carry out their activities of daily living on their own (based on their NYHA class).

Our incidence rate of ADHF was low in comparison with another local study assessing sodium intakes in association with ADHF (Arcand et al., 2009). At an average of 1 year follow-up time, we found a total of 18 ADHF events compared with a total of 27 events in the Arcand et al study. This difference cannot be attributed to difference in the patient population characteristics (including age, BMI, NYHA class, LVEF, etiologies and risk factors of HF) as these were found to be similar between the two studies. However, the majority of our enrolled patients had a pacemaker or an ICD, which may influence their risk of developing an acute episode of HF. The proportion of patients with devices was unavailable in the study by Arcand et al., making a direct comparison of these populations difficult.

Dietary noncompliance, largely related to sodium and fluid restriction, has been implicated as one of the causes of an acute episode of HF (Chin et al., 1997). Our study was not designed to measure dietary sodium or fluid management in patients with HF. It is possible that our patients had better adherence to both sodium and fluid restriction recommendations in comparison with
the population studied by Arcand et al (2009). Dietary noncompliance may also be a factor contributing to the discrepancy in event rates between our patient population and those in the study by Arcand et al (2009).

6.6 (A) Hospital Admissions and Emergency Room Visits in association with ADHF:

ADHF events in this study include both patient visits to the emergency department for the management of ADHF as well as those patients who were hospitalized with a primary diagnosis of ADHF. Hospital admission is recommended for patients presenting with ADHF when the symptoms are severe (Heart Failure Society of America., 2010). Moreover, the risk of readmission is often highest just after hospitalization (Heart Failure Society of America., 2010). Studies have shown that mortality and readmission rates continue to increase in patients diagnosed with ADHF despite medical management (Yancy et al., 2004; Jong et al., 2002; Aghababian et al., 2002). One of the areas that has received little attention is the influence of dietary modification and nutritional support as an adjunct therapy in minimizing the burden of hospitalization and readmission for patients with ADHF (Yancy et al., 2004; Galvao et al., 2006). One randomized controlled trial evaluated the effects of a low-sodium diet (1840 mg/day) versus a normal sodium-diet (2760 mg/day) on readmission rates in 232 patients who had a recent ADHF hospitalization (Paterna et al., 2008). Patients were also assigned a high dose of furosemide (250 or 500 mg twice daily) and prescribed fluid restriction (1-2 L fluid/day) at randomization. These investigators concluded that patients who received a sodium-restricted diet had detrimental neurohormonal effects as well as an increased risk of hospitalization and mortality at 6 months in comparison with patients on a normal-sodium diet (Paterna et al., 2008). However, it is difficult to elucidate the effects of dietary sodium on outcomes because of
the simultaneous assignment of co-interventions (Paterna et al., 2008). Smithline et al. (2007) assessed thiamin supplementation in suspected ADHF cases presenting to the emergency department and subsequently being hospitalized for ADHF. This study did not find any significant change in the rate of hospitalization, dyspnea scores 4-h after enrolment, or length of hospitalization as a result of thiamin supplementation (Smithline et al., 2007). However, this study had several limitations including a small sample size (n=50) as well as having several confounding issues associated with the outcome measures. Future studies are needed to better understand the implications of thiamin supplementation in relationship to factors involved in the development of ADHF.

6.6 (B) Mortality in ADHF:

Patients with ADHF have a high mortality rate of 50% at 5 years (Yancy et al., 2004; Adams et al., 2005). In our study, we identified three out of six patients to have died after an episode of ADHF and three patients to have died from a combination of both ADHF and kidney dysfunction. The reason for the increased mortality rate in patients who develop ADHF has been related to having a low muscle mass, hypoalbuminemia and decreased skeletal muscle glycogen, all of which are markers of severe metabolic wasting and increase the risk for mortality (Diltoer et al., 2004; Sarma et al., 2010). It has also been suggested that the risk of mortality is associated with the choice of therapy (Abraham et al., 2005; Adams et al., 2005). For example, in one study treatment with vasodilators or natriuretic peptides was associated with a significantly lower mortality compared with positive inotropic drugs (Abraham et al., 2005). The use of inotropic drugs has been implicated only for patients with an acute or end-stage HF and their long-term use has been associated with worsened clinical outcomes.
Therefore, further research into alternatives or intermittent therapy should be considered for treating symptoms of ADHF. Another study has shown that the use of loop diuretics is associated with a 37% increase in the risk of arrhythmic death in patients with ADHF (Cooper et al., 1999). Therefore, although loop diuretics are a first line therapy in treating symptoms of ADHF, much uncertainty remains about the risks and efficacy of these drugs at various doses and durations. Thus, larger appropriately powered randomized controlled trials are warranted to further study the risk to benefits ratios associated with the use of loop diuretics as a treatment strategy in patients with ADHF.

6.6 (C) Potential Aetiologies and Risk factors in association with development of ADHF:

It has been suggested that patients who are at an increased risk for the development of ADHF are more to have aetiology of coronary artery disease, hypertension or obstructive pulmonary disease (Adams et al., 2005). One possible explanation for our low observed incidence of ADHF could be related to the relatively low percentage of patients in our initial cohort having HF related to hypertension or obstructive pulmonary disease. Our study was not designed to evaluate differences in HF aetiology or risk factors. In addition to the aetiology of HF, there is also little information characterizing the relationship between comorbidity rates and the incidence rate of ADHF. The exception is chronic renal dysfunction, which has been found to be prevalent (31%) in patients with ADHF (Adams et al., 2005). Although we excluded patients with severely impaired renal function, it is reported that generally patients with HF have some degree of renal dysfunction (McAlister et al., 2004). Therefore, we cannot make empiric observations into the relationship between chronic renal insufficiency, risk of TD and development of ADHF. As discussed in section 2.3.1 (F), TD has been observed to increase the
risk of encephalopathy in patients requiring dialysis. Therefore, it may be worthwhile to further elucidate the relationships between TD, development of severe renal dysfunction and risk of ADHF.

A total of 21 patients (15%) had intensification in their diuretic dose and/or duration. This is similar to the rate of ADHF events (13%) in our cohort of patients (n=138). This supports the use of diuretic therapy as the first line of treatment in treating symptoms of ADHF. However, the safety and efficacy of diuretic therapy for decompensated HF have not been established in randomized, controlled trials and current guidelines are based on extensive observational experiences (Section 2.1.2; Heart Failure Society of America, 2010). Other medications used to treat symptoms of ADHF have their own shortfalls and what data does exist frequently shows marginal or mixed results (Section 2.1.2, Allen et al., 2007). This area of medicine requires urgent demand for novel treatment strategies in modulating worsening symptoms of patients with ADHF. Moreover, patients with ADHF may suffer from elevated levels of cytokines and catecholamines which may contribute to a persistent hypermetabolic state as well as the development of nutrient deficits (Ingwall et al., 1990; Sarma et al., 2010). Therefore the dose and duration of pharmaceutical therapies may contribute to the development of nutrient deficits through increased urinary losses and malabsorption of essential nutrients (Sarma et al., 2010). Although the data is still controversial as to whether diuretic therapy results in an increased urinary excretion of thiamin, it remains possible that the mechanisms behind increased thiamin excretion leading to TD may occur depending on the diuretic dose and duration prescribed to the individual as well as the degree of volume overload present in a patient. Therefore, it becomes
essential to measure nutrient deficits in patients with ADHF who are on increased doses of diuretics.

Data is lacking regarding patients characteristics, co-morbidities, standard care of therapy, efficacy of medications, commonly used treatment strategies, adherence and clinical outcomes of patients with ADHF (Adams et al., 2005; Yancy et al., 2004; Galvao et al., 2006). In view of limited current literature, urgent progress is needed in the management of ADHF.

6.6 (D) Hypothetical relationships between TD and development of ADHF:

Little is known on the nutrient status of patients during the time period between the progression from chronic HF to an acute episode of HF. Considering the significant role of thiamin as a coenzyme in ATP production, TD has been postulated to contribute to the reduction in ATP stores observed in HF and thereby, contribute to cardiac dysfunction and impairments in contractile function. Since several pathophysiologic abnormalities including persistent neurohormonal activation and deterioration of myocardial function may also contribute to the onset of ADHF, it is logical to assume that TD may contribute further to progression of chronic HF to ADHF. It is possible that in the timeframe between chronic HF and the development of ADHF, patients may not be eating well. Some of the factors that may be associated with dietary inadequacy and hence nutritional deficiencies in patients progressing towards ADHF include: 1) inadequate nutrient intake 2) increasing cytokines and catecholamines that may result in a hypermetabolic state 3) malnutrition and 4) chronic venous congestion of gastrointestinal vasculature resulting in malabsorption (Sarma et al., 2010). It would be difficult to measure the extent to which the dietary intakes of nutrients are compromised in patients during their period of deterioration. Patients with chronic HF may already have progressive nutritional wasting and
when they suffer from an acute episode of HF; their metabolic reserve might be depleted even further (Sarma et al., 2010). Therefore, if these patients suffer from TD in the timeframe leading up to an acute episode of HF, it is possible that their deficiency can worsen further due to malabsorption or other factors affecting nutrient requirements, thereby, contributing to exacerbation of worsening symptoms of HF and resulting in progression of chronic HF to ADHF.

Another way that TD might play a role in contributing to the development of an acute episode of HF is through thiamin’s role as an antioxidant. One of the proposed mechanisms responsible for ADHF is an increase in circulating levels of proinflammatory cytokines, which have been shown to be linked to the production of reactive oxygen species (Chen et al., 1998). As discussed in section 2.2.4 (D), thiamin might influence the production of reactive oxygen species and thereby, prevent or reduce an increase in oxidative stress. Considering the novel role of thiamin as an antioxidant, future mechanistic studies of how thiamin might play a role in modulating activated cytokines found in the state of acute HF are needed.

Some studies have reported significant improvements in indices of cardiac function in patients with chronic HF who were taking thiamin supplements (Seligmann et al., 1991; Shimon et al., 1995; Freye et al., 1982). This is an important finding since indices of cardiac function such as LVEF are considered as important prognostic indicators of mortality. However, to date, no study has yet looked at the prevalence of TD or the impact of thiamin replenishment in cases of ADHF. Therefore, larger, adequately powered, more rigorous trials are warranted.

6.7 All-Cause Hospitalizations and Emergency Visits:
We defined all-cause hospitalization as all cases admitted to the hospital for any cause, including, but not limited to, cardiovascular causes. Our inclusion criteria for emergency room visits included patients presenting to the emergency department for cardiovascular and non-cardiovascular causes. We did not find any significant differences between the tertiles in regards to the event rate for either all-cause hospitalizations or visits to the emergency department. Our incidence of all-cause hospitalizations was low (32 events, median= 14 months) compared with 160 events at 3 years as observed in another local study assessing sodium intakes in association with adverse cardiovascular outcomes (Arcand et al., 2011). One of the reasons for the discrepancy in the rates is the relatively short follow-up time period of our study. Other explanations could include the proportion of patients with or without devices as well as the relative level of adherence to dietary sodium and fluid restriction in the respective studies (as discussed in section 6.6). It is also possible that the reason we did not see a high incidence of all-cause hospitalization or emergency room visits for causes related to HF was that our population were relatively stable, well nourished with an adequate thiamin intake and a low prevalence of TD. Therefore, our population were possibly a low-risk group of patients with HF.

6.8 Mortality:

The event rate for mortality was equal across the three thiamin tertiles (33%). We observed that in our cohort of patients, mortality occurred only in males. Literature also shows that mortality is higher in men than in women (p<0.001) (Moskowitz et al., 2003). This may be explained by the higher percentage of men with decreased LV function (25% of men had > 40% LVEF compared with 45% of women; p<0.0001) (Moskowitz et al., 2003). In addition, since the majority of our patients were men, we are likely to make a biased observation of the high
prevalence of mortality in male subjects. Moreover, we are also unable to compare the mortality rates between the two genders considering the small percentage of women (24%) participating in our study. Data from clinical studies have suggested that in a population of patients with stable HF, the average annual mortality rate is 10% (Howlett et al., 2010; Ho et al., 1993). Our population had a relatively lower mortality rate in comparison with the mortality rate reported by Howlett et al. (2010) as well as observed in the study by Ho et al. (1993) and this is likely due to two reasons 1) the short time period of follow-up and 2) optimal care of our patients at the academic hospitals. Due to the relatively low rate of mortality as well as the short follow up time period, we were unable to identify HF aetiologies or risk factors that could potentially contribute to mortality in our population.

Approximately half of the deaths occurred as the result of a combination of renal dysfunction and HF. Renal dysfunction is found to be common in patients with HF and has been implicated as an independent prognostic factor in ventricular dysfunction (McAlister et al., 2004). The reason renal dysfunction might be associated with poorer outcomes in patients with HF is probably attributable, in part, to advanced HF, higher risk of drug toxicity and the presence of multiple comorbidities (McAlister et al., 2004).

6.9 Arrhythmias and Myocardial Infarction (MI)/Angina:

The event rate for arrhythmias and MI/anginas did not differ among tertiles. Moreover, the incidence was relatively low for both arrhythmias and MI/Angina. TD, in its most extreme manifestation, has been postulated to cause arrhythmias (Aldinger, 1965). Moreover, other studies have shown that the incidence of arrhythmias increases as TD progresses (Yoshitoshi et al., 1961; Aldinger, 1965). The decrease of potential cardiac contractions seen in association
with TD has been linked to deficits of myocardial energy production (Aldinger, 1965). It is possible that the low prevalence of TD in our population may also contribute to the low incidence of arrhythmias. However, considering our observational approach to evaluate clinical outcomes in association with erythrocyte [TPP] levels, causality cannot be proven.

Thiamin is hypothesized to be reduced in myocardial cells after an episode of an MI (Wexler et al., 1973). It has also been shown that benfotiamine, a derivative of thiamin, improves functional recovery after an episode of MI through modulation of the neurohormonal response and stimulation of the pentose phosphate pathway enzymes (Katare et al., 2010). Whether TD precedes an episode of MI is yet to be elucidated. Moreover, future studies of changes in stores of thiamin in the myocardial cells and the implications of thiamin therapy in improving functional recovery after an episode of MI should be considered.

6.10 Other Adverse Events:

Similar to the event rate for arrhythmias and MI, we did not find any relationships between other adverse events and biochemical levels of erythrocyte [TPP] levels. Other adverse events included gastrointestinal diseases, kidney dysfunction, cancer and others (e.g. falls, depression). Gastrointestinal diseases included patients with gastritis, peptic ulcers, gastroesophageal reflux disease and others related to the gastrointestinal tract. We did not observe a high number of our patients suffering from gastrointestinal diseases. It is likely that individuals suffering from gastrointestinal diseases have some degree of malabsorption which potentially would contribute to the development of nutrient deficiencies. Malabsorption of thiamin specific to gastrointestinal disease could be, in part, due to recurrent vomiting, poor dietary intake and reduced absorption
capacity of the gastrointestinal mucosa (Uruha et al., 2011). Risk of TD in specific gastrointestinal disorders remains to be further elucidated since data is limited.

Our initial cohort did not include patients with dialysis and during the follow-up time period, very limited numbers of individuals reported being diagnosed with severe chronic renal insufficiency. However, it has been reported that patients with HF generally have some degree of renal dysfunction (McAlister et al., 2004). Therefore, we are unable to make any observational relationships between the level of thiamin and risk of severe renal dysfunction. It is hypothesized that patients with renal insufficiency might have a reduced glomerular filtration rate as a result of reduced renal function, which might be protective against nutrient losses. This is likely due to more time for nutrient reabsorption and less frequent urination and therefore, fewer nutrients are lost. On the other hand, it has also been reported that some patients with renal dysfunction experience more frequent urination. As previously discussed in section 2.3.1 F, data on thiamin status in renal dysfunction is limited. It may be that many interrelated factors play a role in influencing nutrient levels in patients with impaired renal function.

Encephalopathy has been observed in non-alcoholic patients with end-stage renal dysfunction undergoing haemodialysis and peritoneal dialysis (Hung et al., 2001; Ihara et al., 1999; Ueda et al., 2006). It is possible that dialysis patients are at a risk for TD because of poor intake and increased loss of water-soluble vitamins during the procedure of dialysis (Hung et al., 2001). It has been reported that administration of IV thiamin can prevent encephalopathy in patients who are on dialysis (Ueda et al., 2006; Ihara et al., 1999). Therefore, future studies further assessing the prevalence of TD and the implications of routine thiamin supplementation in dialysis patients should be considered.
Malnutrition is an observed issue in patients with cancer and hence, these individuals suffer from widespread nutrient deficiencies. Again, our initial cohort of patients did not include patients with cancer and only 3 patients from 138 individuals were diagnosed with cancer during follow-up. Hence, it is beyond the scope of this thesis to make any empirical insights into cancer and thiamin status.

6.11 Composite Endpoint:

The composite endpoint was defined as the incidence of the first event or follow up, if no event occurred. We did not observe any significant differences in the event rate among tertiles. This is likely due to the short period of follow-up as well as the relatively well-nourished and stable group of ambulatory patients participating in our study.

6.12 Observations on dietary supplements:

We did not find any differences in use of multivitamins or thiamin-containing supplements among the tertiles. We observed two of our patients who were not taking thiamin-containing supplements to start during their follow-up period. At this point, we are unable to make empirical insights as to the advantages of thiamin supplementation in relation to decreased risk of worsening outcomes associated with cardiac dysfunction. Moreover, patients with low erythrocyte [TPP] levels were observed to make less use of a dietitian in comparison with patients who had higher erythrocyte [TPP] levels. Considering patients who were seeing a dietitian had better thiamin status, these findings suggest the importance of a registered dietitian as part of the multidisciplinary team.
7.0 - Study Limitations and Future Directions

We conducted a retrospective investigation of the cardiovascular outcomes in ambulatory patients as a function of their thiamin status. For this study, the sample of HF patients studied was relatively small (where other retrospective cohort studies of drug therapy efficacy have used sample sizes >300) (Graham et al., 2010; Ryan et al., 2009); however, we included a well-characterized group of patients with the direct measurement (HPLC) of erythrocyte [TPP] levels. Our numerical data suggests that those in lowest erythrocyte [TPP] tertile have an increased number of ADHF events but our follow-up time period is relatively short (median 14 months, range 6-24 months). In view of these limitations, we were unable to identify independent predictors of our primary outcome as well as we were unable to do multiple comparisons with stratification. Moreover, larger studies with a longer follow-up time period are recommended to fully understand the scope of TD and implications of thiamin replenishment in improving outcomes for patients with progressive chronic HF.

Our study may be subjected to loss to follow-up bias as subjects that did not agree or that we were unable to contact might be systematically different than those who we tracked till the end. However, our loss to follow up rate was low (7%) compared to the generally acceptable rate for epidemiological cohort studies (50-80%) (Fewtrell et al., 2008). One major disadvantage of our study design is recall bias; where patients are more likely to remember certain events or exaggerate/minimize their symptoms. However, considering that we confirmed all interview responses with a review of the patient’s medical chart and adjudicated them with a cardiologist blinded to the patient’s erythrocyte [TPP] levels, the chances of recall bias is low.

We were unable to find any relationships between diuretic use and TD and this could be related to either the mechanism of diuretics in contributing to nutrient losses, the prescribed dose and
duration of diuretics, the degree of volume overload present within a patient or adherence to medications by patients. Adherence to medical therapies was not measured in our study and therefore we are unable to comment on these. It has been hypothesized that the use of diuretics can influence biochemical levels of thiamin although the data remains controversial. Future studies should try to explore urinary analysis to better understand thiamin excretion in response to diuretics. In addition, more research is needed to understand the mechanics behind potential nutrient losses related to the use of diuretics.

We did not find any relationships between dietary or total thiamin intakes with TD. Despite using a validated sq-FFQ to assess thiamin intake, it is likely that the method of dietary assessment is not the most rigorous for measuring dietary thiamin intake. Moreover, it is possible the use of sq-FFQ underestimated the nutritional inadequacy of some nutrients. The sq-FFQ we used included a section for specified portion sizes. This may have biased our patients estimation of their portion sizes in order to match what they actually consumed. In addition, although we asked about other foods that individuals might consume as part of their diet in the survey, another issue with the use of sq-FFQ might be related to the fact that patient’s responses are limited by our fixed list of foods. Future studies should assess thiamin intake with a more accurate dietary assessment method such as a weighed multiple-days food record. Emphasis on capturing an accurate estimate of carbohydrate intake should also be considered since thiamin plays a role in carbohydrate metabolism. This will help us to understand if thiamin requirements are altered for patients with HF as a result of their carbohydrate intake.

Most of our patients were in the NYHA class I-II which is in line with our findings of fewer outcomes associated with less severe HF. However, the use of NYHA class is subjective and
does not correlate with the objective estimate of functional capacity (Russell et al., 2009). Hence, the lack of relationship between TD and NYHA classification is possible as a result of the error associated with subjectivity of NYHA. As well, the majority of our patients were found to be within Grade III LV function. However, the collection of LV data was dependent upon the assessments made within the last five years. Considering that changes to LV function may have occurred during that time period, our collected LV data may not reflect the true LV functional status of these patients. Future studies are recommended to investigate HF severity with objective assessments of cardiac function as well as ideally collect it at recommended intervals (dependent upon patient’s symptoms and changes to medications therapy) to fully capture the changes in LV function and subsequent outcomes associated with LV function.

This study was performed with patients receiving treatment in a multidisciplinary HF clinic at academic hospitals. Thus, it is possible that the incidence rate of worsening cardiovascular outcomes is lower than the rate that may be observed in patients treated at a community hospital (Thomas et al., 2010). Therefore, the study might be limited in assessing outcomes as well as factors associated with prevalence of TD in patients with HF from non-academic settings. As well, these findings may not be generalized to the larger population of patients with HF due to the differences in treatment strategies between academic-setting and community hospitals (Thomas et al., 2010) as well as the severity of chronic HF. Thus, future studies investigating prevalence of TD as well as incidence of worsening cardiovascular outcomes both in hospitalized and patients at community hospitals should be conducted.
8.0 - Conclusions

The primary objective of our study was to investigate the relationship between the incident rate of adverse cardiovascular outcomes and erythrocyte [TPP] levels. This was a novel and unique undertaking as no study, to our knowledge, has investigated the long term outcomes as related to thiamin status. We also determined the prevalence of TD in ambulatory patients with HF. We found the prevalence of TD to be 8% in our cohort of ambulatory patients with HF. Our patients were also found to be on stable medication regimens, used relatively low doses of diuretics, had less severe HF and were well-nourished based on SGA and dietary assessments. We did not find any significant differences in the incidence of ADHF, all-cause hospitalizations, emergency visits, arrhythmias, myocardial infarctions/angina and other adverse events in relation to thiamin status. Overall, these findings together with our low incidence rate of adverse events suggest that our cohort of ambulatory patients with HF represented a relatively stable population at a low risk of TD. However, we did observe our patients in the lowest erythrocyte [TPP] tertile to have an increased number of ADHF events. In view of these findings, further research is recommended to better understand the relation of thiamin status to the incidence of adverse events.

HF continues to remain a burdensome condition on both the healthcare system as well as on the individual patient. HF can be characterized as a disease of an imbalance between energy demand and supply. Nutritional support in modulating metabolic and energetic efficiency of heart function is a growing area and may offer benefits as an adjunct therapy beyond those of current treatment strategies. Although the data for the role thiamin plays in the management of HF is limited and controversial, the use of thiamin as a supplement has shown promise in improving symptoms and hemodynamic markers in patients with HF. Given the relative safety
as well as tolerability of thiamin supplementation, it would be reasonable to further study the effects of thiamin supplementation as an adjunct therapy on improving outcomes regardless of thiamin status or HF severity.
9.0 – References


Baines M, Bligh JG, Madden JS. Tissue thiamin levels of hospitalised alcoholics before and after oral or parenteral vitamins. *Alcohol Alcohol*. 1988;23(1):49-52.


Lepage S. Acute decompensated heart failure. *Can J Cardiol.* 2008;24 Suppl B:6B-8B.


• Rose G. Assessment of Left Ventricular Systolic Dysfunction. https://docs.google.com/viewer?a=v&q=cache:MtMxHH1hM_YI:www.sangerclinic.com/ppt/LVindices.pps+limitations+of+ejection+fracton&hl=en&gl=ca&pid=bl&srcid=ADGEESg7m2N2GicXmuX1wh4vTJc6CO1vsUg45fisSyY1zuzixQJyaNpn2kgNO7n7J6eNexsXP4cdfsVgsmwMjOAShj627zUD1ysCbltq3tUeV9og-J1QeXAVdADzOdSYASSgeNYosVXp5&sig=AHIEtbQbtbo7APQMtGij8oF5Atv1ZePfNA&pli=1. Published 2005. Accessed February 19, 2012.


• Shils ME, Shike M. Modern nutrition in health and disease. 2006:2069.


• Talwar D, Davidson H, Cooney J, St JO'Reilly D. Vitamin B(1) status assessed by direct measurement of thiamin pyrophosphate in erythrocytes or whole blood by HPLC: Comparison with erythrocyte transketolase activation assay. *Clin Chem*. 2000;46(5):704-710.


10.0 – Appendices

10.1 - Telephone Script

Hello, May I speak to ____________?

Patient answers.

My name is Mavra Ahmed and I am calling from St. Michael’s Hospital. I am calling in regards to the vitamin B1 study you participated in at this hospital on (insert date). The study involved a fasting blood test and some questionnaires regarding your medical history and dietary habits that we filled out together. I am calling to ask whether you will be willing to answer a few questions on events that you might have had since the study visit. These events will pertain to your medical condition and include all cardiovascular and/or noncardiovascular events that you might have had. The questions will be asked on the phone and will take approximately 10-15 minutes of your time. Your participation in this study is completely voluntary. If you choose to participate, you can withdraw at anytime without any effect on your care.

<table>
<thead>
<tr>
<th>Questions/Responses to Patient</th>
<th>Patient responses to Questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thank You/ Thank you for your time.</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Have you had a transplant?</td>
<td></td>
</tr>
<tr>
<td>At which hospital did you have the transplant?</td>
<td></td>
</tr>
<tr>
<td>Has your condition of heart failure worsened?</td>
<td></td>
</tr>
<tr>
<td>Have you started experiencing new or worsening symptoms?</td>
<td></td>
</tr>
<tr>
<td>Are you aware of your NYHA class? What is your NYHA class?</td>
<td></td>
</tr>
<tr>
<td>Have you been to an emergency department due to your condition? How many times have you visited the emergency department in last year?</td>
<td></td>
</tr>
<tr>
<td>Have you been hospitalized for heart failure? How many times have you been hospitalized for heart failure in last year?</td>
<td></td>
</tr>
<tr>
<td>Have you had any other medical conditions?</td>
<td></td>
</tr>
<tr>
<td>Question</td>
<td>Answer</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Do you have a list of your current medication with their dosage with you?</td>
<td>See Appendix A below (page 2).</td>
</tr>
<tr>
<td>(Ask the patient to go over their medication list with you)</td>
<td></td>
</tr>
</tbody>
</table>
| Have you started taking multivitamins? If yes, what is the name of the multivitamin? | Brand: _______________
| Have you started taking other herbal supplements? If yes, what are they? | List:                                                                  |
| Have you started taking thiamin supplements? If yes, what is the thiamin dose in the supplement? | Brand: _______________
|                                                                          | Dose: _______________
| Are you seeing a dietitian? If yes, where is he/she located (e.g which hospital)? |                                                                        |

Alternative options:

Household member of the patient answers the phone.

1) The patient has been deceased.
   
   I am sorry to hear about that. I am calling from St. Michael’s Hospital regarding a study the patient participated in and we were just following up on our participants in regards to their conditions and events.

2) The patient is not home right now.
   
   May I leave a phone number that I can be reached at?
Appendix A:

List of Current Medications:

<table>
<thead>
<tr>
<th>Type</th>
<th>Dosage</th>
<th>Start Date (d/m/y)</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>
## STUDY DATA COLLECTION FORM

**Date of Visit**  
Day  
Month  
Year  
*(Date of screening visit)*

**Date of Contact**  
Day  
Month  
Year  
*(Date of follow-up telephone contact)*

**Date of Chart Review**  
Day  
Month  
Year

**Primary Cardiologist:**

**Patients Current Vital Status**  
☐ Alive  
☐ Alive with Heart Transplantation  
☐ Deceased

*If alive, has patient been contacted regarding occurrence of events:*

☐ Yes  
☐ No

*Date & Outcome: ________________________________*

**Clinic Events Since Screening Visit:**

<table>
<thead>
<tr>
<th></th>
<th>NO</th>
<th>YES</th>
<th>(IF YES, complete CRF#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Death</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>2.</td>
<td>Transplant</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>3.</td>
<td>MI</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>4.</td>
<td>Arrhythmia</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
5. Stroke/TIA
6. Worsening heart failure
7. Hospitalization
8. Emergency Room Visit
9. Other adverse events
10. No events

Total Number of Events (Case Report Forms Attached): __________

Changes in Medication since Date of Entry □ Yes □ No

<table>
<thead>
<tr>
<th>Changes in Medication (Name of Medication)</th>
<th>Commencement Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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</tbody>
</table>

Vitamin/Mineral Supplement Use: □ Yes □ No

Name of Vitamin/Mineral Supplement: __________

Thiamin Containing Supplement: □ Yes □ No

Name of Thiamin-Containing Supplement: ______________

Thiamin dose in the supplement: ______________

Diet Counseling: □ Yes □ No

Center of Diet Counseling: ______________

Review completed by: ________________________ Date: ________________________
### EVENT INFORMATION: DEATH

<table>
<thead>
<tr>
<th>Date of Death</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>Month</td>
<td>Year</td>
<td></td>
</tr>
</tbody>
</table>

**Location of Death:**

- □ Hospital
- □ Home
- □ Work
- □ Other, please specify: __________________________

**If the patient is deceased, did the patient have progressive heart failure within 2 weeks prior to death?**

- □ Yes  
- □ No  
- □ Unknown

**Did this patient have progressive ischemic heart disease within 2 weeks prior to death?**

- □ Yes  
- □ No  
- □ Unknown

**Primary Cause of Death:**

In the opinion of the investigator(s), what is the primary cause of death?

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

Please classify the cause of death:

- **CARDIAC** ...........................................  
  - □ Yes  
  - □ No

- **NON-CARDIAC** ...........................................  
  - □ Yes  
  - □ No
If available, medical Records/Source Documents are listed below. Please refer to the list below and append the documents that are applicable.

☐ HOSPITAL DEATH
   ○ Death Summary/Note
   ○ Emergency Department Notes
   ○ MD & Nurses Notes
   ○ History & Physical
   ○ Procedure Reports
   ○ Consult Reports
   ○ Diagnostic Reports
   ○ Lab Reports
   ○ ECGs
   ○ Autopsy Report, if applicable.

☐ HOME DEATH
   ○ Death Certificate
   ○ If applicable, EMS Notes, MD Narrative, Autopsy report

☐ EXTENDED CARE FACILITY DEATH
   ○ Death Note
   ○ Progress Note
   ○ Medical Records
   ○ Death Certificate

**Sign Off and Enclosures**

I DECLARE THAT THE STATEMENTS AND THE DATA CONTAINED IN THIS CASE RECORD FORM ARE COMPLETE AND CORRECT TO THE BEST OF MY KNOWLEDGE

Reviewer name *(print)*: ____________________________
Reviewer signature: ____________________________

Date of signature: [ ] [ ] [ ]

Day  Month  Year

PLEASE SIGN AND RETURN THIS COMPLETED DATA FORM TO THE PROJECT MANAGER WITHIN 7 WORKING DAYS OF COMPLETION
St. Michael’s
Inspired Care. Inspiring Science.

10.4 CRF 02
THDST Prevalence Follow-up Study
Study ID (THDST Study #)
CRF02

EVENT INFORMATION: Transplant

Date of Transplant

Location of Transplant:
☐ Name of Hospital: ___________________

Primary Cause of Transplant:
In the opinion of the investigator(s), what is the primary cause of transplant?

______________________________
______________________________
______________________________

Sign Off and Enclosures

I DECLARE THAT THE STATEMENTS AND THE DATA CONTAINED IN THIS CASE RECORD FORM ARE COMPLETE AND CORRECT TO THE BEST OF MY KNOWLEDGE

Reviewer name (print): ________________________________

Reviewer signature: ________________________________

Date of signature: 

Day  Month  Year

PLEASE SIGN AND RETURN THIS COMPLETED DATA FORM TO THE PROJECT MANAGER WITHIN 7 WORKING DAYS OF COMPLETION
EVENT INFORMATION: Worsening Heart Failure

Date of Onset

<table>
<thead>
<tr>
<th>Day</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
</table>

Specify NYHA Class: __________

Was patient hospitalized for this heart failure?  Are there any medication changes?

- □ Yes
- □ No
- □ Unknown
- □ Yes
- □ No
- □ Unknown

If NO, where was patient treated?

- □ Emergency Department
- □ Heart Function Clinic
- □ Other: ____________

Did the patient require use of IV diuretics in ER?

- □ Yes
- □ No
- □ Unknown

Did the patient experience new or worsening characteristic symptoms of heart failure?

<table>
<thead>
<tr>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Dyspnea</td>
</tr>
<tr>
<td>2.</td>
<td>Orthopnea</td>
</tr>
<tr>
<td>3.</td>
<td>Paroxysmal nocturnal dyspnea</td>
</tr>
<tr>
<td>4.</td>
<td>Edema</td>
</tr>
<tr>
<td>5.</td>
<td>Jugular Venous Distension</td>
</tr>
</tbody>
</table>

Sign Off and Enclosures

I DECLARE THAT THE STATEMENTS AND THE DATA CONTAINED IN THIS CASE RECORD FORM ARE COMPLETE AND CORRECT TO THE BEST OF MY KNOWLEDGE

Reviewer name (print): ____________________________

Reviewer signature: _____________________________

Date of signature:

<table>
<thead>
<tr>
<th>Day</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
</table>
EVENT INFORMATION:
HOSPITALIZATION OR EMERGENCY ROOM VISIT

What type of visit was this?
☐ Hospital Admission  ☐ Emergency Room Visit

This event was a:
☐ Discharge
☐ Death

Date of Admission  

Day    Month    Year

Date of Discharge Or Date of Death

Day    Month    Year

Name of Hospital: ___________________________________________________

Was patient transferred to or from another facility during this Hospitalization?
☐ Yes  ☐ No  ☐ Unknown

Please provide any known details:
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
Clinical Event

Please choose an event description code from the following code list
(Reminder: only one event per form)

ENTER CODE HERE: 

MEDICAL
011 Myocardial Infarction – NONFATAL
012 Heart Failure Decompensation
013 Angina (primary) excludes angina induced by tachyarrhythmia, hemorrhage, etc...
014 Arrhythmia
015 Stroke/TIA
016 Implantation of defibrillator/ICD
017 Other: specify_____________

Sign Off and Enclosures

I DECLARE THAT THE STATEMENTS AND THE DATA CONTAINED IN THIS CASE RECORD FORM ARE COMPLETE AND CORRECT TO THE BEST OF MY KNOWLEDGE

Reviewer name (print): ________________________________

Reviewer signature: ________________________________

Date of signature: ________________________________

PLEASE SIGN AND RETURN THIS COMPLETED DATA FORM TO THE PROJECT MANAGER WITHIN 7 WORKING DAYS OF COMPLETION