**THE PREVALENCE OF THIAMIN DEFICIENCY IN AMBULATORY PATIENTS
WITH HEART FAILURE**

by

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ABSTRACT

Thiamin is a required coenzyme in the production of energy to fuel myocardial contraction. Therefore, thiamin deficiency (TD) may contribute to myocardial weakness by limiting the available energy for myocyte contraction. Previous studies report a wide range for the prevalence of TD in patients with heart failure (HF) (3% to 91%). These trials are limited by their small sample size, indirect measurement of thiamin status, exclusion criteria, and their focus on hospitalized patients. Therefore, this study determined the prevalence of TD in a large (n=100) group of ambulatory patients with HF, using high performance liquid chromatography. The prevalence of TD ([thiamin pyrophosphate (TPP)] $\leq$ 180 nM/l erythrocytes) was found to be 7%. TD was not related to furosemide use, dietary thiamin intake, severity of the HF, or age. More investigation into the factors that may influence development of TD in ambulatory patients with HF is warranted.

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Table of Abbreviations

αKGDH	α -ketoglutarate dehydrogenase
ACC	American College of Cardiology
AHA	American Heart Association
ATP	Adenosine Tri-Phosphate
BMI	Body Mass Index
BNP	Brain Natriuretic Peptide
CIGMA	Infusion Glucose Model Assessment
CKD	Chronic Kidney Disease
CMR	Cardiac Magnetic Resonance
EAR	Estimated Average Requirement
ECG	Electrocardiogram
EDTA	Ethylenediamine-tetraacetic acid
ECLIA	Electrochemiluminescence Immunoassay
ETKA	Erythrocyte TK Activity
FFQ	Food Frequency Questionnaire
GFR	Glomerular Filtration Rate

GI	Gastrointestinal
HF	Heart Failure
HPLC	High-Performance Liquid Chromatography
IR	Insulin Resistance
ICD	Implantable Cardiac Defibrillator
LV	Left Ventricle
LVEF	Left Ventricular Ejection Fraction
MDA	Malondialdehyde
MDRD	Modification of Diet in Renal Disease
MRI	Magnetic Resonance Imaging
MSH	Mount Sinai Hospital
NE	Norepinephrine
NP-proBNP	biologically inactive by-product of Pro-BNP peptide processing
NYHA	New York Heart Association
PDHC	Pyruvate Dehydrogenase Complex
PPP	Pentose Phosphate Shunt Pathway
RDA	Recommended Dietary Allowance

SGA	Subjective Global Assessment
TBARS	ThioBarbituric Acid Reactive Substances
SMH	St. Michael's Hospital
TCA	Trichloroacetic Acid
TD	Thiamin Deficiency
TK	Transketolase
TMP	Thiamin Monophosphate
TPP	Thiamin Pyrophosphate
TPPE	Thiamin Pyrophosphate Effect
TTP	Thiamin Triphosphate

1.0. INTRODUCTION

Cardiovascular disease accounted for more than 32% of all deaths in Canada in 2004 and is the leading cause of death and hospitalization in this country (The Heart and Stroke Foundation of Canada, 2010). The average morbidity rate for Heart Failure (HF) alone is 10% per year.

Previous studies have found that 50% to 68% of hospitalized patients suffer from malnutrition (Freeman & Roubenoff, 1994) which is directly related to the rate of mortality in patients with HF (Kalantar-Zadeh, Anker, Horwich, & Fonarow, 2008).

Thiamin, a water-soluble B-complex vitamin, acts as a coenzyme in the metabolism of carbohydrates and is involved in the production of ATP (Guyton & Hall, 2006; Shils & Shike, 2006). Reductions in the rate of ATP production may contribute to myocardial weakness and a lower cardiac output by limiting energy available for myocyte contraction (Neubauer et al., 1997). Recent supplementation trials have demonstrated significant improvements in cardiac function following thiamin therapy suggesting that thiamin has the potential to positively influence cardiac remodelling (Shimon et al., 1995).

In addition, several factors have been reported to increase the risk of thiamin deficiency (TD) in patients with heart failure including severe HF (Schwengel, Gottlieb, & Fisher, 1994), malnutrition (Kwok, Falconer-Smith, Potter, & Ives, 1992; Lourenco et al., 2009), advanced age (O'Keeffe, 2000; Pepersack et al., 1999), frequent hospitalization (Cruickshank, Telfer, & Shenkin, 1988; Hanninen, Darling, Sole, Barr, & Keith, 2006; Lemoine et al., 1980; Pepersack et al., 1999) and use of diuretic medications (Lubetsky et al., 1999; Seligmann et al., 1991).

Studies that evaluated thiamin status in human subjects reported a high prevalence of TD in HF patients, ranging from 3% to 91% (Kwok et al., 1992; Levy, Soine, Huth, & Fishbein, 1992; Seligmann et al., 1991; Pfitzenmeyer, Guiland, d'Athis, Petit-Marnier, & Gaudet, 1994; Abou-

Hashem, Maamoun, Hamza, Fahmy, & Mortagy, 2009; Hanninen et al., 2006). However, these studies have been limited by their small sample size, variations in thiamin assay methods, or their use of subjects who were on high dose diuretic therapy. Moreover, previous prevalence studies were mainly focused on the hospitalized patients with HF whereas ambulatory patients have received little attention.

Considering the limitations involved in the previous studies, this study was designed to determine the prevalence of TD in a large group of ambulatory patients with HF, using the direct method of thiamin measurement, and to study the influence of factors that may be associated with the risk of developing TD.

2.0 LITERATURE REVIEW

2.1. Thiamin

2.1.1. Chemistry

Thiamin, also known as vitamin B1 or aneurin, is a water-soluble white crystalline B-complex vitamin (Goodhart, Shils, & Young, 1988; Shils & Shike, 2006). Chemically, thiamin is a pyrimidyl-substituted thiazole (Shils & Shike, 2006) where both the pyrimidine ring and thiazole moiety are essential for biologic activity (Goodhart et al., 1988) (**Figure 2.1-a**). Aqueous solutions of thiamin are stable at an acidic pH, however, they are unstable in alkaline solutions and are sensitive to ultraviolet light and high temperature (Goodhart et al., 1988; Gottschlich & Matarese, 1998; Shils & Shike, 2006).

Thiamin is present as four different compounds in human tissue: free thiamin, thiamin monophosphate (TMP), thiamin pyrophosphate (TPP), and thiamin triphosphate (TTP) (Leslie & Gheorghide, 1996). Total thiamin concentrations in healthy adults are estimated to be between 25-30 mg with 80% as TPP, 10% as TTP, and the rest of it as TMP and free thiamin. Vitamin B1 is mainly stored in skeletal muscles, heart, liver, kidney and brain (Gottschlich & Matarese, 1998; Jeejeebhoy & Kinney, 1988; Shils & Shike, 2006). The half-life of thiamin is between 9 to 18 days, and as a result, body stores can deplete within two to three weeks (Ariaey-Nejad, Balaghi, Baker, & Sauberlich, 1970; Sica, 2007).

2.1.2. Dietary Sources, Absorption, and Excretion

Major dietary sources of thiamin include yeast, unprocessed cereal grains, meat products, and nuts (Goodhart et al., 1988; Leslie & Gheorghiade, 1996; Shils & Shike, 2006), whereas dairy products and seafood are poor sources of thiamin (Shils & Shike, 2006) (**Table 2.1**).

Figure 2.1: a. Thiamin structure, b. Thiamin pyrophosphate (TPP) (Shils & Shike, 2006)

Table 2.1: Thiamin content of common foods (Shils & Shike, 2006)

Food Type	Thiamin Content (mg/100g)
Wheat flour (whole meal)	0.4-0.5
Rice	
Whole rice	0.5
Polished rice	0.03
Rice bran	2.3
Vegetables	
Peas	0.36
Other legumes	0.4-0.6
Potatoes	0.1
Cow's milk	0.04
Meats	
Pork	1.0
Beef	0.3
Lamb	0.2
Poultry	0.1
Refined sugars	Nil

Thiamin is readily absorbed in the small intestine by two distinct mechanisms; at concentrations less than 1 $\mu\text{mol/L}$, thiamin is absorbed by an active, carrier-mediated system. Active transport is greatest in jejunum and ileum. At higher concentrations, passive diffusion acts as the major mechanism for absorption (Goodhart et al., 1988; Jeejeebhoy & Kinney, 1988; Rindi & Laforenza, 2000; Shils & Shike, 2006). Excess thiamin and its metabolites, similar to most water-soluble vitamins, are excreted in the urine (Goodhart et al., 1988). Oral thiamin administration has been shown to have very low (5.3%) bioavailability relative to intravenous administration at a similar dose (Tallaksen, Sande, Bohmer, Bell, & Karlsen, 1993). This low bioavailability is related to saturation of the active transporters in the intestine (Bains et al. 1988)

2.1.3. Coenzyme Function of TPP

The majority of the absorbed thiamin is phosphorylated to thiamin pyrophosphate (TPP) (**Figure1-b**) (Goodhart et al., 1988; Jeejeebhoy & Kinney, 1988; Shils & Shike, 2006). TPP serves as a magnesium-coordinated coenzyme in two general types of reactions, which are involved in the metabolism of aldehyde groups (Jeejeebhoy & Kinney, 1988; Stipanuk, 2000). One is the decarboxylative conversion of α -keto acids to acetyl-CoA derivatives in the Krebs cycle which is catalyzed by multi-enzymatic dehydrogenase complexes (Goodhart et al., 1988). Such enzymes include the pyruvate dehydrogenase complex (PDHC), an enzyme complex located at the point of entry of carbon into the Krebs cycle, and α -ketoglutarate dehydrogenase (α KGDH), a rate-limiting enzyme and a component of Krebs cycle (Shils & Shike, 2006) (**Figure 2.2**).

Figure 2.2: TPP-dependent enzymes.

α-ketoglutarate dehydrogenase (αKGDH); Pyruvate dehydrogenase complex (PDHC); Transketolase (TK) (modified from (Shils & Shike, 2006))

The other reaction involves TPP as a coenzyme for the transketolase (TK) enzyme, a key component of the pentose phosphate shunt pathway (PPP) (Shils & Shike, 2006; Stipanuk, 2000). The PPP serves as an alternate pathway to glycolysis, as a means of generation of NADPH and 5-carbon sugar phosphates, particularly ribose 5-phosphate (Stipanuk, 2000) (**Figure2.2**). TK enzyme catalyzes a series of reversible reactions that convert excess ribose 5-phosphate and xylulose 5-phosphate to the glycolytic intermediates, fructose 6-phosphate and glyceraldehyde 3-phosphate. These intermediates can be channelled back into the glycolytic

pathway and other pathways of glucose metabolism, and subsequently be used for energy production. In addition, the initial pyruvate dehydrogenase-catalyzed step supplies acetyl-CoA for the nervous system, where it acts as a precursor for biosynthesis of lipids and acetylcholine (Goodhart et al., 1988; Stipanuk, 2000).

2.1.4. Thiamin as an Antioxidant

Previous studies suggest that thiamin may also act as a free radical scavenger (G. E. Gibson & Blass, 2007). Two different mechanisms are proposed to explain the direct influence of thiamin as an antioxidant agent. One includes the formation of thiamin disulfide and nitric oxide as a result of the opening of the thiazole ring and the subsequent loss of one hydrogen and one electron ($H^+ + e^-$) (Stepuro, Piletskaya, & Stepuro, 2005). The second pathway includes the separation of two hydrogens and two electrons from the NH_2 group of the pyrimidine ring while thiamin is oxidized to thiochrome (Lukienko, Mel'nichenko, Zverinskii, & Zabrodskaya, 2000) (**Figure 2.3**). It is suggested that the antioxidant effect of thiamin is due to the transfer of H^+ (from the thiazole ring) and $2H^+$ (from the pyrimidine ring) to reactive substances. In a study by Lukienko et al (Lukienko et al., 2000), it has been shown that thiamin inhibits lipid peroxidation in rat liver microsomes while reducing the level of free radical oxidation of unsaturated fatty acids in vivo. In another study, thiamin was considered as a potential therapeutic agent in diseases linked to copper toxicity and oxidative stress, such as Wilson's disease (Sheline & Choi, 2004), which further supported the role of thiamin as an antioxidant.

In this study, the level of oxidative stress was evaluated by measuring the plasma levels of F2-isoprostanes. Measurement of F2-isoprostanes is considered as one of the best available methods to evaluate oxidative stress and lipid peroxidation in vivo (Roberts & Morrow, 2000).

F2-isoprostanes are prostaglandin-like substances that are produced by free radical-catalyzed peroxidation of arachidonic acid in vivo (Morrow et al., 1990). Some of the advantages of using this marker over other quantitative markers of oxidative stress are 1) F2-isoprostanes are very stable, 2) they are specific products of lipid peroxidation, 3) they can be detected in measureable quantities in all normal biological fluids including plasma and urine, 4) their levels increase substantially in animal models of oxidative injury, and 5) their amount is unaffected by the lipid content of the diet (Roberts & Morrow, 2000). One of the most commonly applied assays for evaluation of oxidative stress is through measurement of the end-products of peroxide breakdown, including malondialdehyde (MDA), by means of thiobarbituric acid-reacting substances (TBARS) assay (Halliwell, 2000). However, MDA is not considered as a specific product of lipid peroxidation. Diene conjugation is another common method for the assessment of lipid peroxidation in vivo (Halliwell & Chirico, 1993). Conjugated diene structures are produced as a result of peroxidation of unsaturated fatty acids. Measurement of the amount of ultraviolet (UV) absorbance by these compounds is used as an index of lipid peroxidation. However, this technique has also been criticized in previous studies which have suggested that application of this assay to body fluids can produce artifacts (Halliwell & Chirico, 1993). Some of the limitations involved with other assays are the lack of sufficient sensitivity or specificity, or the influence of external factors, such as lipid content of the diet, on the product being measured (Roberts & Morrow, 2000).

Figure 2.3: Two chemical pathways that account for the antioxidant effects of thiamin.

The pathway on the left (I, II, III, IV) shows the interaction of NO and thiamin. The reactions on the right (II, V, VI) show the interaction with t-butyl hydroperoxide (R. S. Gibson, 2005)

2.1.5. Intake Requirements and Toxicity

The daily required dose for thiamin varies among individuals, as it is directly correlated with the level of carbohydrate intake (Goodhart et al., 1988). For instance, during pregnancy, lactation, increased muscle activity, or abnormal cases of hyperthyroidism, the requirements for thiamin increase. The “Dietary Reference Intakes” (DRI) developed for thiamin are the “Estimated Average Requirement” (EAR) and the “Recommended Dietary Allowance” (RDA) (Institute of Medicine, 1998). EAR estimates a daily intake value that meets the requirement of 50% of the healthy individuals in a group. The EAR for thiamin is set at 1.0 mg/day for adult (≥ 19 y) men

and 0.9 for adult women (≥ 19 y). The RDA is the average daily dietary intake level that is sufficient to meet the nutrient requirements of 97 to 98 percent of the individuals in a particular age and gender group. The RDA for thiamin is defined as the EAR value plus twice the coefficient of variation (CV). Assuming a CV of 10% for thiamin, the RDA is set at 1.2 mg/day for adult (≥ 19 y) men and 1.1 mg/day for adult women. 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 and individuals (Institute of Medicine, 1998).

Oral thiamin administration has not been linked to any toxic effects as excess thiamin is readily excreted by the kidneys (Goodhart et al., 1988; Guyton & Hall, 2006). In rare cases, generalized pruritus and minor irritation in the arm have been reported when very high doses (multiple 100 mg boluses) of intravenous thiamin hydrochloride has been injected (Wrenn, Murphy, & Slovis, 1989).

2.1.6. Human Thiamin Deficiency (TD) Disorders and Risk Factors:

Two classical human disorders resulting from TD include Beriberi and Wernicke's encephalopathy (Thiamine. monograph.2003; Shils & Shike, 2006). In adults, the two major forms of Beriberi include "dry Beriberi", and "wet Beriberi" (Loma-Osorio, Penafiel, Doltra, Sionis, & Bosch, 2008). Beriberi is very rare in developed countries and it is often associated with alcoholism (Gottschlich & Matarese, 1998). Dry Beriberi is mainly characterized by peripheral neuropathy consisting of symmetric impairment of sensory, motor, and reflex functions causing calf muscle tenderness and muscle wasting (Shils & Shike, 2006; Stipanuk, 2000). Clinical signs of wet Beriberi include edema, tachycardia, cardiomegaly (Shils & Shike, 2006) and muscle weakness. A potentially fatal form of wet Beriberi is called Shoshin Beriberi,

presenting with hypotension, tachycardia and circulatory collapse (Loma-Osorio et al., 2008; Shils & Shike, 2006). One study investigating the biochemical lesions observed in wet Beriberi hypothesized that acetyl-CoA deficiency played an important role in this disease (Bakker & Leunissen, 1995). Based on their assumption, TD impairs the function of PDH enzyme resulting in inadequate acetyl-CoA availability for the Krebs cycle and ATP depletion. A lower level of ATP is associated with heart failure, muscle weakness, and peripheral vasodilation (Bakker & Leunissen, 1995; Stipanuk, 2000).

Wernicke's encephalopathy is a common neuropsychiatric reaction to TD, which is associated with chronic alcoholism (Kopelman, Thomson, Guerrini, & Marshall, 2009). It mainly presents with memory disorder, ophthalmoplegia, ataxia (Butterworth, 1993; Kopelman et al., 2009) and neuronal loss (Kopelman et al., 2009). When Wernicke's encephalopathy is diagnosed, it should be treated with high doses of parenteral thiamin to prevent the development of Korsakoff syndrome (Kopelman et al., 2009; Shils & Shike, 2006). This syndrome is generally characterized by amnesia and impairment of conceptual functions (Shils & Shike, 2006). It has been suggested that during the course of Korsakoff syndrome, TD results in decreased activity of α KGDH enzyme, which subsequently results in irreversible neurological damage, including impaired brain energy metabolism and lactic acidosis (Butterworth, 1993).

Immediate parenteral thiamin administration is recommended for patients suspected of having Beriberi (Loma-Osorio et al., 2008; Sheline & Choi, 2004) or Wernicke's encephalopathy (Bridges, Trujillo, & Jacobs, 1999; Cook, Hallwood, & Thomson, 1998; Kopelman et al., 2009). The dosage varies depending on the severity of the condition (Cook et al., 1998); however, the recommended dosage ranges from 50-100 mg/day (Bridges et al., 1999; Loma-Osorio et al., 2008; Sheline & Choi, 2004).

TD is common in under-developed countries where the diet is largely dependent on nonenriched grains such as wheat and white rice (Thiamine. monograph.2003; Stipanuk, 2000). TD in developed countries is mainly associated with other risk factors. Chronic alcoholism is one common risk factor leading to TD (Thiamine. monograph.2003; Stipanuk, 2000) due to low intake of thiamin, reduced absorption of nutrients, malabsorption due to a decrease in active transport of thiamin, and hepatic cirrhosis (Bridges et al., 1999; Leevy, 1982). Consumption of foods that contain thiaminases or antithiamin compounds has also been observed to cause TD (Butterworth, 2001; Shils & Shike, 2006; Vimokesant, Kunjara, Rungruangsak, Nakornchai, & Panijpan, 1982). Thiaminases, which are found in some raw fish, shellfish, and ferns, are not stable in heat, and therefore are inactivated during cooking. In contrast, antithiamin compounds, found in some ferns, teas and betel nuts are thermostable and can influence the levels of thiamin absorption. Other risk factors for TD include HIV-AIDS infection (Butterworth et al., 1991; R. S. Gibson, 2005; Tang et al., 1993), chronic renal failure, long-term renal dialysis (Stipanuk, 2000; Talwar, Davidson, Cooney, & St JO'Reilly, 2000), gastrointestinal and liver diseases (Shils & Shike, 2006). Thiamin requirements are also increased during pregnancy as a result of increased metabolism, loss of appetite, vomiting (Heller, Salkeld, & Korner, 1974), and sequestration of this vitamin by the fetus (Butterworth, 2001; Heller et al., 1974).

2.1.7. Assessment of Thiamin Status

Erythrocytes contain approximately 90% of the total thiamin, mainly in the form of TPP (Lu & Frank, 2008). Consequently, two main methods of thiamin assessment include indirect measurement of TPP status based on the level of activity of the TK enzyme, and the direct measurement of TPP using high-performance liquid chromatography (HPLC) (Shils & Shike, 2006).

2.1.7.1. Erythrocyte Transketolase Activation Assay

The most widely used biochemical index of thiamin status is based on the measurement of erythrocyte TK activity (ETKA) in the absence and presence of excess cofactor TPP (R. S. Gibson, 2005; Shils & Shike, 2006). In the pentose phosphate pathway, TK enzyme, with the assistance of TPP, catalyzes the following reaction:

Xylulose-5-phosphate + Ribose-5-phosphate

Sedoheptulose-7-phosphate + Glyceraldehydes-3-phosphate

The rate of production of the product, hexose, is used as the indicator for TK activity. The method is defined as the thiamin pyrophosphate effect (TPPE) where the basal activity of the endogenous TK in erythrocytes is measured, and then compared with the TK activity after excess TPP coenzyme is added (R. S. Gibson, 2005; Shils & Shike, 2006; Baines & Davies, 1988). When an individual is diagnosed with TD, the basal level of TK enzyme activity is low, but it improves with the addition of excess TPP. Therefore, the relative increase in the ETKA indicates thiamin status. The results are expressed based on the ratio of stimulated to basal TK enzyme activity (R. S. Gibson, 2005). A TPPE 0%-15% indicates normal thiamin status, 16%-25% suggests marginal deficiency, and values greater than 25% indicate TD (H. E. Sauberlich, Herman, Stevens, & Herman, 1979; Stipanuk, 2000; Wood et al., 1980).

One major criticism associated with this method is its dependency on the TK enzyme as several factors, other than the level of TPP, can influence the level or activity of erythrocyte TK enzyme activity (R. S. Gibson, 2005). Some of these interfering factors include a loss of TK activity (Wood, Breen, & Penington, 1977), lower level of TK enzyme in elderly patients (O'Rourke et al., 1990), altered binding affinity of TK species for TPP (Kaczmarek & Nixon, 1983), and low

TK activity during certain disease, such as liver disease (Fennelly, Frank, Baker, & Leevy, 1967). Other limitations include the variation in the interpretation of ETKA because of poor inter-assay precision, difficulty in standardization (Talwar et al., 2000), and instability of the TK enzyme during storage.

2.1.7.2. High-Performance Liquid Chromatography (HPLC)

Several methods for the determination of TPP in erythrocytes by HPLC have been published (Lynch & Young, 2000). HPLC methods involve the oxidation of thiamin compounds to thiochrome, which is detected under fluorescent radiation. Both pre-column (Baines, 1985; Mancinelli et al., 2003; Tallaksen, Bohmer, Bell, & Karlsen, 1991; Warnock, 1982) and post-column (Herve, Beyne, & Delacoux, 1994; Talwar et al., 2000) conversion of thiamin to thiochrome have been reported. Pre-column derivatizations, however, are considered to be more convenient (Herve et al., 1994), with sharper peaks and higher resolution (Lynch & Young, 2000).

HPLC, compared to the ETKA assay, is considered as a more sensitive index of thiamin status (Baines, Bligh, & Madden, 1988; Herve et al., 1994). Moreover, erythrocyte TPP is found to be more stable and less susceptible to factors that alter enzyme activity (Baines & Davies, 1988). Other advantages of HPLC are its direct measurement of TPP with high precision (between-patch precision ranging from 5% to 11%) and high recovery rate (87.4-102%) (Baines et al., 1988; Mancinelli et al., 2003; Tallaksen et al., 1991; Talwar et al., 2000).

For this study, the pre-column HPLC method proposed by Mancinelli et al (Mancinelli et al., 2003) is used to measure erythrocyte TPP levels. This HPLC procedure provides a rapid (15 mins elution time), precise, and sensitive method for the evaluation of erythrocyte TPP. The mean (SD) control values for TPP were found to be at 222.23 ± 56.3 nM (range: 94 – 347 nM). The cut-off for deficiency was set at TPP < 180 nM (25th percentile of the control values).

There are two other alternative methods for determining a TD cut-off. One is through controlled feeding studies with healthy volunteers, in which TD could be defined at the time when clinical symptoms developed. However these studies have only been done using the TPPE assessment method. The second method involves measuring TPP levels in alcoholics (with clinical signs of TD) in comparison with healthy controls (Baines & Davies, 1988; Herve et al., 1995). Therefore, the cut-off for TD ($[TPP] < 180 \text{ nM}$), which was the statistical cut-off point based on data from healthy controls (Mancinelli et al., 2003), was comparable to the previously reported cut off by our group ($78 \text{ ng/ml (TPP)} = 184 \text{ nM (TPP)}$) (Hanninen et al., 2006)

Serum and whole blood TPP status have also been evaluated (Lu & Frank, 2008; Tallaksen et al., 1991), however, since they only contain small portion of the TPP (10%), serum and whole blood are not believed to be as sensitive as erythrocyte TPP assays (Tallaksen et al., 1991) for the assessment of thiamin status. Another limitation involving whole blood analysis is that it requires haemoglobin estimation to correct for any cell volume variability (Talwar et al., 2000).

2.1.7.3. Urine Thiamin

Urinary thiamin excretion has been widely used as a method for evaluation of thiamin status in individuals (Hanninen et al., 2006; Lubetsky et al., 1999; H. E. Sauberlich et al., 1979).

However, it has been suggested that in thiamin deplete individuals, thiamin levels in urine correspond to recent thiamin intakes, rather than being a reflection of thiamin status (R. S. Gibson, 2005). Similarly, in a study by Oldham et al (Oldham, 1962), a positive correlation was found between the dose of thiamin intake (500 and 810 micro gram) and the 24-h urine excretion in young adults on standardized diets. In contrast, at lower intakes (330 and 390 micrograms) the urinary excretion was no longer linear.

Urine thiamin excretion was also measured for the detection of the onset of TD (Wood et al., 1980). In a study by Wood et al study, 19 young healthy volunteers were randomized to receive either a control diet (containing a low thiamin-500µg-diet + 5mg thiamin hydrochloride) or a thiamin depleted diet (only low thiamin-500µg-diet + placebo) for 5 weeks; the thiamin depleted group experienced a rapid decrease in the urinary thiamin excretion between week 1 to 5, whereas urine thiamin excretion rose initially and then remained constant in controls. Significant linear correlation between log of urinary thiamin excretion and TPPE in thiamin depleted subjects was found ($p < 0.01$). Therefore, measurement of urinary thiamin levels can be helpful in evaluation of dietary thiamin intake, but it does not determine the degree of depletion in tissues.

HPLC is suggested as a sensitive, precise and reproducible measurement method of urinary thiamin status (Roser, Andrist, Harrington, Naito, & Lonsdale, 1978). Current guidelines for the interpretation of the results considers urinary thiamin excretion levels of $<27 \mu\text{g/g}$ creatinine in urine as deficient (high risk), 27-65 as low (medium risk), and more than 66 as being acceptable (low risk) (H. E. Sauberlich, 1999).

2.2. Heart Failure (HF)

2.2.1. Definition, Etiology, Pathophysiology

HF is considered as a leading cause of death in industrialized countries (Haldeman, Croft, Giles, & Rashidee, 1999). In 2006, it was estimated that there were 400,000 Canadians living with HF (The Heart and Stroke Foundation of Canada, 2010). The mortality rate for HF is 10% per year while between 40-50% of people die within five years of diagnosis (The Heart and Stroke Foundation of Canada, 2010). HF is defined as a clinical syndrome which requires having two

major or one major and two minor clinical factors based on the modified Framinigham clinical criteria (Senni et al., 1998). The eight major clinical criteria include paroxysmal nocturnal dyspnea, orthopnea, elevated jugular nervous pressure, pulmonary rales (edema), third heart sound, cardiomegaly on chest x-ray, and weight loss ≥ 4.5 kg in 5 days if it occurred during therapeutic interventions for HF. The minor criteria are bilateral leg edema, nocturnal cough, dyspnea on ordinary exertion, hepatpomegaly, pleural effusion, tachycardia (heart rate ≥ 120 beats/min), and weight loss ≥ 4.5 kg in five days.

A variety of disease and conditions can lead to HF including: hypertension, myocardial infarction, valvular heart disease, diabetes, dyslipidemia (Kannel, 2000), obesity, cigarette smoking (He et al., 2001), coronary artery disease, and family history of dilated cardiomyopathy (Hunt et al., 2009).

Evaluation of left ventricular systolic performance is a common indicator of the severity of heart failure (Packer M, Cohn J, Abraham W, et al, 1999). Left ventricular ejection fraction (LVEF) is defined as the fraction of the end-diastolic volume that is pumped from the left ventricle into the aorta (Guyton & Hall, 2006). In patients with cardiac hypertrophy and depressed contractile function, the LVEF is depressed (Chatterjee & Rame, 2008). The LV grade classifies LV performance along a continuum of EF level; Grade I (EF>50%) includes patients with mild LV function, and as the LV grade increases to Grade II (EF 30-50%), Grade III (EF 20-30%), and Grade IV (EF< 20%), so does the severity of the LV dysfunction. An EF of approximately 60% is considered as normal (Guyton & Hall, 2006), whereas patients with an EF of 40% or less are believed to have systolic dysfunction (Packer M, Cohn J, Abraham W, et al, 1999).

The progression of the LV dysfunction results in myocyte hypertrophy and dilation of the left ventricle (Barker et al., 2009; Hunt et al., 2009). Common clinical symptoms associated with

HF include dyspnea, fatigue (Hunt et al., 2009), tachycardia, and exercise intolerance (Barker et al., 2009). An elevated LV end-systolic volume, from a low ejection fraction (EF), contributes to low blood pressure and cardiac output (Anderson, 1993). The impaired ability of the heart to pump results in retention of fluid in the vascular system, thereby, inducing elevated venous pressure and edema (Guyton & Hall, 2006). In a study by Neubauer et al (Neubauer et al., 1997), in which the mortality rate between patients with higher ($39\pm2\%$) vs. lower ($22\pm1\%$) left ventricular ejection fraction (LVEF) was compared, LVEF demonstrated a trend to predict cardiovascular mortality.

2.2.2. Stages of HF

New York Heart Association (NYHA) functional classification is the most widely used scale for quantifying the degree of HF (Hunt et al., 2009). This system places patients in four different groups depending on their degree of limitation during physical activity. **Table 2.2** summarizes the characteristics of patients assigned to each class (Johnstone et al., 1994).

Table 2.2: NYHA classification and characteristics (Johnstone et al., 1994)

NYHA classification	Characteristics
Class I	No symptoms
Class II	Symptoms with ordinary activity
Class III	Symptoms with less than ordinary activity
Class IV	Symptoms at rest

This classification, however, has been criticized for being a subjective assessment with a significant inter-observer variability (Hunt et al., 2009). Another limitation associated with this scale is that treatment options used for patients are not significantly different among the four

categories (Hunt et al., 2009). As a result, in 2005, the American College of Cardiology/American Heart Association (ACC/AHA) proposed a new method for the classification of HF patients. This new approach places patients in one of four stages in the development of HF (A to D), based on their risk factors and the extent of structural heart disease (**Figure 2.4**). Based on this classification, stages A and B identify patients who are only at risk for developing HF vs. stages C and D which include those with current or past symptoms of HF. By including patients with risk factors, this system places an emphasis on therapeutic intervention before the development of LV dysfunction in order to reduce the chances of population mortality and morbidity associated with HF (Hunt et al., 2009).

<u>At Risk for Heart Failure</u>		<u>Heart Failure</u>	
<u>Stage A</u>	<u>Stage B</u>	<u>Stage C</u>	<u>Stage D</u>
At risk for heart failure but structural heart disease and symptoms absent	Structural heart disease but without signs and symptoms of heart failure absent	Structural heart disease with prior or current symptoms of heart failure	Refractory heart failure requiring specialized inventions
Ex. <u>Patients with:</u> - Hypertension - Atherosclerotic disease - Diabetes - Obesity	Ex. <u>Patients with:</u> - LV remodeling including LVH and low EF - Asymptomatic valvular disease	Ex. <u>Patients with:</u> - known structural heart disease and - Shortness of breath and fatigue, reduced exercise tolerance	Ex. <u>Patients:</u> Who have marked symptoms at rest despite maximal medical therapy

Figure 2.4: Stages in development of HF (Adapted from (Hunt et al., 2009))

2.2.3. Diagnostic Tools and Biomarkers

Collection of a detailed clinical history, physical examination as well as assessment of functional and laboratory testing are all recommended for patients who are suspected of having

HF (Arnold et al., 2006). EF, ventricular size, and functional status can be evaluated using several clinical imaging techniques. Echocardiography provides information regarding valvular and ventricular function, and muscle mass (Johnstone et al., 1994). Radionucleic angiography (a MUGA scan) is used as an alternative imaging method in cases where the echocardiogram results are poor, such as in obese patients. Cardiac catheterization, and magnetic resonance imaging (MRI) are among other imaging tools used for the assessment of heart failure (Arnold et al., 2006). Other routine tests performed on HF patients include electrocardiograms (ECG), complete blood work, and chest x-rays (Johnstone et al., 1994).

Patients with HF are shown to have elevated plasma levels of norepinephrine (NE) (Hunt et al., 2009). Initially, an elevation of NE levels improves the performance of the heart by increasing sodium/calcium permeability by acting on the adrenergic receptors, resulting in an elevated heart rate, improved contractile strength, and venous return (Guyton & Hall, 2006). However, long-term activation of the sympathetic nervous system aggravates HF as high NE levels increase hemodynamic stress on the ventricles and induce cardiac hypertrophy (Hunt et al., 2009; Knowlton et al., 1993). Measurement of plasma brain natriuretic peptide (BNP), or NT-proBNP (biologically inactive by-product of Pro-BNP peptide processing) is also used as a marker of left ventricle dysfunction in patients who are suspected of having HF (Arnold et al., 2006; Cheung & Kumana, 1998; Levin, Gardner, & Samson, 1998). BNP is a hormone which is mainly present in the ventricular myocardium and it is released as a result of increased cardiac wall tension and stretching. Therefore plasma levels of BNP and NT-ProBNP elevate during volume overload (Omeland, 2003) in left ventricular systolic dysfunction (it correlated with ventricular function) (Levin et al., 1998; McDonagh et al., 1998). Secretion of BNP improves

myocardial performance by reducing sympathetic tone, enhancing vasodilation (Levin et al., 1998), and lowering of blood pressure by increasing the excretion of salt and water through kidney (Guyton & Hall, 2006). Consequently, BNP and NT-proBNPs are used as markers of ventricular dysfunction (Omland, 2003).

2.2.4. Treatment

2.2.4.1. General Treatment and Prevention Options

In order to reduce symptoms and increase the chances of survival, medical treatment focuses on improving cardiac contractility while preventing ventricular remodelling, vasoconstriction, and congestion (Barker et al., 2009; Johnstone et al., 1994; Kannel, 2000) .

Several classes of drugs are used for the treatment of HF including vasodilators (ACE inhibitors and angiotensin II receptor antagonists) which lower the blood pressure, beta-blockers, which are used to reduce ventricular wall stress and cardiomyocyte remodelling, cardiac glycosides (e.g. digoxin) to control the heart rate, and nitrates to reduce peripheral vascular resistance (Barker et al., 2009; Johnstone et al., 1994).

Surgical treatment options include the insertion of pacemaker or implantable cardiac defibrillator (ICD), coronary angioplasty, and bypass (Liu et al., 2003). Monitoring and controlling of elevated blood pressure, dyslipidemia, avoidance of smoking, and consumption of a healthy diet are recommended as preventative strategies for HF (Kannel, 2000). Patients with symptomatic HF are advised to follow a no-added-salt diet (2 g/day to 3 g/day) to low-salt diet (1 g/day to 2 g/day) (Arnold et al., 2006). Also, patients with fluid retention, congestion, and renal dysfunction should limit their fluid intake to 1.5 L/day to 2 L/day (Arnold et al., 2006).

Moreover, HF patients should receive proper instructions on appropriate alcohol consumption, and physical activity (Liu et al., 2001).

2.2.4.2. Influence of Diuretic Treatment

Diuretics are commonly used to modify volume or composition of body fluids in various clinical situations including hypertension, liver cirrhosis and ascites, nephritic edema, as well as heart failure (Davison, 2005; Johnstone et al., 1994; Padilla, Armas-Hernandez, Hernandez, Israili, & Valasco, 2007). All diuretics target different sites in the nephron to enhance excretion of sodium and water in the urine (Davison, 2005; Padilla et al., 2007). It has been suggested that the long-term administration of diuretic medication may result in lower arterial blood pressure and increase in cardiac output (Wilson & Freis, 1959).

Commonly used groups of diuretics include thiazides, carbonic anhydride inhibitors, potassium-sparing diuretics, and loop diuretics (Rose, 1991). Thiazide diuretics inhibit reabsorption of Na^+ and Cl^- in the distal tubule and carbonic anhydrase in the proximal tubule of nephron (Davison, 2005), and therefore result in excretion of 5-10% of the filtered sodium (Packer M, Cohn J, Abraham W, et al, 1999). Carbonic anhydrase inhibitors, like acetazolamide, increase free water clearance by blocking bicarbonate in the proximal tubule. K^+ -sparing diuretics, such as spironolactone, result in reabsorption of very small percentage of Na^+ (2-3%) at the collecting duct (Davison, 2005; Rose, 1991). Loop diuretics, including furosemide, act on the thick ascending loop of Henle by inhibiting $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ co-transporter (Padilla et al., 2007; Rose, 1991). As a result, loop diuretics lower the osmotic pressure in the medulla, and thereby impair the reabsorption of water in both descending limb and collecting duct (SUKI, RECTOR, & SELDIN, 1965).

Common adverse affects associated with diuretic treatment are hypotension, impaired renal function, electrolyte disturbance, and neurohormonal activation (Johnstone et al., 1994; Packer M, Cohn J, Abraham W, et al, 1999). There is evidence from both animal (Lubetsky et al., 1999; Yui, Itokawa, & Kawai, 1980; Yui, 1980, Lubetsky, 1999) and human (Seligmann et al., 1991; Shimon et al., 1995; Zenuk et al., 2003) studies that diuretic therapy, particularly with furosemide, in patients with HF is associated with TD. Two distinct mechanisms for the action of diuretics are suggested: first, diuretics cause an increase in urinary flow and therefore the urinary excretion of thiamin increases along with it (Yui et al., 1980); and secondly, through furosemide inhibition of thiamin uptake by cardiac cells, which results in decline in TPP production in HF patients (Yui et al., 1980; Zangen, Botzer, Zangen, & Shainberg, 1998). Yui et al (Yui et al., 1980) performed one of the initial studies on the influence of furosemide on thiamin status on rats, and showed that intra-peritoneal administration of furosemide for four weeks resulted in a significantly lower thiamin status, and higher level of urinary thiamin excretion, compared to rats who were not on furosemide. Another study by Lubetsky et al. (Lubetsky et al., 1999) on rats showed that urinary thiamin losses are not specific to furosemide, and that other diuretic drugs, including mannitol, acetazolamide, chlorothiazide, and amiloride, can induce TD due to an increase in urinary flow.

Several studies on patients with HF also found furosemide to increase the risk of developing TD. Zenuk (Zenuk et al., 2003) reported biochemical evidence of TD in 98% of patients with HF who were receiving furosemide doses of 80 mg/d or greater. Similarly, Seligman et al. (Seligmann et al., 1991) showed a 91% prevalence of TD in hospitalized elderly patients with HF. In a study using healthy volunteers, it was found that furosemide induced TD in a dose-

dependent manner, and that there was a significant correlation between the rate of urinary thiamin excretion and urine flow ($r=0.54$, $p < 0.001$) (Rieck et al., 1999).

Therefore it has been suggested that the thiamin status in patients who are on long-term diuretic medication should be monitored for the development of TD, and that thiamin supplementation should be considered if the dietary uptake is below RDA recommendations (Rieck et al., 1999; Yui et al., 1980). Some studies, however, have failed to find a significant relationship between TD and furosemide therapy in patients with HF (Hanninen et al., 2006; Pfitzenmeyer et al., 1994).

2.3. HF and TD

2.3.1. Thiamin and HF

As mentioned in section 2.1.6., wet Beriberi is one manifestation of TD which results in decreased levels of cardiac output, peripheral dilation, and biventricular HF (Guyton & Hall, 2006; Wooley, 2008). Therefore, the symptoms of wet Beriberi can mimic the clinical signs of HF, as well as possibly aggravating the underlying heart failure condition (Hanninen et al., 2006; Seligmann et al., 1991; Shimon et al., 1995). There also seems to be a shift in the focus of TD research from Beriberi, which is mainly common in underdeveloped countries (Stipanuk, 2000), to a broader range of diseases, including heart disease (Leslie & Gheorghiade, 1996; Wooley, 2008). A study by Neubauer et al, suggested that an impaired energy state of the myocardium increases the risk for both the development and progression of HF in patients with dilated cardiomyopathy (Neubauer et al., 1997). In this study, the phosphocreatinine/ATP ratio was used as an index of the energetic state of the heart. The authors found that mean LVEF was significantly lower in patients with a low phosphocreatinine/ATP ratio group compared with a

group of healthy volunteers with a normal phosphocreatinine /ATP ratio group ($P < 0.03$) (Neubauer et al., 1997). Ingwall et al. (Ingwall, Atkinson, Clarke, & Fetters, 1990) also compared the ATP synthesis rates (creatinine kinase activity) and LVEF in biopsy specimens from patients with severe cardiomyopathy in comparison with those without cardiomyopathy and suggested that decreasing the energy reserve for ATP synthesis increases the risk for development of diastolic and systolic HF (Ingwall et al., 1990). Similarly, Hanninen's study on hospitalized patients with HF observed a trend where 40% of patients with advanced HF (NYHA class III/IV; LVEF $<35\%$) had TD, in comparison with only 25% of subjects with NYHA class I/II HF (Hanninen et al., 2006). Considering the significant role of thiamin as a coenzyme in ATP producing reactions (Jeejeebhoy & Kinney, 1988; Stipanuk, 2000), along with ATP's role as the source of energy for cardiac contraction (Ingwall et al., 1990; Neubauer et al., 1997), it is logical to assume that TD would aggravate the rate of ATP production, and therefore, result in myocardial dysfunction.

2.3.2. Common Factors Contributing to the Development of TD in HF

Nutritional analysis of hospitalized patients (using subjective global assessment (SGA)) has showed that up to 68% of patients with HF suffer from significant malnutrition (Freeman & Roubenoff, 1994). In one study, 38% of outpatients with HF were malnourished and the frequency of malnutrition increased in patients with more severe HF condition, ranging from 22% (NYHA II) to 78% (NYHA III/IV) (Schwengel et al., 1994).

In particular, nutritional analysis of the diet of patients with HF, using a food frequency questionnaire, revealed evidence of inadequate daily thiamin intake (Brady, Rock, & Horneffer, 1995). Kwok et al (Kwok et al., 1992) stated that elderly patients with HF with self-reported

poor appetite had significantly lower TPP levels compared with those with good or fair appetite ($P < 0.005$). A recent study reported that 30% of outpatients with HF had inadequate levels of thiamin intake (Lourenco et al., 2009). Anorexia, which is an outcome of gut/hepatic edema, fatigue, dyspnea, nausea, lower food intake, and drug therapy (e.g digoxin), is another factor that may exacerbate malnutrition in patients with HF (Kalantar-Zadeh et al., 2008; Witte & Clark, 2006).

A number of studies have reported an increase in the resting metabolic rate in patients with HF (Obisesan et al., 1996; Poehlman, Scheffers, Gottlieb, Fisher, & Vaitekevicius, 1994; Riley et al., 1991). Hypercatabolism in patients with HF is possibly the result of an increase in catabolic factors such as norepinephrine, epinephrine, and inflammatory cytokines (Bourdel-Marchasson & Emeriau, 2001). Moreover, an increased rate of breathing (Freeman & Roubenoff, 1994), elevated resting peripheral oxygen consumption, and increased cardiac work (Berry & Clark, 2000; Riley et al., 1991) have also been shown to contribute to hypermetabolism. The relationship between HF and metabolic rate was studied by Obessisen et al. (Obisesan et al., 1996) who reported that the increase in metabolic rate corresponded to the level of severity of the HF.

Both inadequate intake and altered metabolic rate may contribute to loss of lean body mass in patients with HF (Freeman & Roubenoff, 1994). Autopsy results of malnourished adults who had lost 40% of their total body weight revealed a 35% reduction in their heart weight. In particular, weight loss, and the loss of cardiac muscle are vital factors that can aggravate cardiac dysfunction and increase the risk of mortality.

Advanced age is another factor that increases the risk of TD in HF patients. Biochemical signs of TD have been reported in elderly inpatients ranging between 40-48% (O'Keeffe, 2000; Pepersack et al., 1999).

Frequent hospitalization in patients with HF has also been reported to increase the risk of TD. The reported prevalence of TD in HF hospitalized patients ranges between 20% to 40% (Cruickshank et al., 1988; Hanninen et al., 2006; Lemoine et al., 1980; Pepersack et al., 1999). Hanninen et al (Hanninen et al., 2006) found evidence of TD in one-third of the hospitalized HF patients (n=100). The high prevalence of TD in hospitalized patients may be related to the severity of the disease, greater number of medications, and loss of appetite and poor intake of thiamin (Lemoine et al., 1980; Pepersack et al., 1999).

Taken together, patients with HF may be at increased risk for TD for different reasons including malnutrition, anorexia, poor dietary thiamin intake, elevated resting metabolic rate, advanced age, and frequent hospitalization.

2.3.3. Prevalence Studies

Several studies evaluating thiamin status in human subjects have reported a high prevalence of TD in HF patients, ranging from 3% to 91% (Kwok et al., 1992; Levy et al., 1992; Seligmann et al., 1991; Pfitzenmeyer et al., 1994; Abou-Hashem et al., 2009; Hanninen et al., 2006). This wide variation is due to differences in the underlying nutrition status of subjects, the concurrent use of medications including loop diuretics, the severity of disease, and the measurement technique used for the assessment of thiamin status (Wooley, 2008).

In a study by Seligmann et al. (Seligmann et al., 1991), the erythrocyte thiamin levels in elderly (mean age 70 years) HF inpatients on furosemide therapy (80-240 mg/day, for at least 3 months) were compared to 16 age-matched controls, using TPPE. The prevalence of TD was found to be

91% (n=23), which was significantly higher compared to the 12% (n=16) of TD prevalence in the control group ($p < 0.001$). In response to the article by Seligmann et al., Levy et al (Levy et al., 1992) investigated the prevalence of TD in a group of relatively younger (mean age 47 ± 10 yrs) HF outpatients (n=38, LVEF<40%), with oral doses of diuretics, predominantly furosemide, in the range of 20-960 mg/day. Using the ETKA method, only 1 out of 38 subjects (3%) was found to be at risk (Levy et al., 1992). Comparing the mean age of subjects between their study's and Seligmann's (47 vs. 70 years), the authors suggested that advanced age could be a risk factor for TD in patients with HF. Kwok et al. (Kwok et al., 1992) compared thiamin levels in 37 elderly hospitalized patients (70% on loop diuretics) with those of 35 non-HF inpatients, using TPPE. The evidence of TD (TPPE>19%) was seen in 13% and 29% of the HF and non-HF groups, respectively. The thiamin status of the elderly group with HF was compared with 41 healthy elderly subjects (mean age 74 ± 6 years), however, no significant difference in the prevalence of TD was found. Therefore, given their findings, Kwok et al. (Kwok et al., 1992) concluded that TD is not common in an unselected group of elderly HF hospitalized patients. Pfitzenmeyer et al. (Pfitzenmeyer et al., 1994) assessed the thiamin levels of 35 elderly (mean age 86 ± 3.1 yrs) HF inpatients against 35 age-matched control subjects without any evidence of HF. Using two distinct methods for the assessment of erythrocyte TPP (TPPE and HPLC), 37% of the HF group had evidence of TD. In a study by Brady et al. (Brady et al., 1995), the thiamin status of 38 patients with HF who were taking a daily dose of at least 80 mg furosemide (NYHA class I-IV) was determined by ETKA; evidence of TD was found in 21% (8 out of 38) of patients with HF (Brady et al., 1995). Yue et al. (Yue, Beermann, Lindstrom, & Nyquist, 1997) evaluated the erythrocyte TPP levels in 81 hospitalized patients with HF (NYHA class II-IV) receiving furosemide treatment in comparison with 18 control subjects (patients with myocardial infarction but no HF), using HPLC. However, no significant difference in the

erythrocyte TPP concentration was seen. In 2006, Hanninen et al. (Hanninen et al., 2006) conducted a large cross-sectional study (in our lab) consisting of 100 hospitalized HF patients (NYHA class I-IV, median dose of 60 mg/day furosemide for 14 months) and 50 matched control subjects to evaluate the prevalence of TD using HPLC (Warnock, 1982). A 33% prevalence of TD was determined among the subjects, which was significantly higher compared to control subjects (12%) ($p = 0.007$). Hanninen's study (Hanninen et al., 2006) had some similarities to our study as all of the subjects were recruited from the St. Michael's Hospital, the HPLC method was used for the assessment of thiamin status, and the same food frequency questionnaire was used for the evaluation of dietary thiamin intake (section 3.1.3.3.). One main difference between the two studies was that the study by Hanninen et al. (2006) focused on hospitalized patients with HF, whereas in our study all subjects were recruited from ambulatory cardiology clinics. Finally, in a recent case control study (Abou-Hashem et al., 2009) thiamin status was compared between 63 elderly HF patients (mean age 67 ± 4.6 years, LVEF $< 50\%$, both inpatients and outpatients), and a control group consisting of 63 age-matched individuals with similar nutritional status, using HPLC. 27% (17/63) of the patients with HF were found to be TD, compared to only a 14% (9/63) deficiency in the control group. This difference, however, was not significant ($p = 0.08$). Moreover, there was a positive correlation between TPP level and LVEF ($r = 0.63$, $p < 0.001$) (Abou-Hashem et al., 2009).

2.3.4. Supplementation Trials

Freye and Hartung (Freye & Hartung, 1982) were among the first investigators who examined the influence of thiamin supplementation through administration of incremental doses of thiamin. In their study, up to a total dose of 50 mg/kg was injected in 6 hospitalized patients (in the ICU unit) with myocardial and pulmonary insufficiency. The results showed that thiamin

supplementation enhanced the elevation of central venous pressure, and that thiamin might act as a mild peripheral vasodilator, which can improve cardiac performance (Freye & Hartung, 1982). In the study by Seligmann et al. (Seligmann et al., 1991), six patients who were earlier identified as being TD, received 100 mg of IV thiamin twice daily for one week (Seligmann et al., 1991). As a result, thiamin status was significantly improved in all six patients ($p < 0.001$) (Seligmann et al., 1991). LVEF was also improved in 4 out of 5 patients and the level of functional capability increased by at least 1 NYHA class in all 6 subjects (Seligmann et al., 1991). In a follow-up study (Shimon et al., 1995), 30 HF patients, who were receiving a daily furosemide dose of >80 mg for at least 3 months, were initially randomized to receive 200 mg/day of IV thiamin or placebo for one week. Echocardiography results revealed a significant improvement in LVEF after 1 week of IV thiamin supplementation ($p < 0.05$). Erythrocyte thiamin, measured by TPPE, as well as plasma thiamin levels increased significantly following IV thiamin administration ($p < 0.01$ and $p < 0.001$, respectively). Following discharge from the hospital, all subjects received 200 mg of oral thiamin supplementation for an additional six weeks. In the 27 patients who received oral thiamin supplementation, plasma thiamin levels, LVEF and mean NYHA class improved significantly compared to the baseline values ($p < 0.001$), ($p < 0.01$), and ($p < 0.01$), respectively). Erythrocyte thiamin levels also increased in 22 out of 27 patients when compared with baseline and the cardiac output improved by an additional 22% ($p < 0.01$). The authors concluded that thiamin supplementation can improve left ventricular function and restore erythrocyte and plasma thiamin levels in patients with HF receiving long-term furosemide therapy (Shimon et al., 1995). A supplementation trial was also conducted by Pfitzenmeyer (Pfitzenmeyer et al., 1994), in which 35 HF inpatients were randomized to receive either 200 mg/day of IV or intra-muscular thiamin supplementation ($n=17$) for one week vs. a second group with no supplementation ($n=18$). The assessment of the

results at day 15 showed a significant improvement in the thiamin status ($p = 0.026$) (Pfitzenmeyer et al., 1994).

Taken together, recent supplementation trials have demonstrated significant improvements in cardiac function, which suggests that thiamin has the potential to positively influence cardiac remodelling.

2.4. Research Question

2.4.1. Rationale

Patients with HF are at an increased risk for TD, for reasons such as malnutrition due to anorexia, increased metabolic rate and the use of diuretic medications. Thiamin is an important cofactor in many energy producing reactions in the body and therefore, TD may contribute to the reduction in ATP stores observed in patients with HF. Reductions in the rate of ATP production may contribute to myocardial weakness and a lower cardiac output by limiting energy available for myocyte contraction. Recent supplementation trials have demonstrated significant improvements in cardiac function following thiamin therapy suggesting that thiamin has the potential to positively influence cardiac remodelling. However, the majority of studies reporting the prevalence of TD were limited by their small sample size utilized, variations in thiamin assay methods, and their reliance on the hospitalized patients who were receiving high doses of diuretic medication, whereas ambulatory HF patients have received little attention. As a result, two possibilities exist for the nutritional status of ambulatory patients with HF; on the one hand, these individuals might be well-nourished with a low prevalence of TD, however, on the other hand, these individuals may be chronically undernourished, yet not sick enough to be hospitalized with a high prevalence of TD. Subsequently, this investigation was undertaken to

clarify the thiamin status of this unique population of patients with HF. Moreover, the influence of diuretic therapy, the level of dietary thiamin intake, the nutritional status, the degree of HF severity, and presence of other comorbidities on thiamin status need a more thorough investigation.

Therefore, we have conducted a cross-sectional study to investigate the prevalence of thiamin deficiency in a large group of ambulatory patients with heart failure, using direct method of thiamin measurement.

2.4.2. Hypothesis

1. Primary Hypothesis

The prevalence of TD will be approximately 20% in our population of ambulatory patients with HF.

2. Secondary Hypotheses

1. In patients with HF, age, the level of thiamin intake, nutritional status, use of diuretic medications, and severity of the HF will be associated with prevalence of TD.
2. The plasma levels of F2-isoprostanes will be inversely related to the level of erythrocyte [TPP]
3. The plasma levels of NT-proBNP and NE will be elevated in patients with severe HF.

2.4.3. Objectives

1. Primary Objectives

To determine the prevalence of TD in a group of ambulatory outpatients with HF using High-Performance Liquid Chromatography (HPLC) method.

2. Secondary Objectives

1. To study the correlation between TD with clinical and demographic variables including HF severity, nutritional status, thiamin intake, and diuretic medication.
2. To evaluate the plasma levels of F2- isoprostanes, as a marker of lipid peroxidation in vivo and investigate its association with TD.
3. To measure the plasma levels of NT-proBNP and norepinephrine to further evaluate the influence of HF severity on thiamin status.

3.0. METHODOLOGY

3.1. Study Subjects

Patients with a history of HF were recruited from cardiology outpatient clinics at St. Michael's Hospital (SMH) and Mount Sinai Hospital (MSH). The majority of patients were recruited from the internal defibrillator (ICD) clinic at the SMH. Patients with a primary diagnosis of ischemic, dilated, idiopathic or valvular HF (NYHA class I-IV) were eligible to participate. We considered patients with HF as those who had previously been diagnosed with HF and who had evidence of left ventricular dysfunction. The measured LVEF was obtained from the results of either a MUGA scan, echocardiogram or during cardiac catheterization, which was performed within the last five years (January/2005 till present). Patients were excluded from the study if they were unable to provide informed consent or had a history of any concurrent condition which would result in TD, namely, gastrointestinal disorders (e.g. Crohn's disease, ulcerative colitis), liver disease, prolonged diarrhoea, dialysis, prolonged fever, infection, or renal failure. Patients were also not eligible if they were rapidly deteriorating, hospitalized in the last two

months, consuming excessive alcohol (>3 drinks per day), had a documented history of alcoholism or alcoholic cardiomyopathy, were pregnant, or were on experimental medications. This study was approved by the St. Michael's Hospital (SMH) and Mount Sinai Hospital (MSH) research ethics boards, and the protocol was registered at <http://clinicaltrials.gov> (Identifier: NCT00953823).

Eligible subjects were given a short explanation of the study along with an information sheet (Appendix, form 1 – Summary sheet) by the study coordinator. An appointment was booked for interested patients to return to SMH at a later date to participate in the study. Participants were offered compensation for their transportation and a \$5 Tim Horton's gift card (registered trademarks owned by The TDL Marks Corporation in Canada).

3.1.2. Collection of Information

Upon the entrance into the study, written informed consent was obtained from every participant and a copy of the consent form was given to the patient (Appendix, Form 2 – Prevalence consent form). Anthropometric and demographic data, including age, gender, ethnicity, height and weight was collected by the study coordinator (Appendix, form 3 – Demographics form). In addition, data regarding the etiology of HF, HF risk factors, and other comorbidities were collected by the study coordinator (Appendix, Form 4 – Case-report-form). The patient's medical chart was used to extract the LV grade, LVEF, NYHA functional class as well as the results from the latest physical examination including heart rate, blood pressure, and history of edema. Patients were also asked to bring a list of their current medications, including the type, dose and duration of any diuretics that they were using. In addition, any information on over-the-counter medications and supplements was also recorded.

3.1.3. Nutritional Assessment

3.1.3.1. BMI

BMI was used as one of the factors to evaluate patient's risk for developing HF. Considering the Health Canada guidelines, BMI in the range of 18.5–24.9 is considered as being normal, 25–29.9 as overweight, and >30 as being obese (Health Canada, 2003) (As was mentioned in section 2.2.1., obesity is one of the major risk factors for HF). In order to measure BMI, the weight of the patients (with no shoes) was measured by the study coordinator using an in-floor stand-on electronic scale (Scale-Tronix, Wheaton, IL). The height of the subjects was obtained from the SMH medical charts from echocardiogram reports. BMI was then calculated by dividing the body weight in kilograms by the square of the height in meters (kg/m^2).

3.1.3.2. SGA

SGA is a clinical technique used for the assessment of nutritional status based on medical history and physical examination (Detsky et al., 1987). It is widely used in different patient populations, including surgical, oncology, renal, and HIV patients for the diagnosis of malnutrition (Makhija & Baker, 2008). Compared to other techniques of nutrition assessment, SGA provides a rapid and simple method for the determination of malnutrition risk (Makhija & Baker, 2008). A significant correlation ($p < 0.001$) was found between the SGA class and the level of weight loss and percent weight loss (Kendall's τ was 0.46 and 0.40, respectively) (Detsky et al., 1987). The SGA classification has also been shown to have a high degree of interobserver reproducibility at 91% (kappa statistic: 0.784) (Detsky et al., 1987). One study also suggested that the SGA classification is a useful predictor of mortality and morbidity in the

undernourished elderly ($p < 0.05$) (Sacks et al., 2000). Nutrition assessment allows clinicians to do an in-depth investigation of adequacy of intake and to better address the severity of the malnutrition (Charney, 2008).

The SGA evaluation was performed by the study coordinator who was trained by a Registered Dietitian. The clinical history section of SGA assessed the amount and rate of weight loss, changes in the usual dietary pattern, presence of any gastrointestinal (GI) symptoms in the last 2 weeks as well as changes in the functional capacity. The physical examination looked for any evidence of subcutaneous fat loss below the eyes, cheeks and biceps as well as for the presence of muscle wasting in different areas including the temple, deltoids, scapula, and ribs. The presence of any signs of edema in the ankles or ascites was also evaluated by the study coordinator (Appendix, Form 5 – SGA), which may be due to HF or malnutrition. Based on the results of history and physical examination, patients were placed in one of the three SGA categories for nutrition status. These categories were: A= well nourished, B= mild to moderately malnourished, C= severely malnourished.

3.1.3.3. Collection and Analysis of Dietary Thiamin

A semi-quantitative food frequency questionnaire (FFQ) was used to estimate recent thiamin and macronutrient intake in the subjects within the past month (Appendix, Form 6 – FFQ). The one month time frame was suitable because body stores can deplete within two to three weeks of a thiamin deficient diet (Ariaey-Nejad et al., 1970; Sica, 2007). The Block Brief 2000 FFQ (70-item) (Block G,) was used, which has been updated from its previous full-length version (98-item) (Block, Dresser, Hartman, & Carroll, 1985). The Brief or reduced version of the questionnaire was designed to provide a valid assessment of the nutrient intake while being less time consuming in comparison with the original version (17 minutes vs. 30-35 minutes) (Block,

Hartman, & Naughton, 1990). For thiamin, the results of the full FFQ assessment and food record were shown to correlate well ($r = 0.41$ to 0.57) (Block, Woods, Potosky, & Clifford, 1990; Sobell, Block, Koslowe, Tobin, & Andres, 1989). Moreover, it was shown that the reduction in correlation between the FFQ and three four-day diet records, as a result of using the reduced version as supposed to the full version of FFQ, was very small to none (r (full-version to reduced version): 0.57 to 0.56 , 0.47 to 0.47 , 0.42 - 0.41) (Block et al., 1990).

The Block FFQ was chosen because it was previously used by Brady et al. (1995) to estimate thiamin intake in both hospitalized and ambulatory patients with HF. Hanninen et al. (2006) later modified this FFQ (Block G) to conduct a more complete evaluation of thiamin intake. Some of these modifications included addition of extra food categories rich in thiamin, grouping foods with similar thiamin content together, addition of questions about caffeine and alcohol intake and thiamin antagonists (e.g. raw fish) which have been found to decrease thiamin bioavailability (Rindi & Laforenza, 2000), and modification of the vitamin/mineral supplementation questions to focus more on the thiamin containing supplements (multivitamins and B-complex vitamins) (Hanninen, 2003). In addition, food categories were reorganized according to the Canada's Food Guid. The modified version of the questionnaire consisted of sixty six different categories of food items. Patients were assisted by the study coordinator to complete the FFQ. A sheet containing photos of different portion sizes (part of the original Block 2000 FFQ) was provided to the patients for better estimation of portion sizes. Besides reporting the regular food intake, patients were asked to report all the supplements they were taking, including B1-containing supplements. Food frequency data was evaluated by entering it into the Food Processor SQL nutrient analysis software (ESHA Research Inc, version 10.4.0, Salem, Oregon, using data from the 2007b Canadian Nutrient File). Data entry was double-checked by a trained FFQ analyst to identify any errors. The total amount of estimated thiamin

intake was reported in gr/day. The level of daily dietary thiamin intake alone as well as the total thiamin intake (dietary + supplements) were calculated and compared to the EAR cut-off limits.

3.2. Collection of Blood Sample

Blood samples were collected by SMH core laboratory technicians following an over-night fast. Four 4.0 ml vacutainer tubes with anticoagulant agent ethylenediamine-tetraacetic acid (EDTA) were collected to be used for the assessment of erythrocyte TPP, plasma norepinephrine, and plasma F2-isoprostanes. Fasting blood samples were used for the assessment of erythrocyte TPP to control for the role of TPP in the metabolic processes discussed in section 2.1.3. One 5.0 ml SST vacutainer (containing clot activator and gel for serum separation) was used by the SMH core lab for the evaluation of plasma NT-proBNP levels as well as creatinine, urea, fasting glucose, and insulin. An HCG blood test was performed for female participants who were at the childbearing age group to confirm that they were not pregnant. This test was done as part of the research ethics board requirements to identify those who were pregnant (and to exclude them from the study).

3.2.1. Evaluation of Erythrocyte TPP Levels

3.2.1.1. Erythrocyte TPP Extraction

Blood samples, which were collected in the EDTA tubes, 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 plasma from erythrocytes. Plasma was aliquoted into labelled eppendorf tubes and stored at -80°C for evaluation of NE and F2-isoprostanes.

Erythrocyte TPP was extracted and analysed according to the method developed by Mancinelli et al. (Mancinelli et al., 2003). Following the separation of plasma, the buffy coat was removed,

and the remaining erythrocytes were all transferred to one 50 ml polypropylene tube, and washed four times with four volumes of saline. The sample was centrifuged (1000 g, 10 minutes, 10 °C) after each wash. Following a final fourth wash, the sample was centrifuged at a higher speed (1400 g, 20 min, 10°C) in order to obtain well-packed red blood cells. Extra saline was then removed to leave a 1:1 cell suspension. The obtained uniform suspension was mixed, and a 2 ml aliquot of the mixture was transferred into a 15 ml polypropylene tube. In order to complete protein precipitation, 250 µl of trichloroacetic acid (TCA) (Sigma-Aldrich Co., Oakville, ON) 40% was added, vortexed , and incubated for 1 h in the dark at room temperature. Following the incubation, samples were centrifuged again (1900 g, 20 min, 22°C) and the supernatant was transferred to another 15 ml tube. The extracted liquid was washed with 5 volumes of water saturated diethyl ether to remove TCA. The extract was filtered through a 1ml syringe, using MINISART RC4 filters 0.45 µm (VWR, Toronto, ON), and stored at –80°C for up to 2 months for later analysis.

3.2.1.2. Erythrocyte TPP Analysis by HPLC

Erythrocyte TPP was determined using HPLC according to the procedure used by Mancinelli et al (Mancinelli et al., 2003). The HPLC instrument was equipped with a Waters 1525 binary HPLC pump power. The 2002 Empower chromatography software was used for processing and storage of data (Waters, Mississauga, Ontario). The column was an Ultra Amino 5µm, 100 Å, 250 x 4.6 mm with an Ultra Amino 5µm, 100 Å, 4.0 x 10mm Guard Cartridges (Restek, Bellefonte, PA). The HPLC was performed by precolumn, alkaline oxidation of TPP to thiochrome. A 2475 Waters Fluorescent Detector (Waters, Mississauga, Ontario) was set at an excitation wavelength of 375 nm and an emission wavelength of 430 nm. The flow rate was 1.0 ml/min (Flow A=0.5ml/min + Flow B=0.5 ml/min). Considering the results from Mancinelli et

al. (Mancinelli et al., 2003), the running time of 15 minutes should have been adequate, however, in our study the running time was set at 20 min, letting the machine more time to flush the column before the next injection. The injection volume was 50uL. The mobile phase consisted of 65% 85mM Potassium Phosphate (pH 7.5) buffer and 35% acetonitrile. Mobile phase was prepared and de-gassed daily.

Working standards were processed first in order to derive a standard curve and to ensure that peaks were sharp and well resolved. 10 mM standard stocks of thiamin, TMP, and TPP (Sigma Co., Toronto, ON) were previously prepared and stored at 4°C. Working standards were mixed and diluted to obtain three concentrations of 100 uM, 300 uM, and 600 uM. The process of injection started by running the most dilute standard, working up to most concentrated.

Prior to the injection, 500 uL of each standard was pipetted into an eppendorf tube, followed by addition of 25 uL Potassium Hexacyanoferrate (30.4 mM) and 25 uL Sodium Hydroxide (0.8 M). The sample was vortexed between each addition and was thereby ready for thiochrome reaction. A gastight # 1001 injector syringe (Hamilton Co, Reno, NV, USA) was rinsed with ddH₂O and used for the injections. 250 mL of standard was injected to make sure at least 50 ml had passed through the column.

Following the processing of working standards, erythrocyte samples were injected into the HPLC machine. Filtered erythrocytes were first thawed to room temperature and similar to the preparation steps for working standards, they were prepared for thiochrome reaction and injected into the machine. The response from the detector was recorded by the empower software. For the working standards, the thiamin, TMP, and TPP peaks, appeared at approximately 4, 8, and 11 minutes respectively, which corresponded to the results from Mancinelli's paper (Mancinelli et al., 2003). Repeated injections resulted in compression of the peaks as the column became contaminated over time. As a result, every four months the column

was replaced with a new one to attain well separated peaks. The standard curve for TPP was constructed by the Empower software using the average concentration of the two runs for each of the three dilutions. The fluorescence results (Area under the curve (AUC)) from the patient samples were plotted against the standard curve to obtain the TPP concentration of the erythrocytes. Based on the results of the study by Mancinelli et al. (Mancinelli et al., 2003), the normal levels of erythrocyte TPP was assumed to be at 222.2 ± 56.3 nmol/l and an erythrocyte TPP < 180 nmol/l was considered thiamin deficient.

In order to verify extraction recovery (Mancinelli et al., 2003), standard solutions (mix of thiamin, TMP, and TPP) at 50, 100, 300, and 600 nmol were analyzed with TCA extraction. The intra-day HPLC precision was evaluated by processing duplicates of individual blood samples separately (n=7). To verify inter-day HPLC precision, same patient samples (n=6) were run on two different days (one month in between). The intra-day and inter-day variability was based on the relative standard deviations (RSD) of the duplicate samples (RSD: (standard deviation of the 2 concentrations/average of the 2 concentrations) x 100). To test for the extraction recovery in the erythrocytes, initially 50 µl of radioactive labelled thiamin [³H(G)] hydrochloride (American Radiolabeled Chemicals, St. Louis, MO) was added to 2 ml aliquots, just prior to TCA extraction. However, later analysis showed that it was not a suitable method as it was found that labelled thiamin was partially lost (approximately 25%) in the protein pellet, which likely contributed to the low rate of recovery. Therefore, a second method was used to evaluate extraction recovery in which washed erythrocytes were spiked with 10 µl of 10 µM TPP standard (Sigma Co., Toronto, ON) before the addition of TCA and deproteinization. The final concentration of the spiking TPP in the 1ml filtered sample was expected to be at 100 nM (10 µl x 10 µM = 1ml x 100 nM). The difference in the erythrocyte TPP concentration between the regular and spiked samples was measured to obtain the % recovery.

3.2.2. Blood Markers

Analysis of routine blood tests including fasting glucose, creatinine, urea, insulin, and HCG were done in the SMH core laboratory using standardized hospital procedures. The Glomerular Filtration Rate (GFR) was calculated based on the serum creatinine levels. The original Modification of Diet in Renal Disease (MDRD) Study equation was used to calculate GFR (Levey et al., 1999):

$$\text{GFR (mL/min/1.73 m}^2\text{)} = 186 \times (\text{S}_{\text{cr}}/88.4)^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African-American) (SI units)}$$

The National Kidney Foundation has used GFR to classify the severity of chronic kidney disease (CKD) into 5 stages (Levey et al., 2003):

- **Stage 1** Kidney damage with normal or high GFR (GFR $\geq$ 90 ml/min)
- **Stage 2** Kidney damage with mild $\downarrow$ GFR (GFR = 60-89 ml/min)
- **Stage 3** Moderate $\downarrow$ GFR (GFR = 30-59 ml/min)
- **Stage 4** Severe $\downarrow$ GFR (GFR = 15-29 ml/min)
- **Stage 5** Kidney failure (GFR <15 ml/min)

The normal GFR in young adults is approximately between 120-130 mL/min per 1.73 m² and it gets slower with age. Individuals with GFR <60 mL/min/1.73 m² are characterized by the loss of 50% or more of normal adult kidney function. The rationale for including individuals with GFR $\geq$ 60 mL/min/1.73 m² is that GFR may remain at normal level despite considerable kidney damage (Levey et al., 2003). As a result, persons with normal GFR may still be at risk of having CKD if other markers of kidney damage were found, including pathologic abnormalities or markers of damage abnormalities in blood or urine tests or imaging studies (Levey et al., 2003).

Serum NT-proBNP levels were also determined by the core lab using an electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics) on a Cobas E601 analyzer (Hitachi High Technology Co, Tokyo, Japan). The normal range for plasma NT-proBNP concentration varies depending on the age of the patients and whether they were hospitalized or not. In this case, all subjects were ambulatory, therefore age was the only factor influencing the normal range. The normal reference ranges for ambulatory patients are as follows: age < 50: NT-proBNP < 125 ng/L, age 50-75: NT-proBNP < 250 ng/L, age > 75: NT-proBNP < 500 ng/L. Plasma norepinephrine was measured using a commercial ELISA kit (LDN Labor Diagnostika Nord, Nordhorn, Germany). Similarly, F2-isoprostanes levels were determined using a commercial ELISA kit (US Biological, Swampscott, MA) and according to manufacturer's protocol.

3.3. Statistical Analysis

The prevalence of TD in patients with HF was estimated to be at 20% (expected proportion (P) = 0.2), based on the study by Brady et al. (Brady et al., 1995) on the prevalence of TD in out-patients with HF. The sample size was calculated using the following formula (Naing L, Winn T, Rusli BN, 2006) which is designed for prevalence studies.

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

Z = Z statistic for a level of confidence

P = expected prevalence or proportion (in proportion of one)

D = precision (in proportion of one)

Considering the level of confidence of 95% ($Z= 1.96$) and the precision value of 7% ($d=0.07$), the required sample size was 126 subjects.

All of statistical analysis was performed using the SPSS software, version 18.0. P-values were two-tailed, and a p value < 0.05 was considered significant. Unlike most of the previous studies ((Brady et al., 1995; Hanninen et al., 2006; Pfitzenmeyer et al., 1994)), the distribution of erythrocyte TPP levels were considered relatively normally distributed (skewness = 1.9). As a result parametric statistics were used for the analysis of erythrocyte TPP concentration.

Besides erythrocyte [TPP], the dietary levels of thiamin intake, GFR, required caloric intake, and the estimated level of caloric intake were all determined to be normally distributed.

Therefore, parametric statistics were applied for their analysis as well. In contrast, the total amount of thiamin intake (from both diet and supplements), plasma levels of F2-isoprostanes, NE, and NT-proBNP as well as the dose of furosemide intake were not normally distributed. As a result, non-parametric statistics were used for their analysis.

Pearson's correlation coefficient was applied to test the association between erythrocyte [TPP] and dietary thiamin intake or GFR. Spearman's rank correlation coefficient was used to determine association between erythrocyte [TPP] and total thiamin intake, plasma concentrations of F2-isoprostanes, NE, and NT-proBNP.

Parametric analysis of the continuous data was performed using the independent samples t-test and the data was presented as mean (SD). One-way ANOVA was utilized to test for difference in three or more groups, including the LV functional class and diuretic types. Non-parametric analysis of continuous data was done by using the Mann-Whitney U test and Kruskal–Wallis test. The data were presented as the median (range).

Categorical data were analyzed using Chi-Squared Test, or Fisher's Exact Test when the number in any of the cells was less than five.

4.0 RESULTS

4.1. Study Subjects

Patients were recruited between September/2009 to October/2010 from the St Michael's Hospital (SMH) and Mount Sinai Hospital (MSH) cardiology clinics. Over 1100 ((SMH (n=1050), MSH (n= 63)) eligible patients with HF were approached to participate. 287 patients initially expressed interest in the study. An appointment was booked right away, or patients were contacted by the research coordinator at a later time. 105 eligible subjects entered into the study. Patients refused to participate for the following reasons: lived far from Toronto area (n= 42), did not return phone calls (n= 37), were no longer interested (n=30), did not show up for their appointment (n=19), were not willing to fast (n=5), passed away (n=3), other (n=48). At admission, information on demographics, HF history and risk factors, medication history, SGA, as well as blood samples were collected from 105 HF patients during their visit to SMH. The erythrocyte TPP concentration was successfully analyzed for 100 subjects. Therefore, the total sample size for this study was 100, which was based on the number of patients we could recruit during the period of this study.

4.1.1. Subject Characteristics

Table 4.1 summarizes the characteristics of HF patients. The majority of the subjects were male (n = 82) and Caucasian (n = 76). The mean age was 65 years. The estimated BMI was mainly in the overweight ($25.0 \leq \text{BMI} \leq 29.9$, n = 41) and obese ($\text{BMI} \geq 30.0$, n = 32) ranges. Only one patient was found to be underweight ($\text{BMI} < 18.5$, n = 1).

Table 4.1: Characteristics of HF patients

Characteristics	HF patients (n=100)
Gender-Male ¹	82 (82)
Ethnicity ¹	
Caucasian	76 (76)
African-American	3 (3)
Asian	8 (8)
Other	13 (13)
Age (Years) ²	65 (11.3)
BMI (kg/m²) ²	28.2 (5.3)
Underweight ^{1,3}	1 (1)
Normal weight ^{1,3}	26 (26)
Overweight ^{1,3}	41 (41)
Obese ^{1,3}	32 (32)

¹ n (%)² Mean (SD)³ Body Mass Index (BMI); Underweight: BMI<18.5, normal weight: 18.5 ≤ BMI ≤ 24.9, overweight: 25.0 ≤ BMI ≤ 29.9, obese: BMI ≥ 30.0

4.1.2. Cardiac Disease Characteristics

Table 4.2 illustrates the etiology and HF risk factors. The principal causes of HF were cardiomyopathy (n = 49), ischemic heart disease (n =7), or both (n=43). In terms of the HF risk factors 42% had a history of smoking and 9% were currently smoking. Other co-morbidities included hypertension (50%), family history of heart disease (68%), hypercholesterolemia (53%), respiratory disease (15%), stroke or mini stroke (TIA) (15%), and diabetes (36%). The distribution of patients in five stages of CKD is presented in **Table 4.2**. 35.7% of subjects had a moderate decrease in their level of kidney function and 2% were found to have a severe decrease in the level of kidney function.

The relationship between the HF and the SGA grade is shown in **Table 4.3**. Almost all subjects were well-nourished (SGA grade A: 99%). 83 patients had an implantable cardioverter-defibrillator (ICD) device. The majority of the subjects had a grade III (n = 64) LV function with mild or no limitation in their ordinary physical activities (NYHA class I-II, n = 73).

Table 4.2: Cardiac disease characteristics of HF patients (n=100)

Characteristics	n (%)
Etiology of HF	
Valvular disease	19 (19)
Cardiomyopathy	92 (92)
Ischemic heart disease	50 (50)
Previous cardiac surgery	46 (46)
ICD	83 (83)
HF Risk Factors	
Smoking	51 (51)
Past	42 (42)
Current	9 (9)
Hypertension	50 (50)
Family history of heart disease	68 (68)
Hypercholesterolemia	53 (53)
Respiratory disease	15 (15)
Stroke/TIA	15 (15)
Diabetes	36 (36)
Chronic Kidney Disease (CKD)¹	
Stage Based on GFR	
Stage 1/2 (normal GFR ≥ 60)	61 (62.2 %)
Stage 3 (Moderate $\downarrow$ in kidney function)	35 (35.7%)
Stage 4 (Severe $\downarrow$ in kidney function)	2 (2%)
Stage 5 (Kidney failure)	0

¹ Data shown for 98 patients (2 samples were lost in SMH Biochemistry Laboratory) the stages of CKD were estimated based on the Glomerular Filtration Rate (GFR) levels: $30 \leq \text{GFR} \leq 50$: stage 3, $15 \leq \text{GFR} \leq 29$: stage 4, $\text{GFR} < 15$: stage 5. Patients with normal GFR (CKD stage 1, 2) may still be at risk of having CKD

Table 4.3: Cardiac disease severity (n=100)

Characteristics	n (%)
Left Ventricle Dysfunction	
Grade II	22 (22)
Grade III	64 (64)
Grade IV	14 (14)
NYHA Classification¹	
Class I-II	73 (73)
Class III-IV	27 (27)
SGA Grade²	
A	99 (99)
B/C	1 (1)

¹**NYHA functional class I-II:** Patients with mild or no limitation in ordinary physical activity, **NYHA functional class III-IV:** patients with severe limitation in activity in ordinary physical activity

²**(SGA):** class A= well nourished, class B/C = mild to severely malnourished
 NYHA, New York Heart Association
 SGA, Subjective Global Assessment

4.1.3. Medication and Supplementation History

Medication history for the subjects is summarized in **Table 4.4**. Fifty two participants were taking furosemide with a median (25th, 75th percentile) daily dose of 40 mg/day (40, 80). Twenty four patients were also taking a second diuretic, either spironolactone (n = 22) at a median (25th, 75th percentile) daily dose of 25 mg/day (12.5, 25) or metolazone (n = 1) at the daily dose of 6.25 mg/day. Six patients took only spironolactone.

Other common HF medications included: cholesterol lowering medications (75%), angiotensin II receptor blockers (19%), ACE inhibitors (74%), β -Blockers (85%), calcium channel blockers (10%), anticoagulants (42%), aspirin (55%), and digoxin (26%).

Twenty eight patients were taking thiamin containing supplements in the form of multivitamins. Five of these patients were also taking a B-complex vitamin. One patient was taking only B-complex vitamins. Some of the other vitamins or herbal supplements included vitamin D (n = 10), vitamin C (n = 9), omega 3(n = 8), calcium carbonate (n = 7), vitamin B₁₂ (n = 6), Co-enzyme Q10 (n = 6), Iron (n = 3), vitamin E (n = 3), potassium (n = 3), glucosamine (n = 3), cod liver oil (n = 3).

Table 4.4: Medications and supplements (n=100)

Medication History	n	(%)
Diuretic Medications		
Furosemide	52	(52)
Spironolactone	28	(28)
Metolazide	1	(1)
Statins (Cholesterol-lowering drugs)	75	(75)
Angiotensin II Receptor Blockers	19	(19)
ACE Inhibitors	74	(74)
Beta Blockers	85	(85)
Calcium Channel Blockers	10	(10)
Anticoagulants	42	(42)
Aspirin	55	(55)
Digoxin	26	(26)
Thiamin containing supplements ¹	29	(29)
Other supplements ²	47	(47)

¹ n (%), HF patients taking B1 supplements (e.g., multivitamin or B-complex)

² n (%), HF patients taking any vitamins, minerals, or herbal supplements (with or without B1)

4.1.4. Blood Work

The results for blood work of interest are shown in **Table 4.5**. 65.7 % (n=99) of the subjects had a normal glucose level (within the 4.0-6.0 normal range). The fasting insulin results (n=99) were within the normal range (13-161 pmol/L) for 86.9% of patients.

Table 4.5: Selected blood parameters

Blood parameters	HF patients
Insulin (pmol/L)¹	55 (37, 93)
Glucose Fasting (mmol/L)²	6.1 (1.9)
Urea (mmol/ L)³	7.9 (3.9)

¹ Median (25th, 75th percentile). Data shown for 99 subjects (One samples was missing)

² Mean (SD). Data shown for 99 subjects (One sample was missing)

³ Mean (SD). Data shown for 100 patients.

4.2. Erythrocyte TPP Status and Its Relationship with Other Factors

4.2.1. Prevalence of TD

As was mentioned in section 3.2.1.2., working standards were processed first in order to derive a standard curve and to ensure that peaks were sharp and well resolved. Working standards were injected at three concentrations of 100uM, 300uM, and 600uM. The mean $\pm$ SD for the standard curve r-values (for thirty two HPLC runs) was 0.99 ± 0.008 . Prevalence of TD is presented in **Table 4.6**. Seven percent of subjects had TD. Only one out of 7 patients with TD was taking thiamin-containing supplements (multivitamins). The average recovery for four dilutions of TPP was at 102% (range 96-110%). The range of recoveries for the aqueous standard solutions of thiamin and TMP were (96-124%) and (83-94), respectively. The mean $\pm$ SD recovery of the

TPP for the patient samples was at $51.62\% \pm 15.25$. The intra- and inter-day precisions median (range) were 0.56% (0.03-2.17%) and 3.18% (0.30-8.63%), respectively. **Figure 4.1** shows the distribution of erythrocyte TPP values among the 100 subjects. The mean (SD) of [TPP] was 341.91(148.29) nmol/L. **Figure 4.2** presents the distribution of erythrocyte TPP values in B1-supplement users only (n=29). Twenty three patients were taking only multivitamins, 3 patients were taking B-complex (50mg) and a multivitamin, 2 patients were taking B-complex (100 mg) and a multivitamin, and one patient was taking B-complex (100mg) or B1 supplement (100mg), daily. The mean (SD) of [TPP] for B1-supplement users was at 355.92(177.92) nmol/L. There was no significant difference in the erythrocyte [TPP] for B1-supplement users (mean=355.92, SD=177.92) compared to those who were not taking any B1-containing supplements (mean=335.17, SD=135.32) ($p = 0.5$) (**Figure 4.3**). No relationship was found in the age of those who were TD compared to those without deficiency ($p = 0.58$, n=100).

Table 4.6: Prevalence (%) of TD in HF subjects

	n (%)
All subjects	7 (7%)
Excluding B1-containing supplement users	6 (8.5%)

¹ n=100

² n=71, subjects taking thiamin-containing supplements including multivitamins, B-complex vitamins or B1 vitamins were excluded from analysis

Figure 4.1: Distribution of erythrocyte [TPP] in all subjects

Distribution of erythrocyte TPP concentration (nmol/L) in patients with HF. The mean (SD) TPP concentration was 341.91 (148.29) nmol/L. The horizontal line indicates the TD cut-off line at 180 nM.

Figure 4.2: Distribution of erythrocyte [TPP] in thiamin-containing supplement takers

Distribution of erythrocyte TPP concentration in HF patients who were taking thiamin-containing supplements (n = 29). 6 subjects (▲) were taking B-complex supplements. The mean (SD) TPP concentration was 355.92(177.92) nmol/L. The horizontal line indicates the TD cut-off line at 180 nM.

Figure 4.3: Mean erythrocyte [TPP] and supplementation

Erythrocyte [TPP] (nmol/L) in those taking B1-containing supplements (n=29), and those not taking any B1 containing supplements (n=71).

Figure show Mean (SD) for each variable (p = 0.5).

4.2.2. Relationship Between TD and Estimated Thiamin Intake

The relationship between erythrocyte [TPP] and estimated dietary / (dietary + supplement)

thiamin intake is shown in **Figure 4.4** and **4.5**, respectively. There were no relationships

between erythrocyte [TPP] and estimated thiamin intake from diet or between erythrocyte [TPP]

and total thiamin intake (diet + B1-containing supplements) ($r = -0.01$, $p = 0.91$, $n = 100$ and $r_s =$

0.11 , $p = 0.25$, $n = 100$, respectively). After excluding B1-supplement users, no relationship was

found between erythrocyte [TPP] and estimated dietary thiamin intake ($r = 0.04$, $p = 0.7$, $n = 71$).

There was no significant difference in the dietary thiamin intake between the TD (Mean = 1.09,

SD = 0.54) and non-deficient groups (Mean = 1.36, SD = 0.733) ($p = 0.3$). Similarly, there was

no significant difference in the total thiamin intake (Median, range) between the TD (1.11,

(0.24-2.70)) and non-deficient (1.53, (0.45-105.65)) groups (Mann-Whitney U = 208; p = 0.11, n=100).

The estimated dietary thiamin intake and total thiamin intake (diet + B1-containing supplement) were compared to the EAR cut-off for adults (male: 1.0 mg/day, female: 0.9 mg/day) (**Figure 4.6** and **Figure 4.7**). 65% (n=100) of the subjects had a dietary thiamin intake of $\geq$ EAR cut-off. When the thiamin intake from both diet and supplements was considered, 72% (n=100) of the patients had a total thiamin intake of $\geq$ EAR cut-off. There was no significant difference in the mean erythrocyte [TPP] between those with adequate ($\geq$ EAR) vs. inadequate ($<$ EAR) thiamin intakes (both dietary and total intakes) (**Figure 4.6** and **Figure 4.7**, respectively). No difference was found in the prevalence of TD between those with total thiamin intake $<$ ERA compared to those with total thiamin intake $\geq$ EAR (p = 0.4).

The estimated median (range) of the daily caloric intake based on the FFQ results was 1396 (377, 3958) kcal/day. The normal resting energy expenditure for each participant was estimated using the Harris- Benedict (Harris JS & Benedict FG, 1919) equation. The median and range was found to be 1945 (1084, 5476). There was a significant positive correlation between the level of dietary thiamin intake (mg/day) and the level of reported caloric intake (kcal/day) (**Figure 4.8**). No relationship was found between the reported caloric intake (kcal/day) and the mean erythrocyte TPP concentration (mg/day) (**Figure 4.9**)

Figure 4.4: Erythrocyte [TPP] and dietary thiamin intake

Relationship between Erythrocyte [TPP] (nmol/L) and estimated dietary thiamin intake in mg/day ($r = -0.01$, $p = 0.91$, $n = 100$). Supplement users were included

¹The EAR cut-off line is based on the thiamin cut-off for adult male at 1.0 mg/day

Figure 4.5: Erythrocyte [TPP] and estimated total thiamin intake

Relationship between Erythrocyte [TPP] (nmol/L) and estimated thiamin intake (diet + B1 containing supplements) in mg/day ($r_s = 0.11$, $p = 0.25$, $n = 100$)

¹The EAR cut-off line is based on the thiamin cut-off for adult male at 1.0 mg/day

Figure 4.6: Mean erythrocyte [TPP] and dietary thiamin intake compared to EAR

Erythrocyte [TPP] (nmol/L) in subjects with dietary thiamin intake of < EAR (n=35) compared to those with dietary thiamin intake of ≥ EAR (n= 65). Figure shows Mean (SD) for each variable (p = 0.65)

Figure 4.7: Mean erythrocyte [TPP] and total thiamin intake compared to EAR

Erythrocyte [TPP] (nmol/L) in subjects with total thiamin intake of < EAR (n=28) compared to the total thiamin intake of ≥ EAR (n=72). Figure shows mean (SD) for each group (p = 0.8)

Figure 4.8: Dietary thiamin intake and reported caloric intake

Relationship between dietary thiamin intake (mg/day) and the level of reported caloric intake (kcal/day) ($r = 0.82$, $p < 0.001$, $n = 100$)

*Correlation was Significant at p value < 0.05

Figure 4.9: Mean erythrocyte [TPP] and reported caloric intake

Relationship between reported caloric intake (kcal/day) and the mean erythrocyte TPP concentration (mg/day) ($r = 0.05$, $p = 0.6$, $n = 100$)

4.2.3. Relationship Between TD and Cardiovascular Disease Characteristics

Mean erythrocyte [TPP] decreased with an increase in heart failure severity; however this was not significant (**Figure 4.10**). There was also no difference in the prevalence of TD between patients with grade II LV function and those with grade III-IV LV function (**Table 4.7**).

Subjects with NYHA class III-IV had a significantly higher erythrocyte [TPP] compared to those with class I-II symptoms (**Figure 4.11**). 86% of subjects with TD (n=7) had NYHA class I-II symptoms. Furthermore, there was no difference in the prevalence of TD between subjects with NYHA class I-II vs. those with class III-IV symptoms (**Table 4.7**).

Figure 4.10: Mean erythrocyte [TPP] and LV grade

Erythrocyte [TPP] (nmol/L, Mean (SD)) in patients with grade II (n=22), grade III (n=64), and grade IV (n=14) LV function ($F(2, 97) = 1.65, p = 0.2$)

Figure 4.11: Mean erythrocyte [TPP] and NYHA functional class

Erythrocyte [TPP] (Mean (SD)) in patients with NYHA functional class grade I-II (n=73) and III-IV (n=27) ($p = 0.04$)

Table 4.7: Cardiovascular disease characteristics

	Erythrocyte TPP	Erythrocyte TPP	p-value
	<180 nmol/l (TD) (n=7)	≥180 nmol/l (n=93)	
NYHA class¹			
I-II	6(86%)	67(72%)	NS(0.7)
III-IV	1(14%)	26(28%)	
LV grade¹			
II	1(14%)	27(29%)	NS(0.5)
III-IV	6(86%)	72(71%)	

¹Values are represented as n (%)

NS, non-significant

NYHA, New York Heart Association functional class

4.2.4. Relationship Between TD and Blood Markers

There was a significant positive relationship between erythrocyte [TPP] and plasma F2-isoprostane concentration (**Figure 4.12**). There was no difference in the plasma F2-isoprostane concentration (Median, range) between TD (12.20, (4.12-21.90)) vs. non-deficient patients (10.94, (1.98-72.40)) (Mann Whitney U= 305.50, $p = 0.8$, $n=100$). There was a significant correlation between the plasma F2-isoprostane concentration and age ($r = 0.29$, $p = 0.03$, $n=100$) as well as F2-isoprostane concentration and GFR ($r = -0.45$, $p < 0.001$, $n=98$).

Plasma NT-proBNP levels between LV grades approached statistical significance ($p=0.055$) for the $50 \leq \text{age} \leq 75$ year old age group and achieved significance ($p=0.04$) for those > 75 years of age (**Figure 4.13**). Plasma NT-proBNP levels were measured in 99 subjects. There was no relationship between erythrocyte [TPP] and plasma levels of NT-proBNP (**Figure 4.14**).

However, there was a significant correlation between plasma NT-proBNP concentration and GFR ($r = -0.30$, $p = 0.002$, $n=98$) as well as age ($r = 0.37$, $p < 0.001$, $n=99$).

No difference was found in the plasma levels of NE among the LV functional grade groups (**Figure 4.15**). Similar to the results for plasma NT-proBNP levels, there was not any relationship between the plasma NE concentration and erythrocyte [TPP] (**Figure 4.16**).

Figure 4.12: Erythrocyte [TPP] and plasma F2-isoprostane levels

Relationship between Erythrocyte [TPP] (nmol/L) and the concentration of plasma F2-isoprostanes (pg/mL) ($r_s = 0.22$, $p = 0.03$, $n=100$)

*Correlation was Significant at p value < 0.05

Figure 4.13: Plasma NT-proBNP levels and LV grade

Plasma NT-proBNP (ng/L) in subjects < 50 years old (Kruskal-Wallis: $\chi^2 = 0.66$, $df=2$, $p = 0.72$, $n=12$), $50 \leq \text{age} \leq 75$ years old (Kruskal-Wallis: $\chi^2 = 5.80$, $df=2$, $p = 0.055$, $n=68$), and age > 75 years old (Kruskal-Wallis: $\chi^2 = 6.43$, $df=2$, $p = 0.04$, $n=19$)

Figure shows median (25th, 75th percentile) for each variable

Figure 4.14: Erythrocyte [TPP] and plasma NT-proBNP levels

Relationship between Erythrocyte [TPP] (nmol/L) and the concentration of plasma NT-ProBNP (ng/L) ($r_s = 0.08$, $p = 0.43$, $n = 99$ (one sample was lost in the Biochemistry Laboratory))

Figure 4.15: Erythrocyte [TPP] and plasma NE levels

Plasma norepinephrine concentration (nmol/L) in subjects with grade II (n=22), grade III (n=63), and grade IV (n=13) LV function (Kruskal-Wallis: $\chi^2 = 2.47$, df=2, p=0.3)

Figure shows median (25th, 75th percentile) for each variable (n=98)

Figure 4.16: Erythrocyte [TPP] and the plasma NE levels

Relationship between erythrocyte [TPP] (nmol/L) and the plasma norepinephrine levels (nmol/L). ($r_s = -0.07$, p = 0.45, n= 98)

4.2.5. Relationship Between TD and Diuretic Intake

The relationship between erythrocyte [TPP] and single or multiple diuretic use is shown in **(Figure 4.17)**. There was no relationship between the erythrocyte [TPP] among patients who were taking furosemide only, spironolactone only, or two diuretics. TD was not related to either the use or to the dose of furosemide (**Table 4.8**). Likewise, there was no relationship between TD and spironolactone use, or the use of multiple diuretics (**Table 4.8**). The erythrocyte [TPP] was not different between diuretic users vs. those who were not taking any type of diuretics ($p = 0.3$, $n=100$).

As previous literature (Zenuk et al., 2003) had investigated patients with HF with furosemide doses of > 80 mg/day, we decided to look at this relationship and found that there was no significant difference. (**Figure 4.18**)

Figure 4.17: Mean erythrocyte [TPP] and diuretic use

Erythrocyte [TPP] (nmol/L, Mean (SD)) in plasma taking furosemide only (n=29), or spironolactone only (n=6), or 2 diuretics at the same time (Furosemide + spironolactone (n=22) or furosemide + metolazone (n=1))
(F (2, 55) = 1.20, p = 0.3)

Table 4.8: Relationship between TD and diuretic use

	Erythrocyte TPP	Erythrocyte TPP	p-value
	<180 nmol/l (TD)	≥180 nmol/l	
Furosemide ¹			
Yes	2(29%)	50(53%)	NS(0.3)
No	5(71%)	43(47%)	
Furosemide dose			
(mg/day) ²			
1-80	2(100%)	42(84%)	NS(1.0)
>80	0(0%)	8(16%)	
Spirolactone ¹			
Yes	1(14%)	27(29%)	NS(0.7)
No	6(86%)	66(71%)	
Multiple diuretics ¹			
0/1 diuretic	7(100%)	70(75%)	NS(0.4)
>1 diuretic	0(0%)	23(25%)	

¹ n (%), (TD) (n=7), Non-deficient group (n=93)² n (%), (TD) (n=2), Non-deficient group (n=50)

NS, non-significant

Figure 4.18: Mean erythrocyte [TPP] and furosemide dose

Erythrocyte [TPP] (nmol/L, Mean (SD)) in subjects taking furosemide at a dose of ≤ 80 mg/day (n=44) vs. those taking furosemide at a dose of > 80 mg/day (n=8) (p = 0.5)

5.0 DISCUSSION

5.1. Thiamin status

5.1.1. Prevalence of TD

The primary objective of this study was to determine the prevalence of TD in ambulatory patients with HF. Our results revealed that the prevalence of TD was 7% (n=100), determined by HPLC.

The results of previous studies on the prevalence of TD revealed a wide range of rates from 3% to 91% (Kwok et al., 1992; Levy et al., 1992; Seligmann et al., 1991; Pfitzenmeyer et al., 1994; Abou-Hashem et al., 2009; Brady et al., 1995; Hanninen et al., 2006). Our results are comparable to those of Levy et al. (1992) who found a prevalence of 3% (n=38) in ambulatory patients with HF (NYHA class II-IV), using the TPPE technique. The HF population in this study, however, was relatively younger with mean (SD) age of 47(10) (Levy et al., 1992). In contrast, our findings were not consistent with a number of other studies where a higher prevalence of TD was observed. Seligmann et al. (1991) found a 91% prevalence of TD in a group (n=23) of hospitalized patients with HF and similar symptoms. For Pfitzenmeyer et al. (1994), 37% of hospitalized elderly (Mean (SD): 86(31) y) patients with HF (n=35) had TD. Hanninen et al. (2006) also found evidence of TD in one-third of the hospitalized HF patients (n=100), using the direct method (HPLC). Kwok et al. (1992), however, concluded that the prevalence of TD in patients with HF was uncommon after they found only a 13% prevalence of TD in a small group of hospitalized (n=37) patients with HF (NYHA class III-IV). The 13% prevalence rate of TD in Kwok et al. (1992) was close to double our findings, using TPPE. The patient population in two of the previous studies consisted of a mix of both ambulatory and hospitalized patients with HF

(Brady et al., 1995; Abou-Hashem et al., 2009). The first study by Brady et al. (1995) had 71% (27/38) of the study population being ambulatory patients (NYHA class II-III) and having a TD prevalence of only 21%. In the second study by Abou-Hashem et al. (2009), 27% (n=63) of the ambulatory and hospitalized patients (the proportion of ambulatory group vs. the hospitalized was not mentioned) were deficient, using the HPLC technique. Taken together, studies to date suggest an association between prevalence and the proportion of the population that were hospitalized patients. The prevalence of TD in studies of hospitalized patients ranged between 33-91% (Hanninen et al., 2006; Pfitzenmeyer et al., 1994; Seligmann et al., 1991) and was higher compared to the 21% and 27% observed in those with a combination of hospitalized and ambulatory subjects (Abou-Hashem et al., 2009; Brady et al., 1995). The prevalence of TD was lowest in Levy study (3%) (Levy et al., 1992) which consisted of ambulatory patients only. A more detailed comparison of Brady's (Brady et al., 1995) and Levy's (Levy et al., 1992) studies showed that these two studies were similar in many aspects including the sample size (n=38), the severity of heart failure (mean NYHA class III-IV and LV grade < 40%), the measurement method that was used to evaluate the prevalence of TD (TPPE > 15%), and the mean (SD) age of the subjects (55(14) y vs. 47 (10) y, respectively). Therefore, the use of a combination of hospitalized and outpatient population vs. an exclusively ambulatory population of patients was one of the main factors differentiating these two studies. The results of our study fits well with these two previous studies as the prevalence of TD in our group of all ambulatory patients were only 7%.

There have been several reports of hospitalized elderly patients with biochemical signs of TD (Pepersack et al., 1999; O'Rourke et al., 1990; Lemoine et al., 1980). In a study by Pepersack et al. (1999), the prevalence of TD in a large group (n=118) of patients admitted to a geriatric ward

(mean (SD), 83(7) y) was found to be at 44% compared to only 20% in an age-matched outpatient control group (n=30). O'Rourke et al. (1990) reported that a group of (n=13) hospitalized elderly (67-92 y) patients had significantly lower thiamin levels ($p<0.01$, TPPE) compared to 10 healthy controls (10-82 y).

One possible explanation for a high prevalence of the TD in hospitalized patients is a low level of daily thiamin intake prior to hospitalization. One study at a teaching hospital in the UK has demonstrated that 25% of the meals given to elderly inpatients contained less than the RDA requirement for thiamin (Anita J. Thomas, Paul Finglas, & Valda W. Bunker, 1988). Lemoine et al. (1980) also concluded that based on the results of a dietary interview, 57% of the inpatients (n=656) had thiamin intakes lower than the RDA. Poor appetite (Lemoine et al., 1980) and a changed meal environment (Elmstahl, Blabolil, Fex, Kuller, & Steen, 1987) were also attributed to a higher TD rate in hospitalized patients. Therefore, it was not surprising to have a lower rate of TD in our population compared to the earlier studies on hospitalized patients.

Patients' state of health and diet (including the thiamin status) prior to the hospitalization and duration of hospitalization may also influence TD in hospitalized patients. Therefore, future investigations into the factors that may influence TD both before and during the course of hospitalization is recommended.

No relationship was found between age (mean (range) 63 (34-87 y)) and TD. Our finding was consistent with other prevalence studies in patients with HF (Brady et al., 1995; Hanninen et al., 2006). Kwok et al (1992) also failed to find any difference between the thiamin status of a young and healthy group (mean $28 \pm \text{SD } 6$ y) compared to healthy elderly individuals (mean $74 \pm 6 \text{ SD y}$). However, one study (O'Rourke et al., 1990) found a lower level of erythrocyte TPP

concentration in a group of healthy elderly persons (mean(range): 78(70-82)) compared to a healthy young population (mean(range): 26(19-37)). Hanninen (Hanninen, 2003) suggested that having a wider age range (approximately 60 years vs. only 40 years) may have accounted for a significant relationship between age and TD in O'Rourke study (O'Rourke et al., 1990).

Overall, the clinical research studies which have reported the prevalence of TD in HF patients to date suffered from a number of limitations. Most studies had a small sample size (Brady et al., 1995; Kwok et al., 1992; Levy et al., 1992; Seligmann et al., 1991; Pfitzenmeyer et al., 1994). With the exception of the Hanninen et al (n=100) (Hanninen et al., 2006), Yue et al (n=81)(Yue et al., 1997), and Abou-Hashem et al (n=63) studies (Abou-Hashem et al., 2009), the most others used a sample size of <40 patients (n(range): 23-38)(Brady et al., 1995; Kwok et al., 1992; Levy et al., 1992; Seligmann et al., 1991; Pfitzenmeyer et al., 1994) . Another limiting factor is the inconsistency of the measurement methods for determination of thiamin status. With the exception of the three recent prevalence studies, where the direct measurement of TPP was performed by means of HPLC, the remaining studies used indirect measurement of thiamin status (Brady et al., 1995; Kwok et al., 1992; Seligmann et al., 1991; Pfitzenmeyer et al., 1994). As was mentioned in section **2.1.7.1.**, the indirect method is influenced by factors other than thiamin that may alter the activity of TK enzyme including loss of the activity of TK, lower level of TK enzyme in elderly patients, altered binding affinity of TK species for TPP, low TK activity during certain disease, instability of the TK enzyme for storage, and etc. For that reason, ETKA/TPPE is not considered a sensitive or suitable indicator of TD (Baines & Davies, 1988). In contrast, our sample size consisted solely of a large group of ambulatory patients-a group that has not previously been investigated. In addition, our sample size of 100 outpatients was considerably greater than previous studies on ambulatory patients (Brady et al., 1995; Levy et

al., 1992). The erythrocyte TPP level was measured directly using the HPLC technique which provides a more rapid and sensitive method compared with ETKA and TPPE (Baines et al., 1988; Herve et al., 1994). Our subjects were chosen from a wide range of patients with HF, with NYHA functional class ranging between II-IV, and an LV grade of II-IV. We did not exclude anyone based on the type or dosage of diuretic treatment, medications, or supplements, including B1-containing supplements.

5.1.2. The erythrocyte [TPP] Assessment Technique

The range of recoveries for the aqueous standard solutions of thiamin, TMP, and TPP were comparable to Mancinelli's (2003) (section 4.2.1.) However, the mean recovery rate of TPP for washed erythrocyte samples was lower than what was reported by Mancinelli et al. (2003) (51.62% vs. 100%). The recovery rates in the Mancinelli et al. (2003), however, were based on the results from healthy subjects. In addition, in a follow up and ongoing project, healthy control subjects are being recruited to validate the current HPLC technique and the proposed cut-off for TD. Preliminary data from these investigations revealed higher recovery rates from healthy controls. To date, the mean (SD) recovery of TPP in washed erythrocytes is at 75% (30.1) (n=10) which is almost 50% higher compared to the mean (SD) recovery rates (52%) for our patients group (n=100). Following the correction of TPP concentration for the recovery rate, the prevalence of TD substantially decreased (from approximately 50% to 7%). The difference in recovery rates between healthy and HF subjects suggests that there is something, possibly a medication, in the blood of patients with heart failure that influences the recovery of TPP or enhances its precipitation with TCA. More healthy control subjects will be screened to assist us in better understanding the recovery of TPP in this technique. To our knowledge, there has not been any other study on the prevalence of TD in patients with HF in which a % recovery was

reported (Abou-Hashem et al., 2009; Brady et al., 1995; Hanninen et al., 2006; Kwok et al., 1992; Levy et al., 1992; Pfitzenmeyer et al., 1994; Seligmann et al., 1991). Therefore the interpretation of data in these studies was limited to uncorrected levels of thiamin and difficult to compare them.

5.2. Relationship Between TD and Diuretic Intake

Whether diuretic therapy is linked to TD in patients with HF is still a subject of controversy.

While some studies have reported a high prevalence of TD in furosemide users (Seligmann et al., 1991; Zenuk et al., 2003), others have failed to find a relationship between TD and furosemide therapy (Hanninen et al., 2006; Pfitzenmeyer et al., 1994).

58% of our subjects were using one or more types of diuretic medications. There was no association between TD and furosemide use (**Table 4.8**). Our results are in agreement with a number of previous studies in which no relationship was found between TD and furosemide use in patients with HF ((Hanninen et al., 2006; Pfitzenmeyer et al., 1994; Brady et al., 1995; Yue et al., 1997). The median (range) dose of the daily furosemide use was 40 (20-320 mg/day). Fifteen percent (6/52) were taking furosemide at a dose greater than 80 mg/day. The erythrocyte [TPP] was not different between those who were taking furosemide at a dose of ≤ 80 mg/day vs. >80 mg/day (**Figure 4.18**). These results are consistent with previous studies (Pfitzenmeyer et al., 1994; Hanninen et al., 2006) as well as in those with subjects taking relatively high doses of furosemide, including Brady et al. (1995) (mean (SD): 242(216), >80 mg/day) and Yue et al. (1997) (2 groups of patients with HF, median(range): group 1: (80 (10-750) mg/day), group 2: (120 (40-1500)mg/day).

In contrast, some previous studies reported a relationship between the use of furosemide and the development of TD in patients with HF (Zenuk et al., 2003) (Seligmann et al., 1991) or have suggested that the dose of furosemide could induce TD in a dose-dependent manner in healthy subjects (Rieck et al., 1999). It has been proposed that furosemide intake increases the risk of TD by either enhancing the urinary flow (and therefore the urinary excretion of thiamin) (Lubetsky et al., 1999; Yui et al., 1980) or through inhibition of thiamin uptake by cardiac cells (Zangen et al., 1998). However, the applicability of these studies was limited, due to their use of rat models (Lubetsky et al., 1999; Yui et al., 1980) or rat heart cells in vitro (Zangen et al., 1998).

Six percent of the subjects were taking spironolactone alone while 22% were taking spironolactone along with furosemide (**Table 4.8**). Similar to our results for furosemide, there was no relationships between TD and spironolactone use. However, we did find that the level of erythrocyte [TPP] was lower in those who were taking spironolactone alone compared to either those who were on furosemide alone or multiple diuretics (**Figure 4.17**), although this difference was not statistically significant. Similarly, Hanninen et al. (2006) observed that spironolactone use prior to hospitalization was significantly associated with TD ($p < 0.05$). Hanninen et al. (2006) supported this finding by arguing that spironolactone acts on the distal tubules and collecting duct, a possible location of thiamin metabolism/phosphorylation in the kidney (Hanninen, 2003).

Considering our results for both furosemide and spironolactone use, one explanation for the lack of diuretic effect is related to the mechanism and site of action of these diuretics. Loop diuretics, including furosemide, act on the thick ascending loop of Henle by inhibiting $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ co-transporter (Padilla et al., 2007; Rose, 1991), while potassium-retaining diuretics, including

spironolactone, hinders the reabsorption of Na^+ (2-3%) at the distal tubules and collecting duct (Davison, 2005; Rose, 1991). The site for thiamin reabsorption, however, is considered to be in the proximal tubules by means of a thiamine/ H^+ antiporter, based on the results of two studies on rats (Gastaldi et al., 2000; Laforenza, Orsenigo, & Rindi, 1998). In addition, one study on human derived renal epithelial cells showed that the reabsorption of thiamin through the thiamine/ H^+ antiporters is sodium-independent (Gastaldi et al., 2000), but energy, and pH dependent (with higher uptake at alkaline or neutral pH)(Ashokkumar, Vaziri, & Said, 2006). Therefore, there is a possibility that the actions of furosemide and spironolactone on the ascending loop of Henle and collecting duct, respectively, do not influence the reabsorption of thiamin at the proximal tubules. However, there is still a need for more research on the underlying mechanism and influence of a broader group of diuretics (and perhaps including those which act on the proximal tubules and alter the pH such as carbonic anhydrase inhibitors) to see whether there is a link between diuretic use and thiamin status.

Aside from furosemide and spironolactone, the only other type of diuretic used by the patients was metolazone (n=1). Twenty three percent of the subjects were taking multiple (two) diuretics. Multiple diuretic use did not have any association with the prevalence of TD (**Table 4.8**). Likewise, Hanninen et al. (2006) did not find any association between multiple diuretic use and TD in hospitalized patients.

5.3. Relationship Between TD and HF Severity

No relationship was found between TD and NYHA functional class (**Table 4.7**). In fact, the mean erythrocyte TPP levels were significantly higher in those with NYHA class III-IV compared to those with NYHA class I-II (**Figure 4.11**). Our findings are comparable to Hanninen et al. (2006) where they also did not discover any relationship between NYHA

classification and TD (Hanninen, 2003). However, the NYHA functional classification has been criticized in the research literature for its subjective nature (Raphael et al., 2007; Hunt, American College of Cardiology, & American Heart Association Task Force on Practice Guidelines, 2005). It has also been argued that NYHA classification has a high degree of inter-observer variability and is not sensitive enough to identify small but significant changes in the clinical status (Packer, 1987).

Similar to NYHA classification system, we did not find any sign of difference in the prevalence of TD between patients with grade II-LV function compared to those with grade III-IV LV function (**Table 4.7**). However, comparing the erythrocyte TPP level among the patients with grade II-IV LV function (**Figure 4.10**), subjects with higher LV grade (more aggravated HF) had a numerically lower level of erythrocyte [TPP], however, this was not statistically significant. Previous research has also supported the association between thiamin status and LV function. Abou-Hashem et al. (2009) found a highly significant positive correlation between [TPP] level and LVEF ($r = 0.63$, $p < 0.001$). Brady et al. (1995) and Hanninen et al. (2006) found a trend for patients with TD to have a lower LV ejection fraction and worsened LV functional grade, respectively. However, their findings were not statistically significant. Overall, the findings of Abou-Hashem et al. (2009), Brady et al. (1995), and Hanninen et al. (2006) suggest that patients with more severe HF, as indicated by the LVEF and LV functional grade, are more likely to be at risk of TD. The association between LV functional grade and TD in patients with HF has been linked to elevated resting metabolic rate (Obisesan et al., 1996; Poehlman et al., 1994; Riley et al., 1991), hypercatabolism and an increase in catabolic factors like norepinephrine and inflammatory cytokines (Bourdel-Marchasson & Emeriau, 2001), increased rate of breathing (Freeman & Roubenoff, 1994), and elevated cardiac work (Berry & Clark, 2000; Riley et al., 1991).

We did not attempt to match patients' symptoms with the Framingham clinical criteria at the time of enrolment. As a result, we recruited patients with a documented history of HF, but who may not currently have overt clinical symptoms of heart failure due to ongoing medical intervention. In addition, we used the results of latest available MUGA, echocardiogram, or cardiac catheterization tests within the last five years (January/2005 till present) to obtain the LVEF and LV functional grades.

It has been suggested that pharmacological interventions may result in rapid changes in hemodynamic variables, and therefore, signs and symptoms of HF may change over weeks or months. Thus, repeat of LVEF assessment has been recommended after 4-6 months of optimal medical therapy (Hunt et al., 2009). Subsequently, while we recruited relatively stable patients into our study, the data may still be limited by the LVEF data.

5.4. Relationship Between TD and Thiamin Intake

Based on the analysis of the dietary B1 intake, 35% of subjects had daily thiamin intakes of below the EAR cut-off limits (**Figure 4.6**). Even after the addition of the thiamin intake from supplements to the dietary intakes, 28% of subjects remained below the EAR cut-off limits (**Figure 4.7**). In addition, there was no significant difference in the total thiamin intake between the TD and non-deficient groups. Our findings are in contrast to the study by Hanninen et al (2006) in which the mean intake of thiamin was found to be adequate compared to EAR in a group of hospitalized patients with HF. Using the same semi-quantitative FFQ, Hanninen (Hanninen, 2003) reported that only 12% (11/94) of the subjects had dietary thiamin intakes < EAR, although 50% of their subjects were malnourished and reported decreased appetite. However, similar to this study (**Figure 4.4** and **Figure 4.5**), Hanninen did not find a significant

relationship between erythrocyte TPP concentration and estimated total or dietary thiamin intake (Hanninen, 2003). One possible reason behind our high frequency (35%) of patients with inadequate thiamin intakes is underreporting of food intake, which has been considered as a recurring problem with food frequency questionnaires.

We initially decided to use FFQ in this study since it was a screening project and this FFQ had already been used and modified by a previous study for the assessment of dietary thiamin intake (Hanninen et al., 2006). However, aging and the increased risk of problems with short-term memory has been suggested as some of the factors that can affect assessment methods that rely on retrospective reports of food intake rather than on direct records (Sawaya et al., 1996). It has also been suggested that differences in ethnicity and level of education can also influence reporting of the dietary intake (Kristal, Feng, Coates, Oberman, & George, 1997). One study has considered sex as an influencing factor where older women (66 ± 6 y) tend to underreport energy intake to a greater extent than older men (66 ± 6 y) (Johnson, Goran, & Poehlman, 1994). It is true that a 3 to 7 day weighed food record, which is considered the gold standard for the assessment of energy and nutrients intake (R. S. Gibson, 1990; Willett, 1998), may result in a more accurate estimate of food intake. However, collection of food records in an observation study, which involved only one visit to the hospital, was not as feasible as using an FFQ. In addition, food records have been shown to have their own limitations including underestimation of the usual intake due to undereating or underreporting during the period of data collection (Sawaya et al., 1996). 24-hour recall is another common tool for the assessment of intake, in which the chances of underreporting was shown to be lower compared to FFQ (Schatzkin et al., 2003). However, 24-hour recall does not reflect the routine intake within a period of time. Therefore, utilization of FFQ was considered to be a suitable method for the assessment of thiamin intake from the habitual diet.

The estimated median (range) for daily caloric intake based on the FFQ results was 1396 (376, 3958) kcal/day, while the normal resting energy expenditure (predicted by the Harris- Benedict (Harris JS & Benedict FG, 1919) equation) was found to be at 2020 (1084, 5476). No relationship was found between the reported caloric intake and mean erythrocyte TPP concentration (**Figure 4.9**). However, the level of dietary thiamin intake was positively associated with the level of daily caloric intake (**Figure 4.8**). Therefore, there is a possibility that the level of energy intake, and perhaps thiamin intake, was underreported. Moreover, analyzing the required thiamin intake per kcal intake of food, rather than by the EAR cut-off, revealed that only 3% (3/100) of the patients were taking inadequate levels of thiamin, based on the recommendation of 0.5 mg thiamin/1000 caloric intake for adults (Shils & Shike, 2006).

We did not find any association between dietary or total thiamin intakes in either of the TD or the non-deficient groups (section **4.2.2**). However, a greater proportion of patients with total thiamin intakes of <EAR had TD (3/28, 11%) compared to those with thiamin intakes of $\geq$ EAR (4/72, 5%). This difference, however, was not significant ($p = 0.4$). The same non-statistical trend was seen between TD and inadequate thiamin intake in the Hanninen et al (2006) study. Likewise, 50% (4/8) of the patients with TD in Brady et al. (1996) had thiamin intakes of less than two thirds of the RDA levels. Therefore, considering these results, it is reasonable to assume that the level of thiamin intake may influence the prevalence of TD in patients with heart failure.

There were no relationships between erythrocyte [TPP] and estimated thiamin intake from diet or between erythrocyte [TPP] and total thiamin intake. Erythrocyte [TPP] levels were not different between those who were taking B1-containing supplements in comparison with those who were not taking supplements (**Figure 4.3**). These findings were consistent with those of

Hanninen's (2006) in which they found no relationship between estimated intake and TPP level. In a study on 206 healthy subjects (age range, 25-64 y), dietary factors (which were evaluated using a face to face FFQ) were found to be a poor predictor of blood thiamin level, using HPLC (Bovet, Larue, Fayol, & Paccaud, 1998). Nichols et al. (Nichols & Basu, 1994) also failed to find any correlation between dietary thiamin intakes (using 3-nonconsecutive-day food record) and blood thiamin levels, based on TPPE measurements on 60 healthy (age ≥ 65 y) subjects. The lack of a positive relationship between thiamin intake and blood thiamin levels in patients with heart failure has been linked to the influence of other factors on the thiamin levels including elevated resting metabolic rate (Obisesan et al., 1996; Poehlman et al., 1994; Riley et al., 1991), hypercatabolism (Bourdel-Marchasson & Emeriau, 2001), elevated rate of breathing (Freeman & Roubenoff, 1994), and increased cardiac work (Berry & Clark, 2000; Riley et al., 1991). These factors can influence the requirements for thiamin in patients with HF. Therefore further research is needed to define an acceptable intake for these subjects.

Except for one (SGA grade = B) subject, the rest of the sample (99%) were found to be well-nourished (SGA grade = A). The prevalence of malnutrition was considerably lower compared to what was reported by Hanninen et al. (2006) (50% malnourishment) or by Freeman et al. (1994) (68% malnourishment) in hospitalized patients with HF. This is likely related to an improved health status of our participating out-patients in relationship to hospitalized patients.

5.5. Relationship Between TD and Biomarkers

We did not find any relationship between TD and the level of plasma F2-isoprostanes. Since thiamin acts as an antioxidant, we expected to see elevated levels of F2-isoprostanes in patients with lower levels of erythrocyte [TPP] relative to those with higher erythrocyte [TPP].

Surprisingly there was a positive association between F2-isoprostanes concentration and erythrocyte [TPP] levels (**Figure 4.12**). Several factors, including advanced age (Wang et al., 1995), diminished renal function (Cottone et al., 2009), and smoking (Sakano et al., 2009) are suggested to increase lipid peroxidation, and as a result elevate the level of F2-isoprostanes. In fact, we found a strong positive correlation between age and plasma F2-isoprostanes as well as a negative correlation between GFR and F2-isoprostane concentration. However, the results of linear regression analysis (controlling for age and GFR) revealed that plasma F2-isoprostane concentration was still a significant predictor of erythrocyte TPP levels ($p = 0.012$). Plasma level of F2-isoprostanes were also suggested to increase in patients with more severe heart failure (NYHA class III compared to class II) ($p = 0.001$) (Polidori et al., 2004). However, we did not find a significant relationship between plasma F2-isoprostanes and LV grade (II, III, IV) ($p = 0.4$). Although F2-isoprostanes are considered as being suitable markers of lipid peroxidation, lack of validated methods of assessment and variations in different immunoassay techniques makes it difficult to compare results from different studies (Morrow & Roberts, 1996). One source of variation is in the type of body fluid that is used for the assessment of F2-isoprostanes (plasma, urine, or serum) (Halliwell & Lee, 2010). Also, the lack of proper validation of commercial kits against appropriate mass spectrometric methods (Halliwell & Lee, 2010) results in variations in the interpretation of the results. And finally, to our knowledge, no other study has looked at the relationship between thiamin status and F2-isoprostane. Therefore, more research is needed to confirm whether there is a relationship between plasma F2-isoprostane levels and thiamin status.

The mean plasma levels of NT-proBNP levels were found to increase as the severity of the heart failure increases in the elderly population with HF (≥ 75 years old) (**Figure 4.13**). This was in agreement with data presented in section **2.2.3**, suggesting the application of NT-proBNPs as a

marker of LV dysfunction in patients with HF. However, no significant relationship was found between plasma NT-proBNP concentration and erythrocyte TPP levels (**Figure 4.14**). Previous research has shown that factors other than HF including age, gender, and renal function influence NT-proBNP levels (Omland, 2003; Pfister et al., 2010). We also found a significant positive correlation between age and plasma NT-proBNP concentration as well as a negative correlation between GFR and NT-proBNP. Although the influence of age has already been accounted for in defining a normal cut-off for NT-proBNP (section **3.2.2.**), the effect of renal function has not been considered. This could explain the lack of association observed between erythrocyte [TPP] and those with normal or elevated NT-proBNP levels. Moreover, it has been suggested that a variety of other cardiac conditions including diastolic dysfunction of the left ventricle, pulmonary hypertension, or valvular heart disease can affect the NT-proBNP concentration (Omland, 2003) and result in a false-positive rate of NT-proBNP elevation. Similarly, plasma levels of norepinephrine were not found to be associated to the erythrocyte [TPP] concentration (**Figure 4.16**) or the LV grade (**Figure 4.15**).

Overall, plasma levels of F2-isoprostanes, NE, and NT-proBNPs are influenced by variety of factors. Therefore, it would likely be more reasonable to utilize these methods in thiamin supplementation trials and study the change between the baseline and post-supplementation levels of these biomarkers. By doing this, we can control for most of the other influencing factors, such as age and renal function.

6.0. CONCLUSION AND FUTURE DIRECTIONS

The primary objective of our study was to determine the prevalence of TD in 100 ambulatory patients with HF. The prevalence of TD in our population of outpatients was 7%. This was a novel finding in a large group of ambulatory patients for which the magnitude of the problem

was not known. Several factors made this project unique including its large sample size of ambulatory patients with HF, the direct measurement of erythrocyte thiamin levels by using HPLC method, and a comprehensive look at the influence of other risk factors on the development of TD.

The prevalence estimate of TD found in our study was comparable to that observed in previous studies on ambulatory patients and lower than those previously reported for hospitalized patients with HF. To our knowledge, very few studies have evaluated TD in outpatients with HF and no study has ever compared the prevalence of TD between ambulatory and hospitalized patients with HF. Also, our population of ambulatory patients with HF was limited to two teaching hospitals. Therefore, it would be interesting to look at TD in ambulatory patients from urban teaching centre in comparison with community hospital study.

Unlike many studies, we had few exclusion criteria for patients and therefore the thiamin status was measured in patients with varying ranges of HF severity, types and doses of diuretic medications, diet, and supplement intake. The impact of diuretic medication on thiamin losses and subsequently on thiamin status has received much attention however remains controversial. Previous studies that reported a relationship between diuretic use and TD have suggested a relationship between the rate of urinary flow and TD. We, like several other previous studies found no relationship between TD and furosemide intake. The lack of relationship between diuretics and thiamin status may be related to the site and mechanism of thiamin reabsorption in the kidney. Therefore, there is a need for more research on the underlying mechanism of action of diuretics on the kidney and its relationship to thiamin metabolism. In addition, we found a tendency towards a lower level of erythrocyte [TPP] with spironolactone use. This relationship

was also observed in hospitalized patients with HF. Therefore, further investigation into the relationship between TD and spironolactone use in particular is warranted.

We also found no relationship between the dietary thiamin intake or total thiamin intake and thiamin status. Our findings were comparable to previous studies in which no association was found between thiamin intake and thiamin status. The lack of relationship between thiamin intake and TD may be due to underreporting, and therefore, underestimation of the dietary thiamin intake. Despite some of the limitations involved with the FFQ, it was still considered as the most feasible method for the assessment of habitual food intake in an observational compared to other methods including a 3 to 7 day food record or 24-hour recall. For future prevalence studies (in which subjects agree to make an extra visit to the research centre), application of a weighted food record may provide a more precise estimation of the intake. Furthermore, TD was not associated with the presence of malnutrition as all our participants were well-nourished, possibly due to having a less restrictive diet and better appetite compared to the hospitalized patients. Having the fact that most of the participants were well nourished further supports our relatively low prevalence of TD.

Age and NYHA functional class were not related to TD, which was also consistent with previous findings. We did not find any relationship between TD and severity of HF, based on LV function. Our findings were in contrast to previous research where severity of HF was found to relate to TD. Since our data for LV function was limited to the assessments within the last five years, it may not have reflected the true status of LV function at the time of thiamin evaluation. Future studies should investigate the influence of HF severity on TD using a LVEF assessment results which were performed ideally within the last six months.

We did not find any association between TD and plasma levels of F2-isoprostanes. Surprisingly there was a positive association between the level of F2-isoprostanes and erythrocyte [TPP].

This may indicate that factors other than thiamin deficiency or cardiac function have influenced the plasma levels of F2-isoprostanes. Moreover, considering the variations in the techniques for assessment of interpretation of F2-isoprostanes, there is a need for a validated and universal method for the evaluation of this marker in vivo. Plasma NT-proBNP levels were significantly correlated with HF severity, however, similar to LV grades, they were not related to TD. NE, on the other hand, was not found to be related to either heart failure severity or TD. Therefore, these markers may be more useful in future supplementation studies where the baseline and post-supplementation plasma levels are being compared, while the other factors remain constant.

In summary, we found that the prevalence of TD in ambulatory patients with HF is 7%. Age, NYHA functional class, furosemide use, severity of the HF, and intake of thiamin were not associated with TD. The influence of these potential TD risk factors should further be investigated in future studies. Our data suggests that the population of ambulatory patients investigated were relatively well nourished with a low prevalence of thiamin deficiency. These findings can not be generalized to the larger population of HF patients as there was significant selection bias in those participants who chose to return for the screening study (100 out of 1100 approached patients with HF) as well as the fact that majority of the participants (82%) had an ICD. Future studies, designed to collect data on a wider cross section of ambulatory patients would provide a more generalizable estimation of the true prevalence of TD in this group.

7.0 REFERENCES

- Abou-Hashem, R. M., Maamoun, M. M., Hamza, S. A., Fahmy, H. M., & Mortagy, A. K. (2009). Thiamine level in hospitalized elderly egyptian patients with congestive heart failure and left ventricular systolic dysfunction. *Journal of the American Geriatrics Society*, 57(11), 2165-2166.
- Anderson, R. M. (1993). The gross physiology of the cardiovascular system. (pp. 19-24). Tucson, Arizona: Racquet Press.
- Anita J. Thomas, Paul Finglas, & Valda W. Bunker. (1988). The B vitamin content of hospital meals and potential low intake by elderly inpatients. *Journal of Human Nutrition and Dietetics*, 1, 309-320.
- Ariaey-Nejad, M. R., Balaghi, M., Baker, E. M., & Sauberlich, H. E. (1970). Thiamin metabolism in man. *The American Journal of Clinical Nutrition*, 23(6), 764-778.
- Arnold, J. M., Liu, P., Demers, C., Dorian, P., Giannetti, N., Haddad, H., et al. (2006). Canadian cardiovascular society consensus conference recommendations on heart failure 2006: Diagnosis and management. *The Canadian Journal of Cardiology*, 22(1), 23-45.
- Ashokkumar, B., Vaziri, N. D., & Said, H. M. (2006). Thiamin uptake by the human-derived renal epithelial (HEK-293) cells: Cellular and molecular mechanisms. *American Journal of Physiology.Renal Physiology*, 291(4), F796-805.
- Baines, M. (1985). Improved high performance liquid chromatographic determination of thiamin diphosphate in erythrocytes. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, 153(1), 43-48.
- Baines, M., Bligh, J. G., & Madden, J. S. (1988). Tissue thiamin levels of hospitalised alcoholics before and after oral or parenteral vitamins. *Alcohol and Alcoholism (Oxford, Oxfordshire)*, 23(1), 49-52.

- Baines, M., & Davies, G. (1988). The evaluation of erythrocyte thiamin diphosphate as an indicator of thiamin status in man, and its comparison with erythrocyte transketolase activity measurements. *Annals of Clinical Biochemistry*, 25 (Pt 6)(Pt 6), 698-705.
- Bakker, S. J., & Leunissen, K. M. (1995). Hypothesis on cellular ATP depletion and adenosine release as causes of heart failure and vasodilatation in cardiovascular beriberi. *Medical Hypotheses*, 45(3), 265-267.
- Barker, J., Williams, C., & Tulloh, R. (2009). Understanding and treatment of heart failure. *Paediatrics and Child Health*, 19(1), 1-8.
- Berry, C., & Clark, A. L. (2000). Catabolism in chronic heart failure. *European Heart Journal*, 21(7), 521-532.
- Block G. *Block brief questionnaire 2000 (unpublished)*. www.nutritionquest.com
- Block, G., Dresser, C. M., Hartman, A. M., & Carroll, M. D. (1985). Nutrient sources in the american diet: Quantitative data from the NHANES II survey. I. vitamins and minerals. *American Journal of Epidemiology*, 122(1), 13-26.
- Block, G., Hartman, A. M., & Naughton, D. (1990). A reduced dietary questionnaire: Development and validation. *Epidemiology (Cambridge, Mass.)*, 1(1), 58-64.
- Block, G., Woods, M., Potosky, A., & Clifford, C. (1990). Validation of a self-administered diet history questionnaire using multiple diet records. *Journal of Clinical Epidemiology*, 43(12), 1327-1335.
- Bourdel-Marchasson, I., & Emeriau, J. P. (2001). Nutritional strategy in the management of heart failure in adults. *American Journal of Cardiovascular Drugs : Drugs, Devices, and Other Interventions*, 1(5), 363-373.

- Bovet, P., Larue, D., Fayol, V., & Paccaud, F. (1998). Blood thiamin status and determinants in the population of seychelles (indian ocean). *Journal of Epidemiology and Community Health*, 52(4), 237-242.
- Brady, J. A., Rock, C. L., & Horneffer, M. R. (1995). Thiamin status, diuretic medications, and the management of congestive heart failure. *Journal of the American Dietetic Association*, 95(5), 541-544.
- Bridges, K. J., Trujillo, E. B., & Jacobs, D. O. (1999). Alcohol-related thiamine deficiency and malnutrition. *Critical Care Nurse*, 19(6), 80-85.
- Butterworth, R. F. (1993). Pathophysiologic mechanisms responsible for the reversible (thiamine-responsive) and irreversible (thiamine non-responsive) neurological symptoms of wernicke's encephalopathy. *Drug and Alcohol Review*, 12(3), 315-322.
- Butterworth, R. F. (2001). Maternal thiamine deficiency: Still a problem in some world communities. *The American Journal of Clinical Nutrition*, 74(6), 712-713.
- Butterworth, R. F., Gaudreau, C., Vincelette, J., Bourgault, A. M., Lamothe, F., & Nutini, A. M. (1991). Thiamine deficiency and wernicke's encephalopathy in AIDS. *Metabolic Brain Disease*, 6(4), 207-212.
- Charney, P. (2008). Nutrition screening vs nutrition assessment: How do they differ? *Nutrition in Clinical Practice : Official Publication of the American Society for Parenteral and Enteral Nutrition*, 23(4), 366-372.
- Chatterjee, K., & Rame, J. E. (2008). Systolic heart failure: Chronic and acute syndromes. *Critical Care Medicine*, 36(1 Suppl), S44-51.

- Cheung, B. M., & Kumana, C. R. (1998). Natriuretic peptides--relevance in cardiovascular disease. *JAMA : The Journal of the American Medical Association*, 280(23), 1983-1984.
- Cook, C. C., Hallwood, P. M., & Thomson, A. D. (1998). B vitamin deficiency and neuropsychiatric syndromes in alcohol misuse. *Alcohol and Alcoholism (Oxford, Oxfordshire)*, 33(4), 317-336.
- Cottone, S., Mule, G., Guarneri, M., Palermo, A., Lorito, M. C., Riccobene, R., et al. (2009). Endothelin-1 and F2-isoprostane relate to and predict renal dysfunction in hypertensive patients. *Nephrology, Dialysis, Transplantation : Official Publication of the European Dialysis and Transplant Association - European Renal Association*, 24(2), 497-503.
- Cruickshank, A. M., Telfer, A. B., & Shenkin, A. (1988). Thiamine deficiency in the critically ill. *Intensive Care Medicine*, 14(4), 384-387.
- Davison, A. M. (2005). *Oxford textbook of clinical nephrology* (3rd ed.). Oxford ; New York: Oxford University Press.
- Detsky, A. S., McLaughlin, J. R., Baker, J. P., Johnston, N., Whittaker, S., Mendelson, R. A., et al. (1987). What is subjective global assessment of nutritional status? *JPEN. Journal of Parenteral and Enteral Nutrition*, 11(1), 8-13.
- Elmstahl, S., Blabolil, V., Fex, G., Kuller, R., & Steen, B. (1987). Hospital nutrition in geriatric long-term care medicine. I. effects of a changed meal environment. *Comprehensive Gerontology. Section A, Clinical and Laboratory Sciences*, 1(1), 29-33.
- Fennelly, J., Frank, O., Baker, H., & Leevy, C. M. (1967). Red blood cell-transketolase activity in malnourished alcoholics with cirrhosis. *The American Journal of Clinical Nutrition*, 20(9), 946-949.

- Freeman, L. M., & Roubenoff, R. (1994). The nutrition implications of cardiac cachexia. *Nutrition Reviews*, 52(10), 340-347.
- Freye, E., & Hartung, E. (1982). The potential use of thiamine in patients with cardiac insufficiency. *Acta Vitaminologica Et Enzymologica*, 4(4), 285-290.
- Gastaldi, G., Cova, E., Verri, A., Laforenza, U., Faelli, A., & Rindi, G. (2000). Transport of thiamin in rat renal brush border membrane vesicles. *Kidney International*, 57(5), 2043-2054.
- Gibson, G. E., & Blass, J. P. (2007). Thiamine-dependent processes and treatment strategies in neurodegeneration. *Antioxidants & Redox Signaling*, 9(10), 1605-1619.
- Gibson, R. S. (1990). *Principles of nutritional assessment*. New York: Oxford University Press.
- Gibson, R. S. (2005). *Principles of nutritional assessment* (2nd ed.). New York: Oxford University Press.
- Goodhart, R. S., Shils, M. E., & Young, V. R. (1988). *Modern nutrition in health and disease* (7th -- ed.). Philadelphia: Lea & Febiger.
- Gottschlich, M. M., & Matarese, L. E. (1998). *Contemporary nutrition support practice : A clinical guide*. Philadelphia, Pa.: W.B. Saunders Co.
- Guyton, A. C., & Hall, J. E. (2006). *Textbook of medical physiology* (11th ed.). Philadelphia: Elsevier Saunders.
- Haldeman, G. A., Croft, J. B., Giles, W. H., & Rashidee, A. (1999). Hospitalization of patients with heart failure: National hospital discharge survey, 1985 to 1995. *American Heart Journal*, 137(2), 352-360.
- Halliwell, B. (2000). Lipid peroxidation, antioxidants and cardiovascular disease: How should we move forward? *Cardiovascular Research*, 47(3), 410-418.

- Halliwell, B., & Chirico, S. (1993). Lipid peroxidation: Its mechanism, measurement, and significance. *The American Journal of Clinical Nutrition*, 57(5 Suppl), 715S-724S; discussion 724S-725S.
- Halliwell, B., & Lee, C. Y. (2010). Using isoprostanes as biomarkers of oxidative stress: Some rarely considered issues. *Antioxidants & Redox Signaling*, 13(2), 145-156.
- Hanninen, S. A. (2003). *The prevalence of thiamin deficiency in hospitalized patients with congestive heart failure* (Master's thesis). University of Toronto, Toronto.
- Hanninen, S. A., Darling, P. B., Sole, M. J., Barr, A., & Keith, M. E. (2006). The prevalence of thiamin deficiency in hospitalized patients with congestive heart failure. *Journal of the American College of Cardiology*, 47(2), 354-361.
- Harris JS, & Benedict FG. (1919).
A biometric study of basal metabolism in man.
- He, J., Ogden, L. G., Bazzano, L. A., Vupputuri, S., Loria, C., & Whelton, P. K. (2001). Risk factors for congestive heart failure in US men and women: NHANES I epidemiologic follow-up study. *Archives of Internal Medicine*, 161(7), 996-1002.
- Health Canada. (2003). *Body mass index (BMI) nomogram*.http://www.hc-sc.gc.ca/fn-an/nutrition/weights-poids/guide-ld-adult/bmi_chart_java-graph_imc_java-eng.php
- Heller, S., Salkeld, R. M., & Korner, W. F. (1974). Vitamin B1 status in pregnancy. *The American Journal of Clinical Nutrition*, 27(11), 1221-1224.
- Herve, C., Beyne, P., & Delacoux, E. (1994). Determination of thiamine and its phosphate esters in human erythrocytes by high-performance liquid chromatography with isocratic elution. *Journal of Chromatography.B, Biomedical Applications*, 653(2), 217-220.

- Herve, C., Beyne, P., Letteron, Ph, Delacoux, E. (1995). Comparison of erythrocyte transketolase activity with thiamine and thiamine phosphate ester levels in chronic alcoholic patients. *Clinica Chemica Acta*. 234, 91-100.
- Hunt, S. A., Abraham, W. T., Chin, M. H., Feldman, A. M., Francis, G. S., Ganiats, T. G., et al. (2009). 2009 focused update incorporated into the ACC/AHA 2005 guidelines for the diagnosis and management of heart failure in adults: A report of the american college of cardiology Foundation/American heart association task force on practice guidelines: Developed in collaboration with the international society for heart and lung transplantation. *Circulation*, 119(14), e391-479.
- Hunt, S. A., American College of Cardiology, & American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure). (2005). ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: A report of the american college of Cardiology/American heart association task force on practice guidelines (writing committee to update the 2001 guidelines for the evaluation and management of heart failure). *Journal of the American College of Cardiology*, 46(6), e1-82.
- Ingwall, J. S., Atkinson, D. E., Clarke, K., & Fetters, J. K. (1990). Energetic correlates of cardiac failure: Changes in the creatine kinase system in the failing myocardium. *European Heart Journal*, 11 Suppl B, 108-115.
- Institute of Medicine . Panel on Folate, Other B Vitamins, and Choline, Institute of Medicine . Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, & Institute of Medicine . Subcommittee on Upper Reference Levels of Nutrients. (1998). *DRI: Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6s, folate, vitamin B12s, pantothenic acid, biotin, and choline*. Washington, D.C.: National Academy Press.

- Jeejeebhoy, K. N., & Kinney, J. M. (1988). *Nutrition and metabolism in patient care*. Philadelphia, PA: Saunders.
- Johnson, R. K., Goran, M. I., & Poehlman, E. T. (1994). Correlates of over- and underreporting of energy intake in healthy older men and women. *The American Journal of Clinical Nutrition*, 59(6), 1286-1290.
- Johnstone, D. E., Abdulla, A., Arnold, J. M., Bernstein, V., Bourassa, M., Brophy, J., et al. (1994). Diagnosis and management of heart failure. canadian cardiovascular society. *The Canadian Journal of Cardiology*, 10(6), 613-31, 635-54.
- Kaczmarek, M. J., & Nixon, P. F. (1983). Variants of transketolase from human erythrocytes. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, 130(3), 349-356.
- Kalantar-Zadeh, K., Anker, S. D., Horwich, T. B., & Fonarow, G. C. (2008). Nutritional and anti-inflammatory interventions in chronic heart failure. *The American Journal of Cardiology*, 101(11A), 89E-103E.
- Kannel, W. B. (2000). Incidence and epidemiology of heart failure. *Heart Failure Reviews*, 5(2), 167-173.
- Knowlton, K. U., Michel, M. C., Itani, M., Shubeita, H. E., Ishihara, K., Brown, J. H., et al. (1993). The alpha 1A-adrenergic receptor subtype mediates biochemical, molecular, and morphologic features of cultured myocardial cell hypertrophy. *The Journal of Biological Chemistry*, 268(21), 15374-15380.
- Kopelman, M. D., Thomson, A. D., Guerrini, I., & Marshall, E. J. (2009). The korsakoff syndrome: Clinical aspects, psychology and treatment. *Alcohol and Alcoholism (Oxford, Oxfordshire)*, 44(2), 148-154.

- Kristal, A. R., Feng, Z., Coates, R. J., Oberman, A., & George, V. (1997). Associations of race/ethnicity, education, and dietary intervention with the validity and reliability of a food frequency questionnaire: The women's health trial feasibility study in minority populations. *American Journal of Epidemiology*, 146(10), 856-869.
- Kwok, T., Falconer-Smith, J. F., Potter, J. F., & Ives, D. R. (1992). Thiamine status of elderly patients with cardiac failure. *Age and Ageing*, 21(1), 67-71.
- Laforenza, U., Orsenigo, M. N., & Rindi, G. (1998). A thiamine/H⁺ antiport mechanism for thiamine entry into brush border membrane vesicles from rat small intestine. *The Journal of Membrane Biology*, 161(2), 151-161.
- Leevy, C. M. (1982). Thiamin deficiency and alcoholism. *Annals of the New York Academy of Sciences*, 378, 316-326.
- Lemoine, A., Le Devehat, C., Codaccioni, J. L., Monges, A., Bermond, P., & Salkeld, R. M. (1980). Vitamin B1, B2, B6, and C status in hospital inpatients. *The American Journal of Clinical Nutrition*, 33(12), 2595-2600.
- Leslie, D., & Gheorghiade, M. (1996). Is there a role for thiamine supplementation in the management of heart failure? *American Heart Journal*, 131(6), 1248-1250.
- Levey, A. S., Bosch, J. P., Lewis, J. B., Greene, T., Rogers, N., & Roth, D. (1999). A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. modification of diet in renal disease study group. *Annals of Internal Medicine*, 130(6), 461-470.
- Levey, A. S., Coresh, J., Balk, E., Kausz, A. T., Levin, A., Steffes, M. W., et al. (2003). National kidney foundation practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. *Annals of Internal Medicine*, 139(2), 137-147.

- Levin, E. R., Gardner, D. G., & Samson, W. K. (1998). Natriuretic peptides. *The New England Journal of Medicine*, 339(5), 321-328.
- Levy, W. C., Soine, L. A., Huth, M. M., & Fishbein, D. P. (1992). Thiamine deficiency in congestive heart failure. *The American Journal of Medicine*, 93(6), 705-706.
- Liu, P., Arnold, J. M., Belenkie, I., Demers, C., Dorian, P., Gianetti, N., et al. (2003). The 2002/3 canadian cardiovascular society consensus guideline update for the diagnosis and management of heart failure. *The Canadian Journal of Cardiology*, 19(4), 347-356.
- Liu, P., Arnold, M., Belenkie, I., Howlett, J., Huckell, V., Ignazewski, A., et al. (2001). The 2001 canadian cardiovascular society consensus guideline update for the management and prevention of heart failure. *The Canadian Journal of Cardiology*, 17 Suppl E, 5E-25E.
- Loma-Osorio, P., Penafiel, P., Doltra, A., Sionis, A., & Bosch, X. (2008). Shoshin beriberi mimicking a high-risk non-ST-segment elevation acute coronary syndrome with cardiogenic shock: When the arteries are not guilty. *The Journal of Emergency Medicine*,
- Lourenco, B. H., Vieira, L. P., Macedo, A., Nakasato, M., Marucci Mde, F., & Bocchi, E. A. (2009). Nutritional status and adequacy of energy and nutrient intakes among heart failure patients. *Arquivos Brasileiros De Cardiologia*, 93(5), 541-548.
- Lu, J., & Frank, E. L. (2008). Rapid HPLC measurement of thiamine and its phosphate esters in whole blood. *Clinical Chemistry*, 54(5), 901-906.
- Lubetsky, A., Winaver, J., Seligmann, H., Olchovsky, D., Almog, S., Halkin, H., et al. (1999). Urinary thiamine excretion in the rat: Effects of furosemide, other diuretics, and volume load. *The Journal of Laboratory and Clinical Medicine*, 134(3), 232-237.

- Lukienko, P. I., Mel'nichenko, N. G., Zverinskii, I. V., & Zabrodskaia, S. V. (2000). Antioxidant properties of thiamine. *Bulletin of Experimental Biology and Medicine*, 130(9), 874-876.
- Lynch, P. L., & Young, I. S. (2000). Determination of thiamine by high-performance liquid chromatography. *Journal of Chromatography.A*, 881(1-2), 267-284.
- Makhija, S., & Baker, J. (2008). The subjective global assessment: A review of its use in clinical practice. *Nutrition in Clinical Practice : Official Publication of the American Society for Parenteral and Enteral Nutrition*, 23(4), 405-409.
- Mancinelli, R., Ceccanti, M., Guiducci, M. S., Sasso, G. F., Sebastiani, G., Attilia, M. L., et al. (2003). Simultaneous liquid chromatographic assessment of thiamine, thiamine monophosphate and thiamine diphosphate in human erythrocytes: A study on alcoholics. *Journal of Chromatography.B, Analytical Technologies in the Biomedical and Life Sciences*, 789(2), 355-363.
- McDonagh, T. A., Robb, S. D., Murdoch, D. R., Morton, J. J., Ford, I., Morrison, C. E., et al. (1998). Biochemical detection of left-ventricular systolic dysfunction. *Lancet*, 351(9095), 9-13.
- Morrow, J. D., Hill, K. E., Burk, R. F., Nammour, T. M., Badr, K. F., & Roberts, L. J.,2nd. (1990). A series of prostaglandin F₂-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proceedings of the National Academy of Sciences of the United States of America*, 87(23), 9383-9387.
- Morrow, J. D., & Roberts, L. J.,2nd. (1996). The isoprostanes. current knowledge and directions for future research. *Biochemical Pharmacology*, 51(1), 1-9.
- Naing L, Winn T, Rusli BN. (2006). Practical issues in calculating the sample size for prevalence studies *Archives of Orofacial Sciences*, 1, 9-14.

- Neubauer, S., Horn, M., Cramer, M., Harre, K., Newell, J. B., Peters, W., et al. (1997). Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. *Circulation*, 96(7), 2190-2196.
- Nichols, H. K., & Basu, T. K. (1994). Thiamin status of the elderly: Dietary intake and thiamin pyrophosphate response. *Journal of the American College of Nutrition*, 13(1), 57-61.
- Obisesan, T. O., Toth, M. J., Donaldson, K., Gottlieb, S. S., Fisher, M. L., Vaitekevicius, P., et al. (1996). Energy expenditure and symptom severity in men with heart failure. *The American Journal of Cardiology*, 77(14), 1250-1252.
- O'Keeffe, S. T. (2000). Thiamine deficiency in elderly people. *Age and Ageing*, 29(2), 99-101.
- OLDHAM, H. G. (1962). Thiamine requirements of women. *Annals of the New York Academy of Sciences*, 98, 542-549.
- Omland, T. (2003). N-terminal ProBNP: Marker of systolic dysfunction or nonspecific indicator of cardiac disease? *Heart Drug*, 3, 122-124.
- O'Rourke, N. P., Bunker, V. W., Thomas, A. J., Finglas, P. M., Bailey, A. L., & Clayton, B. E. (1990). Thiamine status of healthy and institutionalized elderly subjects: Analysis of dietary intake and biochemical indices. *Age and Ageing*, 19(5), 325-329.
- Packer M, Cohn J, Abraham W, et al. (1999). The consensus recommendation for the management of chronic heart failure. *The American Journal of Cardiology*, 83(2, Supplement 1), 1A-38A.
- Packer, M. (1987). How should we judge the efficacy of drug therapy in patients with chronic congestive heart failure? the insights of six blind men. *Journal of the American College of Cardiology*, 9(2), 433-438.

- Padilla, M. C., Armas-Hernandez, M. J., Hernandez, R. H., Israili, Z. H., & Valasco, M. (2007). Update of diuretics in the treatment of hypertension. *American Journal of Therapeutics*, 14(2), 154-160.
- Pepersack, T., Garbusinski, J., Robberecht, J., Beyer, I., Willems, D., & Fuss, M. (1999). Clinical relevance of thiamine status amongst hospitalized elderly patients. *Gerontology*, 45(2), 96-101.
- Pfister, R., Muller-Ehmsen, J., Hagemeister, J., Hellmich, M., Erdmann, E., & Schneider, C. A. (2010). NT-pro-BNP predicts worsening renal function in patients with chronic systolic heart failure (NT-pro-BNP and renal function). *Internal Medicine Journal*,
- Pfitzenmeyer, P., Guillard, J. C., d'Athis, P., Petit-Marnier, C., & Gaudet, M. (1994). Thiamine status of elderly patients with cardiac failure including the effects of supplementation. *International Journal for Vitamin and Nutrition Research. Internationale Zeitschrift Fur Vitamin- Und Ernahrungsforschung. Journal International De Vitaminologie Et De Nutrition*, 64(2), 113-118.
- Poehlman, E. T., McAuliffe, T., & Danforth, E., Jr. (1990). Effects of age and level of physical activity on plasma norepinephrine kinetics. *The American Journal of Physiology*, 258(2 Pt 1), E256-62.
- Poehlman, E. T., Scheffers, J., Gottlieb, S. S., Fisher, M. L., & Vaitekevicius, P. (1994). Increased resting metabolic rate in patients with congestive heart failure. *Annals of Internal Medicine*, 121(11), 860-862.
- Polidori, M. C., Pratico, D., Savino, K., Rokach, J., Stahl, W., & Mecocci, P. (2004). Increased F2 isoprostane plasma levels in patients with congestive heart failure are correlated with antioxidant status and disease severity. *Journal of Cardiac Failure*, 10(4), 334-338.
- Raphael, C., Briscoe, C., Davies, J., Ian Whinnett, Z., Manisty, C., Sutton, R., et al. (2007). Limitations of the new york heart association functional classification system and self-reported walking distances in chronic heart failure. *Heart (British Cardiac Society)*, 93(4), 476-482.

- Rieck, J., Halkin, H., Almog, S., Seligman, H., Lubetsky, A., Olchovsky, D., et al. (1999). Urinary loss of thiamine is increased by low doses of furosemide in healthy volunteers. *The Journal of Laboratory and Clinical Medicine*, 134(3), 238-243.
- Riley, M., Elborn, J. S., McKane, W. R., Bell, N., Stanford, C. F., & Nicholls, D. P. (1991). Resting energy expenditure in chronic cardiac failure. *Clinical Science (London, England : 1979)*, 80(6), 633-639.
- Rindi, G., & Laforenza, U. (2000). Thiamine intestinal transport and related issues: Recent aspects. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.)*, 224(4), 246-255.
- Roberts, L. J., & Morrow, J. D. (2000). Measurement of F₂-isoprostanes as an index of oxidative stress in vivo *Free Radical Biology and Medicine*, 28(4), 505-513.
- Rose, B. D. (1991). Diuretics. *Kidney International*, 39(2), 336-352.
- Roser, R. L., Andrist, A. H., Harrington, W. H., Naito, H. K., & Lonsdale, D. (1978). Determination of urinary thiamine by high-pressure liquid chromatography utilizing the thiochrome fluorescent method. *Journal of Chromatography*, 146(1), 43-53.
- Sacks, G. S., Dearman, K., Replogle, W. H., Cora, V. L., Meeks, M., & Canada, T. (2000). Use of subjective global assessment to identify nutrition-associated complications and death in geriatric long-term care facility residents. *Journal of the American College of Nutrition*, 19(5), 570-577.
- Sakano, N., Wang, D. H., Takahashi, N., Wang, B., Sauriasari, R., Kanbara, S., et al. (2009). Oxidative stress biomarkers and lifestyles in japanese healthy people. *Journal of Clinical Biochemistry and Nutrition*, 44(2), 185-195.

- Sauberlich, H. E., Herman, Y. F., Stevens, C. O., & Herman, R. H. (1979). Thiamin requirement of the adult human. *The American Journal of Clinical Nutrition*, 32(11), 2237-2248.
- Sauberlich, H. E. (1999). *Laboratory tests for the assessment of nutritional status* (2nd ed.). Boca Raton: CRC Press.
- Sawaya, A. L., Tucker, K., Tsay, R., Willett, W., Saltzman, E., Dallal, G. E., et al. (1996). Evaluation of four methods for determining energy intake in young and older women: Comparison with doubly labeled water measurements of total energy expenditure. *The American Journal of Clinical Nutrition*, 63(4), 491-499.
- Schatzkin, A., Kipnis, V., Carroll, R. J., Midthune, D., Subar, A. F., Bingham, S., et al. (2003). A comparison of a food frequency questionnaire with a 24-hour recall for use in an epidemiological cohort study: Results from the biomarker-based observing protein and energy nutrition (OPEN) study. *International Journal of Epidemiology*, 32(6), 1054-1062.
- Schwengel, R. H., Gottlieb, S. S., & Fisher, M. L. (1994). Protein-energy malnutrition in patients with ischemic and nonischemic dilated cardiomyopathy and congestive heart failure. *The American Journal of Cardiology*, 73(12), 908-910.
- Seligmann, H., Halkin, H., Rauchfleisch, S., Kaufmann, N., Motro, M., Vered, Z., et al. (1991). Thiamine deficiency in patients with congestive heart failure receiving long-term furosemide therapy: A pilot study. *The American Journal of Medicine*, 91(2), 151-155.
- Senni, M., Tribouilloy, CM., Rodeheffer, RJ., Jacobsen, SJ., Evans, JM., Bailey, KR., Redfield, MM. (1998). Congestive heart failure in the community: a study of all incident cases in Olmsted County, Minnesota, in 1991. *Circulation*, 98(21):2282-9

- Sheline, C. T., & Choi, D. W. (2004). Cu²⁺ toxicity inhibition of mitochondrial dehydrogenases in vitro and in vivo. *Annals of Neurology*, 55(5), 645-653.
- Shils, M. E., & Shike, M. (2006). *Modern nutrition in health and disease* (10th ed.). Philadelphia: Lippincott Williams & Wilkins.
- Shimon, I., Almog, S., Vered, Z., Seligmann, H., Shefi, M., Peleg, E., et al. (1995). Improved left ventricular function after thiamine supplementation in patients with congestive heart failure receiving long-term furosemide therapy. *The American Journal of Medicine*, 98(5), 485-490.
- Sica, D. A. (2007). Loop diuretic therapy, thiamine balance, and heart failure. *Congestive Heart Failure* (Greenwich, Conn.), 13(4), 244-247.
- Sobell, J., Block, G., Koslowe, P., Tobin, J., & Andres, R. (1989). Validation of a retrospective questionnaire assessing diet 10-15 years ago. *American Journal of Epidemiology*, 130(1), 173-187.
- Stepuro, A. I., Piletskaya, T. P., & Stepuro, I. I. (2005). Role of thiamine thiol form in nitric oxide metabolism. *Biochemistry.Biokhimiia*, 70(3), 339-349.
- Stipanuk, M. H. (2000). *Biochemical and physiological aspects of human nutrition*. Philadelphia: W.B. Saunders.
- SUKI, W., RECTOR, F. C., Jr, & SELDIN, D. W. (1965). The site of action of furosemide and other sulfonamide diuretics in the dog. *The Journal of Clinical Investigation*, 44, 1458-1469.
- Tallaksen, C. M., Bohmer, T., Bell, H., & Karlsen, J. (1991). Concomitant determination of thiamin and its phosphate esters in human blood and serum by high-performance liquid chromatography. *Journal of Chromatography*, 564(1), 127-136.

- Tallaksen, C. M., Sande, A., Bohmer, T., Bell, H., & Karlsen, J. (1993). Kinetics of thiamin and thiamin phosphate esters in human blood, plasma and urine after 50 mg intravenously or orally. *European Journal of Clinical Pharmacology*, 44(1), 73-78.
- Talwar, D., Davidson, H., Cooney, J., & St JO'Reilly, D. (2000). Vitamin B(1) status assessed by direct measurement of thiamin pyrophosphate in erythrocytes or whole blood by HPLC: Comparison with erythrocyte transketolase activation assay. *Clinical Chemistry*, 46(5), 704-710.
- Tang, A. M., Graham, N. M., Kirby, A. J., McCall, L. D., Willett, W. C., & Saah, A. J. (1993). Dietary micronutrient intake and risk of progression to acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus type 1 (HIV-1)-infected homosexual men. *American Journal of Epidemiology*, 138(11), 937-951.
- The Heart and Stroke Foundation of Canada. (2010). *The heart and stroke foundation of canada-statistics*. <http://www.heartandstroke.com/site/c.ikiQLcMWJtE/b.3483991/k.34A8/Statistics.htm>
- Thiamine. monograph.(2003). *Alternative Medicine Review : A Journal of Clinical Therapeutic*, 8(1), 59-62.
- Vimokesant, S., Kunjara, S., Rungruangsak, K., Nakornchai, S., & Panijpan, B. (1982). Beriberi caused by antithiamin factors in food and its prevention. *Annals of the New York Academy of Sciences*, 378, 123-136.
- Wang, Z., Ciabattini, G., Creminon, C., Lawson, J., Fitzgerald, G. A., Patrono, C., et al. (1995). Immunological characterization of urinary 8-epi-prostaglandin F2 alpha excretion in man. *The Journal of Pharmacology and Experimental Therapeutics*, 275(1), 94-100.
- Warnock, L. G. (1982). The measurement of erythrocyte thiamin pyrophosphate by high-performance liquid chromatography. *Analytical Biochemistry*, 126(2), 394-397.

- Willett, W. (1998). *Nutritional epidemiology* (2nd ed.). New York ; Oxford: Oxford University Press.
- WILSON, I. M., & FREIS, E. D. (1959). Relationship between plasma and extracellular fluid volume depletion and the antihypertensive effect of chlorothiazide. *Circulation*, 20, 1028-1036.
- Witte, K. K., & Clark, A. L. (2006). Micronutrients and their supplementation in chronic cardiac failure. an update beyond theoretical perspectives. *Heart Failure Reviews*, 11(1), 65-74.
- Wood, B., Breen, K. J., & Penington, D. G. (1977). Thiamine status in alcoholism. *Australian and New Zealand Journal of Medicine*, 7(5), 475-484.
- Wood, B., Gijbbers, A., Goode, A., Davis, S., Mulholland, J., & Breen, K. (1980). A study of partial thiamin restriction in human volunteers. *The American Journal of Clinical Nutrition*, 33(4), 848-861.
- Wooley, J. A. (2008). Characteristics of thiamin and its relevance to the management of heart failure. *Nutrition in Clinical Practice : Official Publication of the American Society for Parenteral and Enteral Nutrition*, 23(5), 487-493.
- Wrenn, K. D., Murphy, F., & Slovis, C. M. (1989). A toxicity study of parenteral thiamine hydrochloride. *Annals of Emergency Medicine*, 18(8), 867-870.
- Yue, Q. Y., Beermann, B., Lindstrom, B., & Nyquist, O. (1997). No difference in blood thiamine diphosphate levels between swedish caucasian patients with congestive heart failure treated with furosemide and patients without heart failure. *Journal of Internal Medicine*, 242(6), 491-495.
- Yui, Y., Itokawa, Y., & Kawai, C. (1980). Furosemide-induced thiamine deficiency. *Cardiovascular Research*, 14(9), 537-540.
- Zangen, A., Botzer, D., Zangen, R., & Shainberg, A. (1998). Furosemide and digoxin inhibit thiamine uptake in cardiac cells. *European Journal of Pharmacology*, 361(1), 151-155.

Zenuk, C., Healey, J., Donnelly, J., Vaillancourt, R., Almalki, Y., & Smith, S. (2003). Thiamine deficiency in congestive heart failure patients receiving long term furosemide therapy. *The Canadian Journal of Clinical Pharmacology = Journal Canadien De Pharmacologie Clinique*, 10(4), 184-188.

Thiamin and Your Heart!

A Study Examining the Prevalence of Thiamin Deficiency in Ambulatory Patients with Heart Failure

What is this study about?

We invite you to participate in a study on the prevalence of thiamin deficiency in heart failure patients. Thiamin is a B vitamin that plays an important role in the production of energy in your body. Therefore, low levels of thiamin may limit the amount of energy available for the heart to pump blood. Poor appetite and use of diuretic drugs can increase the risk of thiamin deficiency. Since most of the information on thiamin deficiency has come from hospitalized patients, we would like to look at whether thiamin deficiency is found in non-hospitalized people with heart failure.

What does this study involve?

If you choose to participate in this study you will be asked to come in for 1 study visit (about 30 min), and you will be asked to do the following:

1. Provide us with information about your medical and dietary history
2. Provide single blood sample (about one and a half tablespoons or 25 ml)

Why participate in this study?

If you have a low level of thiamin in your blood, you will be contacted by study personnel. This information will allow you and your doctor to determine whether a thiamin supplement would be a good idea. In addition, if you are found to have a low blood level of thiamin, you may be invited to participate in a follow-up study of thiamin supplementation.

Contact information:

If you are interested in participating or would like more information, please contact:

Parastoo Azizi, BAsC, MSc(c) Study Coordinator

Tel: 647-836-4583

E-mail: AziziP@smh.toronto.on.ca

Form 2 – Prevalence consent form

The Prevalence of Thiamin Deficiency in Ambulatory Patients with Heart Failure

Consent to Participate in a Research Study

Before agreeing to participate in this research study, it is important that you read and understand this research consent form. This form provides all the information we think you will need to know in order to decide whether you wish to participate in the study. If you have any questions after you read this form, ask your doctor or study personnel. You should not sign this form until you are sure you understand everything on this form.

Title of Research Study

The Prevalence of Thiamin Deficiency in Ambulatory Patients with Heart Failure

Principal Investigator:

Dr. Mary Keith, Ph.D., RD.
Division of Nutrition - Supply Chain and Support Services,
Tel: 416-864-5551
Monday – Friday 8:30 – 5:00

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Study Sponsors

Heart and Stroke Foundation of Canada

Purpose of the Research

The purpose of this study is to determine the number of ambulatory patients with heart failure that have low blood levels of thiamin (vitamin B1). Heart failure is a condition in which the heart is not able to pump as strongly as it should and therefore less oxygen and blood is able to get to the tissues. Thiamin is a water soluble B vitamin that plays an important role in the production of

energy in your body. Therefore, low levels of thiamin may limit the amount of energy available for the heart. Reduction in energy production may contribute to cardiac muscle weakness and subsequently impair cardiac function. Previous studies done on heart failure patients with thiamin deficiency found that thiamin supplementation was associated with improvement in heart function. Poor appetite and reduced interest in eating may increase your risk of thiamin deficiency. In addition, since thiamin is a water soluble vitamin, the use of diuretic medications (the ones that help you to get rid of extra water by urinating more often) may result in losses of thiamin into the urine. Our group has measured levels of thiamin in hospitalized patients with heart failure and found that about one third of these patients have low levels of thiamin. Since there is currently no information on non-hospitalized patients with heart failure, we wish to determine the rate of low thiamin levels in this group. Thiamin supplementation for people with low levels of thiamin may be a simple way help the heart pump. This study is the first step in a larger study looking at the potential benefits of thiamin supplementation.

Description of the Research

You are being invited to participate in this study because you are being treated for heart failure on an outpatient basis and may have low thiamin levels in your blood.

If you consent to participate in this study, you will be asked to come in for 2 study visits, which may or may not coincide with your regular clinic visits at the Cardiology Clinic. The first visit may take about 30 minutes of your time.

During the first study visit, you will be asked to do the following:

1. Provide us with information about your medical history. Medical information will also be obtained from your medical and electronic charts at the hospital
2. Provide information about the foods you normally eat (dietary history)
3. Provide information about medications you are currently taking
4. Fill out a questionnaire about the foods you eat at home
5. Provide single blood sample (about one and a half tablespoons or 25 ml) to be tested for the following:
 - a. Level of thiamin in your blood
 - b. Blood hormones
 - c. Markers of metabolic stress – these are substances which are normally in your blood but increase during periods of stress.

The information obtained from the above will help us determine how many people with heart failure but who are not hospitalized, have low thiamin levels. Information obtained from your blood work will help us see if there is any relationship between these blood markers and thiamin levels in the blood.

If you have a low level of thiamin in your blood, you will be contacted by study personnel. This information will allow you and your doctor to determine whether a thiamin supplement would be a good idea. In addition, if you are found to have a low blood level of thiamin, you may be invited to participate in a follow-up study of thiamin supplementation.

Potential Harms (Injury, Discomforts or Inconvenience)

There may be a small amount of discomfort, redness or bruising associated with any collection of blood samples, however, this is temporary and will disappear in a few days.

Women as Research Participants

If you are female, you may participate in this study. Pregnant women will be excluded from the study as pregnancy will influence your levels of thiamin that are not related to heart failure. If you are a woman in the childbearing age group, you will need to have either a urine or blood pregnancy test done to confirm that you are not pregnant.

Potential Benefits

There are no direct benefits to participating in this study.

Confidentiality and Privacy

The records from this research study, as well as your identity, will be kept confidential and will not be given to anyone who is not helping on this study unless required by law. Your medical records and information will not be transferred electronically. The study coordinator will protect your records and keep confidential all the information, including your name, address and telephone number. The chance that this information will accidentally be given to someone else is small. No information from the study will be shared with Jamieson Laboratories. Prevalence of thiamin deficiency findings will be shared with the Heart and Stroke Foundation in the form of annual reports which are necessary to continue funding. These will not make reference to any individuals.

However, the results of your blood tests can be made available to your family doctor and cardiologist if you desire. Your medical records and information obtained during the study may be reviewed by doctors or medical personnel involved in the study, or by the Health Protection Branch of Canada or other regulatory agencies as required by law. In addition, a representative of the St. Michael's Hospital Research Ethics Board (REB) may inspect your medical/research records as part of their monitoring of the study.

Reimbursement

If you chose to participate in the study, we will be able to reimburse your parking/travel expenses related to your visit to St. Michael's Hospital to a maximum of \$15.00.

Compensation for Injury:

If you suffer a physical injury as a direct result of the study, medical care may be obtained by you in the same manner as you would ordinarily obtain any other medical treatment. In no way does signing this form waive your legal rights nor relieve the investigator, sponsors or involved institutions from their legal and professional responsibility.

Publication of Results

The results of your blood thiamin levels and symptom reports will be reported in medical journals or at conferences. Your name and identity will be kept secret in these reports by using a code number. No information that discloses your identity may be released or published without your consent.

Participation and Withdrawal:

Your participation in this study is completely voluntary. By signing this form, you are agreeing to participate in the study. If you choose not to participate, you and your family will continue to have access to customary care at St. Michael's Hospital. If you choose to participate, you can withdraw at any time without any effect on the care you or your family will receive at St. Michael's Hospital.

Contact Information:

If you have any questions about the study, please call the principal investigator, Dr. Mary Keith, at (416) 864-5551. If you have any questions about your rights as a research participant, please call the Chair of the Research Ethics Board of St. Michael's Hospital, Dr. Julie Spence, at 416.864-6060 x2557.

Consent to Participate in a Research Trial

I acknowledge that the research study described above have been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw without compromising the quality of medical care at St. Michael's Hospital for me and for other members of my family. As well, the potential risks, harms and discomforts have been explained to me and I also understand the benefits (if any) of participating in the research study.

I understand that I have not waived my legal rights nor released the investigators, sponsors, or involved institutions from their legal and professional duties. I know that I may ask now, or in the future, any questions I have about the study or the research procedures. I have been assured that records relating to me and my care will be kept confidential and that no information will be released or printed that would disclose personal identity without my permission unless required by law. I have been given sufficient time to read and understand the above information.

I hereby consent to participate, and will be given a signed copy of this consent form.

Participant's Signature	Participants Name (Printed)	Date
-------------------------	-----------------------------	------

Witness to Consent Process	Witness Name (Printed)	Date
----------------------------	------------------------	------

Person Obtaining Consent Signature	Person Obtaining Consent Printed	Date
---------------------------------------	-------------------------------------	------

I _____ (printed name of investigator) am one of the investigators responsible for the conduct of this study at St. Michael's Hospital, and I have delegated the explanation of this study of this patient to _____ (name of person obtaining consent).

Signature of Investigator	Date
---------------------------	------

I, _____ (patient name) would like the study investigators to share the results of all of the tests done for this study with my family doctor and cardiologist.

☐ I agree ☐ I disagree

Case Report Form (1)

1.0 Participant Demographics

Subject Identifier: _____ Visit Date (d/m/y): ____ / ____ / ____

Randomization #: _____ D.O.B. (d/m/y): ____ / ____ / ____

Age _____ Gender ☐ M ☐ F

Referring Cardiologist _____

Height (cm): _____ Weight (kg): _____ BMI: _____

Ethnicity: ☐ Caucasian ☐ Asian ☐ African American ☐ Hispanic

☐ Other (specify): _____

Home Tel#: () _____ Work # () _____

() _____

Cell #: () _____ Email address: _____

Date Informed Consent Signed (d/m/y): ____ / ____ / ____

Form 4 – Case report form

Randomization # _____ Date (d/m/y): ____ / ____ / ____

Case Report Form (2)***Baseline Visit***

Subject Identifier: _____ Visit Date (d/m/y): ____ / ____ / ____

2.0 Cardiac History

- Date Heart Failure First Diagnosed (d/m/y): ____ / ____ / ____

Condition	NO	YES	IF YES....
1. Valvular Disease			DATE (d/m/y): _____
2. Cardiomyopathy			DATE (d/m/y): _____
3. Ischemic Heart Disease			DATE (d/m/y): _____
4. Previous Cardiac Surgery			DATE (d/m/y): _____ Type: _____
5. Arrhythmia			DATE (d/m/y): _____
6. Pacemaker			DATE (d/m/y): _____
7. Hospitalized for acute decompensated HF (last 2 months)			DATE (d/m/y): _____

3.0 Other Comorbidities

Condition	NO	YES	IF YES....
1. Cigarette Smoking			Past current within
2. HTN			Medication No Yes
3. Family History Heart Disease			Primary relative < 65 years
5. Hypercholesterolemia/TG			Medication No Yes
6. PVD			
7. Respiratory Disease			Asthma COPD
8. Stroke or TIA			Specify: _____

Randomization # _____ Date (d/m/y): ____ / ____ / ____

9. Diabetes			Insulin	Oral agent
10. Iron Deficiency				
11. Folate Deficiency				
Other:				

4.0 Cardiac Functional Class and CCS Class

CLASS	CCS		NYHA
Class II			
Class III			
Class IV			

LV ASSESSMENT	DATE	LVEF (%)
MUGA		
ECHO		
CATH		

LV GRADE	
Grade II (35-50%)	
Grade III (20-35%)	
Grade IV (<20%)	

5.0 Medications

List of other medications and/or supplements;

Type	Reason	Dosage	Start Date (d/m/y)	End Date (d/m/y)

Randomization # _____ Date (d/m/y): ____ / ____ / ____

Furosemide				
Bumetanide				
Ethacrynic acid				
Hydrochlorothiazide (Thiazide)				
Metolazone (Thiazide)				
Spironolactone				
Amiloride				
Type	Reason	Dosage	Start Date (d/m/y)	End Date (d/m/y)

Randomization # _____ Date (d/m/y): ____ / ____ / ____

6.0 Food Frequency Questionnaire: ☐ Complete ☐ Incomplete

7.0 Routine Blood Test: ☐ Complete ☐ Incomplete

Test	Results	Date of Results (d/m/y)	Within the Normal Range
Na (135 - 145 mmol/L)			. Yes ☐ No
K (Serum: 3.5 - 5.0 mmol/L)			. Yes ☐ No
Glucose Fasting: (4.0 - 6.0 mmol/L) Random (4.0 - 7.8 mmol/L)			. Yes ☐ No
Blood Urea (3.0 - 7.0 mmol/L)			. Yes ☐ No
Serum Creatinine Male: (52-112 µmol/L) Female: (42-102 µmol/L)			. Yes ☐ No

Randomization # _____ Date (d/m/y): ____ / ____ / ____

AST (7 - 40 U/L)			☒ Yes ☐ No
ALT (10 - 45 U/L)			☒ Yes ☐ No
ALP (35 - 125 U/L)			☒ Yes ☐ No
PO4 (0.80 - 1.35 mmol/L)			☒ Yes ☐ No
Mg (0.63 - 0.94 mmol/L)			☒ Yes ☐ No
Hgb Male: (130 - 170 g/L) Female: (115 - 155 g/L)			☒ Yes ☐ No
Albumin (35 - 50 g/L)			☒ Yes ☐ No
WBC (4 - 11 x 10 ⁹ /L)			☒ Yes ☐ No
Insulin (13 - 161 pmol/L)			☒ Yes ☐ No
Temperature ($\leq 37^{\circ}$)			☒ Yes ☐ No

- **Heart Rate:** _____
- **Blood Pressure:** _____
- **Edema (peripheral)** Yes ☐ No
- **JVP (jugular venous pressure):** _____
- **Crackles:** _____

Randomization # _____ Date (d/m/y): ____ / ____ / ____

8.0 Specific Blood Tests:

	Result	Within the Normal Range	If NO...
Erythrocyte TPP		Yes ☐ No	TPP < 180 nmol/l? Yes ☐ No
Plasma NE		Yes ☐ No	
Plasma BNP/ NT-proBNP		Yes ☐ No	
Plasma F2- isoprostanes		Yes ☐ No	

STATUS AT STUDY CLOSE OUT

☐ completed study protocol

☐ prematurely dropped out of trial

Date (d/m/y) of drop out _____

☐ deceased Date (d/m/y) patient died _____

Person completing this form: _____

Name of Investigator (print): _____

Investigator's Signature: _____

Date (d/m/y) form signed: _____

Date:
Subject ID:

Appendix 9.9: SUBJECTIVE GLOBAL ASSESSMENT RATING FORM

HISTORY:		SGA SCORE		
		A	B	C
1.	Current dry weight _____ Usual dry weight _____ Overall dry weight change in past ____ months _____ % Loss of usual weight: _____ < 5% _____ 5-10% _____ > 10% Change in past 2 weeks: _____ Increase _____ No change _____ Decrease			
2.	Dietary Protein/Energy Intake _____ Intentional Change Overall change: _____ No change _____ Undesirable/Change due to decreased appetite Duration: _____ Days/Weeks Diet change: _____ Suboptimal solids/fluids _____ Hypocaloric fluids _____ NPO			
3.	Gastrointestinal Symptoms (persisting > 2 weeks) _____ None _____ Diarrhea _____ Nausea/Vomiting _____ Constipation _____ Anorexia			
4.	Functional Impairment (nutrition related) Overall impairment: _____ None _____ Mild (consider anemia) _____ Severe Function in past two weeks: _____ Increase _____ No change _____ Decrease			

PHYSICAL EXAMINATION:	SGA SCORE		
	A	B	C
Muscle Wasting: _____ Temple			
_____ Clavicle			
_____ Deltoid (upper arm)			
_____ Scapula			
_____ Ribs			
_____ Quadriceps (thigh)			
_____ Knee			
_____ Calf			
_____ Interosseous			
Loss of subcutaneous fat: _____ Below Eye/cheeks			
_____ Triceps			
_____ Biceps			
Edema: (related to undernutrition) _____ Ascites _____ Ankles			

OVERALL SGA RATING

- ☐ **A =** Well Nourished (“A” rating in most Categories, or Significant and sustained improvement).
- ☐ **B =** Mild to Moderately Malnourished (neither “A” or “C” is Clearly included)
- ☐ **C =** Severely Malnourished (“C” rating in most Categories, Significant physical signs)

Form 6 – Food frequency questionnaire

ID#: _____

Diet Assessment

Date: _____

Type of Food	How often in the past month (or since your appetite has decreased) have you eaten the following foods at home, in restaurants, or take-out?								How Much Each Time? See Portion Size Pictures or Food Models For A-B-C-D				
	N ever	Once per month	2-3 times per month	Once per week	Twice per week	3-4 times per week	5-6 times per week	Daily					
Fruits and Vegetables – including fresh, frozen, canned or in stir-fry (where applicable)?													
<i>Example for apple</i>					X				<i>How many each time</i>	½	I	2	3
Oranges, tangerines, not including juice									How many	½	1	2	3
Banana									How many	½	1	2	3
Apples or pears									How many	½	1	2	3
Applesauce, fruit cocktail, or other canned fruit									How much	A	B	C	D
Any other fruits like grapes, melons, strawberries, peaches									How much or How many	A-1	B-2	C-3	D-4
Orange juice									How many glasses	1	2	3	4
Other fruit juices like grapefruit, apple, or grape.									How many glasses	1	2	3	4
French fries, fried potatoes or hash browns									How much	A	B	C	D
Potatoes not fried, like boiled, baked, mashed, and potato salad									How much	A	B	C	D
Green peas									How much	A	B	C	D
Green salad									How much	A	B	C	D
Raw tomatoes, including in salads									How much	¼	½	1	2
Carrots, or stews, or mixed vegetables containing carrots.									How much	A	B	C	D
Vegetable soup, vegetable beef, chicken vegetable, or tomato soup									Which bowl		B	C	D
Any other vegetable such as squash, broccoli, spinach, green beans, cole slaw, cabbage, green peppers, onions									How much	A	B	C	D

Type of Food	How often in the past month (or since your appetite has decreased) have you eaten the following foods at home, in restaurants, or take-out?								How Much Each Time? See Portion Size Pictures or Food Models For A-B-C-D				
	N e v e r	Once per month	2-3 times per month	Once per week	Twice per week	3-4 times per week	5-6 times per week	D a i l y					
Meats and Alternates													
Baked beans, chili with beans, pintos, any other dried beans									How much	A	B	C	D
Eggs									How many	1	2	3	4
Hamburgers, cheeseburgers, meat loaf									How much meat	2 oz	4 oz	8 oz	12 oz
Tacos, burritos, enchiladas									How much	A	B	C	D
Beef steaks, roasts, pot roast, including in frozen dinners or sandwiches									How much	A	B	C	D
Pork, including chops, roasts, or dinner ham									How much	A	B	C	D
When you eat beef or pork, do you ☐ Avoid eating the fat ☐ Sometimes eat the fat ☐ Often eat the fat ☐ I don't eat meat													
Mixed dishes with meat or chicken, like stew, chicken pot pie, or frozen meals.									How much	A	B	C	D
Fried chicken									# med pieces	1	2	3	4
Chicken or turkey not fried, such as baked, grilled, or on sandwiches (e.g. chicken salad sandwich)									How much	A	B	C	D
When you eat chicken, do you ☐ Avoid eating the skin ☐ Sometimes eat the skin ☐ Often eat the skin ☐ N/A													
Fried fish or fish sandwich									How much	A	B	C	D
Any other fish or shellfish not fried, including tuna									How much	A	B	C	D
Hot dogs									How many	1	2	3	4
Bacon									How many pieces	1	2	3	4
Sausage, such as breakfast, Polish, Italian									How many	1	2	3	4
Sliced ham luncheon meat									How many slices	1	2	3	4
Other lunch meats such as roast turkey, chicken etc.									How many slices	1	2	3	4
When you eat lunch meats, are they ☐ Usually low-fat ☐ Sometimes ☐ Rarely low-fat ☐ N/A													

Type of Food	How often in the past month (or since your appetite has decreased) have you eaten the following foods at home, in restaurants, or take-out?								How Much Each Time? See Portion Size Pictures or Food Models For A-B-C-D				
	N e v e r	Once per month	2-3 times per month	Once per week	Twice per week	3-4 times per week	5-6 times per week	D a i l y					
Grain Products													
Cooked cereals like oatmeal, cream of wheat									Which bowl		B	C	D
Cold cereals like corn flakes, Cheerios, Special K, fibre cereals									Which bowl		B	C	D
Which cereal do you eat most often? Mark only one: ☐ Total, Just Right ☐ Corn Flakes, Cheerios, Special K, etc. ☐ Bran Buds, Raisin Bran, Fruit-n-Fiber, other fibre cereal Namebrand : _____													
Cereal bars or supplement bars									How many	1	2	3	4
Which kind do eat most often? ☐ Nutrigrain ☐ Quaker ☐ Other Namebrand: _____													
Spaghetti, lasagna, or other pasta <u>with tomato sauce</u>									How much	A	B	C	D
Rice or dishes made with rice									How much	A	B	C	D
Cheese dishes <u>without tomato sauce</u> , like macaroni and cheese									How much	A	B	C	D
Pizza, including take-out									How many slices	1	2	3	4
Biscuits or muffins									How many	1	2	3	4
Crackers									How many	2-3	4-5	6-8	9+
Rolls, hamburger or hot dog buns, English muffins, bagels									How many	½	1	2	3
White bread or toast, including in sandwiches									How many slices	1	2	3	4
Dark bread like rye or whole wheat, including in sandwiches									How many slices	1	2	3	4
Tortillas									How many	1	2	3	4
Milk Products													
Glasses of milk									How many glasses	1	2	3	4
When you drink milk what kind do you <u>usually</u> drink? Mark only one: ☐ Whole milk ☐ 2% milk ☐ 1% milk ☐ Skim milk ☐ Rice milk ☐ Soy milk ☐ I don't drink milk or soy milk													
Cheese, sliced cheese or cheese spread, including on sandwiches.									How many slices	1	2	3	4
Yogurt (not frozen)									How much	A	B	C	D

Type of Food	How often in the past month (or since your appetite has decreased) have you eaten the following foods at home, in restaurants, or take-out?								How Much Each Time? See Portion Size Pictures or Food Models For A-B-C-D				
	N e v e r	Once per month	2-3 times per month	Once per week	Twice per week	3-4 times per week	5-6 times per week	D a i l y					
Other Foods													
Potato chips, corn chips, popcorn									How much	A	B	C	D
Cookies									How many	1-2	3-5	6-7	8+
Ice Cream, frozen yogurt, ice cream bars									How much	A-1	B-2	C-3	D
When you eat ice cream or frozen yogurt, is it ☐ Usually low-fat ☐ Sometimes ☐ Rarely low-fat ☐ N/A													
Doughnuts, cake, pastry, pie									How many pieces	1	2	3	4
Chocolate candy, candy bars									How many bars	1 sm	2	1 lrg	2
Instant breakfast milkshakes like Carnation® or liquid supplements like Ensure®									How many glasses/cans	1	2	3	4
Fruit drinks and punches, such as fruit punch, ice tea, Kool-Aid, Tang									How many glasses/bottles	1	2	3	4
Regular soft drinks (Not diet drinks)									How many bottles/ cans	1	2	3-4	5+
Beer									How many bottles/cans	1	2	3-4	5+
Wine or wine coolers									How many glasses	1	2	3-4	5+
Liquor or mixed drinks									How many drinks	1	2	3-4	5+
Cream, Half-and-Half, or non-dairy creamer in coffee or tea									How many Tbsps.	1	2	3-4	5+
Margarine or butter on bread, potatoes, or vegetables									How many pats (Tsp.)	1	2	3	4
Peanuts or peanut butter									How many Tbsps.	1	2	3	4
Salad dressing or mayonnaise (Not low-fat)									How many Tbsps.	1	2	3	4
Fat or oil in cooking									How many Tsp.	1	2	3	4
Sugar in coffee or tea									How many Tsp.	1	2	3	4
Jellies, jams, honey									How many Tsp.	1	2	3	4
Catsup, salsa, or chile peppers									How many Tbsp.	1	2	3	4

2. Do you regularly drink caffeinated beverages such as coffee, tea, cola beverages, or chocolate drinks?

☐ Yes ☐ No

If Yes, how much per day/week/month/year? _____

3. Do you regularly eat raw shellfish, raw fresh water fish (bass, pike, catfish) or raw fermented fish (e.g. Pla raa)?
Do you regularly chew betel nuts?

☐ Yes ☐ No

4. Do you drink /1-2 alcoholic drinks a day? (1 drink is 12 oz beer, 1.5 oz liquor, 4 oz wine)

☐ Yes ☐ No

If Yes, how many? _____

5. Are there any other foods that are a regular part of your diet that have not been mentioned in this survey?

If Yes, what food? _____ how frequently? _____

☐ Yes ☐ No

6. Are you currently taking any vitamin, mineral, or herbal supplements?

☐ Yes ☐ No

If Yes, what do you take?

Supplement(s)	Do not take	A few days per month	1-3 days per week	4-6 days per week	Every Day
Regular Once-A-Day tablets such as Centrum®					
Stress tablets or B-Complex vitamins					
Vitamin B-1 (Thiamin)					
Other					