Metabolic Mapping and Cardiac Resynchronization (Aim 1)

NCT# 03420833

June 20, 2021

STUDY OVERVIEW

Title	Metabolic Mapping and Cardiac Resynchronization (Aim 1)
Running Title	Metabolic Mapping and CRT
IRB Protocol Number	17-003359
Phase	Pilot
Principal investigator	Yong-Mei Cha, MD Mayo Clinic
Objective	To determine early alterations in metabolic pathways and metabolomics biomarkers in patients with mild heart failure (HF) and left bundle branch block (LBBB), and their role in the prediction of outcomes by early CRT.
Study Design	Single-center, randomized cross-over study
Overall Study Design	We plan to complete subject enrollment by the end of Year 3. Data validation, adjudication and analysis will be finished by the end of Year 5.
Subject Participation Duration	Approximately 12 months
Primary Objectives	**Primary efficacy objectives (composite endpoint)** The study primary endpoint is a reduction in the LV end-systolic volume index (LVESVI, reverse LV remodeling) in the CRT-ON gorup as compared to the CRT-OFF group. **Primary Safety Objective** • Freedom from system-related complications in randomized subjects will be calculated at 1, 6, and 12 months post implant to determine the safety profile, including the device pocket hematoma, pneumothorax, myocardial performation, lead dislodgement, lead revision, and device-related infection.. This safety profile will be balanced against efficacy to quantify the risk-benefit ratio.
Secondary Objectives	**Secondary efficacy objectives** • Hospital admission for HF • Increase in NT pro-BNP level by 30% • Sustained ventricular tachycardia or fibrillation greater than 30 seconds • Decrease in LVEF to ≤34% • Death
Device	BIOTRONIK Edora HF-T QP, or Ilivia HF-T QP DF4
Number of Subjects	100 subjects (of these 30 will be female)
Subject Inclusion Criteria	• LVEF 35%-50% • NYHA class I-II • QRS duration of ≥120ms • Left bundle branch block (LBBB) • Patient is able to receive a transvenous pectoral CRT implant

	• Patient is able to sign informed consent • Two echocardiograms are required to confirm LVEF≤ 50% (one echo must be within 12 months of enrollment) • Patient is on optimal and stable medical therapy (ACE inhibitor or AT1 blocker, beta blocker, etc. over the last 6 months)
Subject Exclusion Criteria	• Advanced comorbid conditions with life expectancy <1 year • Patient is <18 of years of age • Patient has a CRT device • Female patients who is pregnant or not on a reliable form of birth control. Women of childbearing potential are required to have negative pregnancy test within the 7 days prior to device implant • Unwilling or unable to return for required follow-up visits • Patient decides study participation is cost-prohibited
Duration of Exposure	At the 12-month follow-up, when each subject completes his/her cross-over 6-month therapy, the CRT (Edora HF-T QP, or Ilivia HF-T QP DF4) function will be programmed on to provide chronic benefit from CRT for a battery life of 8.6 years.
Reference therapy	Patients are self-referenced as a cross-over design.
Statistical Methodology	The primary endpoint is the change in LVESVI. We will compare the changes in LVESVI between CRT-ON and CRT-OFF groups. We will explore 5-10 metabolomic factors as principal components to predict group differences (using a 2-sample t-test), and response to CRT therapy. In particular, we will relate these principal components to response to CRT using multiple linear regression (for the LVESVI endpoint) and Cox Regression (for the Clinical endpoint).

RESEARCH STRATEGY

A. Background and Significance

Heart failure is a progressive disease with high mortality and morbidity. Heart failure (HF) affects 5.7 million American adults and claims nearly 300,000 lives annually.[1] HF progresses along a continuum from asymptomatic (Stage A and B) to symptomatic (Stage C and D).[2] Nearly three times as many people with left ventricular (LV) systolic dysfunction are in Stage B as in Stages C and D combined.[3,4] In the Framingham Heart Study, total mortality rates within four years of symptomatic HF development were 24% in women and 55% in men.[5] In the community, the prevalence was 6% for those with a left ventricular ejection fraction (LVEF) of 50% or less and 2% for those with an LVEF of 40% or less.[6] This translates to 4% of the population who have mild LV systolic dysfunction with LVEF between 41%-50%.[7] Although the risk of hospitalization or death declines as LVEF increases, an LVEF in the range of 36% to 50% still confers a considerable risk of adverse outcomes. For instance, the CHARM study showed that patients with an LVEF between 33%-52% had annual cardiovascular mortality rates in the range of 4.0%-6.0% and HF hospitalization rates of 5.7%-7.2%.[8]

Left bundle branch block (LBBB) and ventricular dyssynchrony. LBBB is frequently (>20%) accompanied by progression of LV systolic dysfunction, with the consequence of delayed electrical conduction to the LV lateral wall.[9,10] This recognized ventricular dyssynchrony further reduces myocardial efficiency and cardiac output, worsening cardiac performance. Compared with normal ventricular conduction, the presence of LBBB is associated with a near two-fold increase in all-cause mortality.[11] Cardiac resynchronization therapy (CRT) resynchronizes electrical and mechanical coupling by simultaneously exciting the right and left ventricles. It improves chronic LV systolic dysfunction and reverse LV remodeling in patients with LVEF of 35% or lower.[12-14] The benefit of CRT is more favorable in patients with a widened QRS complex and LBBB.[12,14-23] Yet, the metabolomics signature of LBBB and metabolic pathways affected have not been well determined. Based on our own preliminary data (see below), we propose that early CRT, in patients with LVEF of 35%-50% and LBBB, may have potential clinical impact that reverses the initial stages of LV metabolic and structural remodeling and prevents HF progression to an advanced stage.[24]

Metabolomics and biomarkers in HF and CRT. HF is characterized by perturbations in the metabolic processes including impaired oxidative phosphorylation and compromised ATP production.[25-33] Cardiac remodeling in HF involves ventricular electrical and mechanical dyssynchrony, which results in metabolic heterogeneity and energy insufficiency.[23,25-27,34] Heterogeneous myocardial oxidative metabolism has been characterized, especially in patients with ischemic cardiomyophaty and LBBB.[35] As such, metabolomics profiling, when used in combination with standard HF biomarkers (e.g., B-type natriuretic peptide [BNP]), has significant diagnositic and prognostic values, and facilitates understanding the mechanisms of metabolic derrangements and assessing therapeutic outcomes.[36-52] One of the mechanistic benefits of CRT is to reduce mechanical dyssynchrony within the left ventricle with subsequent improvement in LV efficiency and oxygen consumption.[36] However, how CRT affects the myocardial energy and substrate metabolism, and which metabolic state and pathways facilitate myocardial recovery, are unknown. Metabolomic analysis demonstrates increasing potential to provide individualized predictive information for disease progression and precision of treatment.[32,37-45,49-52] Determining the associations between metabolomic changes and CRT is of importance to understand how this therapy improves cardiac metabolism.[53-55] Our preliminary investigations have discovered an energetic and metabolomic signature in HF, and potential therapeutic effects of CRT on the metabolomic profile.[28,52,56] Thus, assessment of the metabolomic state before CRT may provide new biomarkers for evaluation of treatment efficacy from CRT.[45,51]

Gender difference in CRT outcome. Although 50% of patients with HF are women, they constitute less than 25% of the patients enrolled in CRT trials. Despite their underrepresentation in clinical trials, women may benefit more than men from CRT.[18,19,57-59] The more favorable benefit of CRT for women may be associated with differences in baseline disease characteristics. A greater proportion of women have favorable factors such as a substrate of nonischemic cardiomyopathy (NICM) and a LBBB pattern, whereas

a greater proportion of men have ischemic cardiomyopathy (ICM) and renal dysfunction, which are associated with a poor prognosis. In the proposed study, we will particularly enroll a higher proportion of female patients than the clinical trials to address whether sex is a biological variable in CRT outcome. We aim to determine the cardiac metabolomic signature and active metabolic pathways of women, which may differ from that of men, as an underlying mechanism by which women have a more favorable outcome.[45,60]

B. Innovation

Discovery of novel metabolomic biomarkers and transcardiac metabolic signature. Application of unbiased metabolomics and new stable isotope technologies to address metabolomic dysregulation in HF and metabolic reprogramming by effective CRT is an innovative approach, which will permit underpinning metabolic pathways and mechanisms that allow to improve CRT efficacy. This translational study will combine cutting-edge metabolomic technologies available at Mayo Clinic to allow, for the first time, the determination of whole blood metabolite dynamics using ^{18}O stable isotopes along with plasma and transcardiac metabolomic signatures and substrate utilization patterns to uncover the mechanistic link in HF and CRT. These measurements will permit mapping cardiac metabolic heterogenity and the homogenizing effects of CRT critical for assessing treatment efficacy.[35] On the basis of preliminary data, the array of metabolomic data collected will reflect the patient's HF and therapeutic metabolic status, which is an integral outcome of the upstream genomic, transcriptomic, and proteomic events, and will allow the identification of the exact phenotype, potential biomarkers, and mechanisms of underlying diseases.

Discovery of cardiac metabolomics pathways that facilitate favorable CRT outcomes in women. We will evaluate the metabolic reserve, which is the capacity to increase metabolism of energy providing substrates in response to an increase in the workload of the heart.[61] Here the innovation lays in discovering a potential gender difference in metabolic traits and its mechanistic association with CRT outcomes through metabolic pathways using advanced metabolomic technologies. Specific focus will be paid to transcardiac metabolite differences and whole blood phosphometabolite turnover and dynamics of substrate shuttles and pathways, such as acyl-carnitine and branched-chain amino acid metabolism to detect subtle changes in metabolic state.

Effective treatment of HF in the early stage by CRT. This study proposes to investigate the novel use of CRT in patients who have mild HF, with a decreased LVEF of 35% to 50% and a LBBB. This patient population is at high risk for progression from mild to severe HF, which represents an increasing burden to the community and the health care system. Reversal of myocardial dysfunction in the early stage, before it becomes irreversible, is a sound and innovative strategy.

C. Approach

Preliminary results

Mild HF and clinical outcomes

We screened the Mayo Clinic electrocardiography (ECG) database for patients with a diagnosis of LBBB from 1994 through 2014. Among 12,879 patients identified, 1,436 patients met the criteria of having transthoracic echocardiography (TTE) within one-year of the ECG diagnosis of LBBB and a LVEF of 36% to 50% (**Figure 1**). These study patients were matched 1:1 to 1,436 patients without LBBB on the basis of age, sex, baseline LVEF, and year of TTE. The mean (SD) age in both groups was 67 (13) years, and 54% were men. Clinical outcomes were compared between the LBBB and control groups.

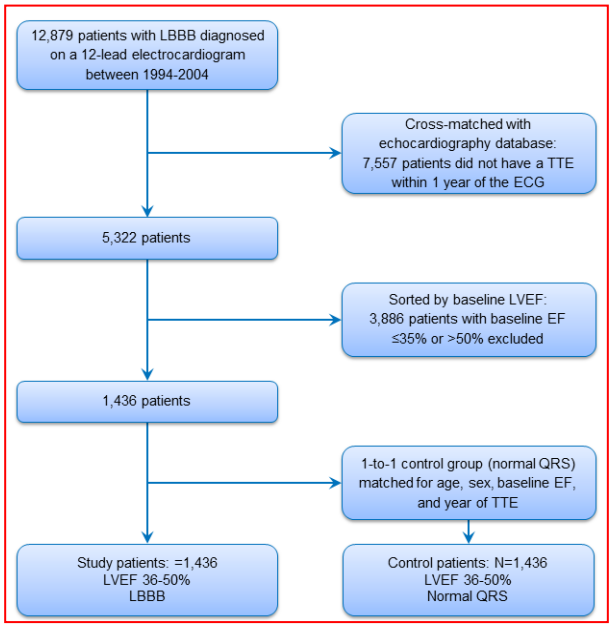

Figure 1: Clinical database screening process for patients with LVEF 36%-50% and LBBB, and matched-control group.

Long-term survival. The Kaplan-Meier 20-year probability of survival in both groups is shown in **Figure 2**. Patients with mild HF (LVEF 36%-50%) and LBBB had lower survival rates at 5 (72% vs. 76%; *P*=.04), 10 (53% vs 61%; *P*<.001), and 20 years (27% vs 46%; *P*<.001) compared with the controls who had a normal QRS duration.

LVEF deterioration to <35%. The event-free rates of the LVEF reduction to <35% at 1 year (83% vs 89%) and 5 years (63% vs 70%) (hazard ratio, 1.34; 95% CI, 1.09-1.63; *P*=.005—were lower in the LBBB group than the control group. This finding suggests that, compared with a normal QRS duration, the presence of LBBB in patients with mild HF led to a greater LVEF deterioration with time, which could be attributed to ventricular dyssynchrony.

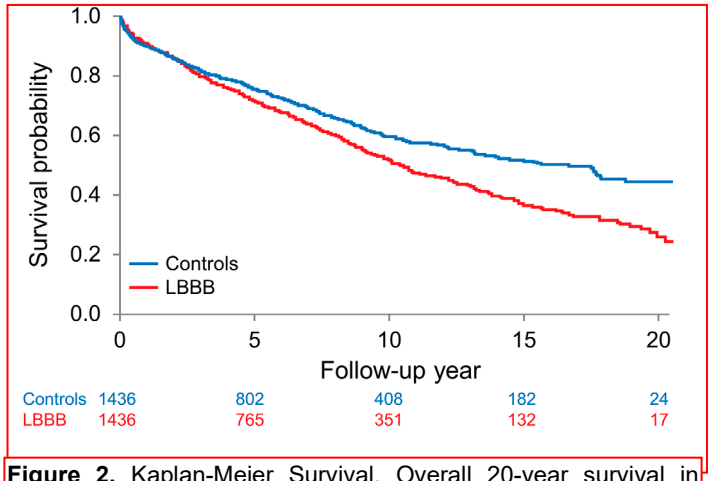

Figure 2. Kaplan-Meier Survival. Overall 20-year survival in patients with LBBB and controls. LBBB, left branch bundle block

Implantable cardioverter-defibrillator and CRT-defibrillator implantation. The survival free of an implantable cardioverter-defibrillator (ICD) or CRT-defibrillator (CRT-D) placement in the LBBB group was lower than the control group (87% vs 92% at 1 year; 80% vs 88% at 5 years) (hazard ratio, 1.73; 95% CI, 1.30-2.30; P<.001). Thus, patients in the LBBB group received ICD/CRT-D more often than the non-LBBB control group. This finding is consistent with evidence of greater LVEF deterioration in the LBBB group.

Progression of LV dysfunction in mild HF with LBBB. Among the 1,436 patients with LVEF of 36%-50% and LBBB, 485 had follow-up echocardiography within the first two-years. The mean (SD) patient age was 65 (14) years, 57% were men, and mean LVEF was 42% (13%). Of these 485 patients, 281 (58%) had no decrease in LVEF, 103 (21%) had an LVEF decrease of 1% to 5%, 43 (9%) had a decrease of 6% to 10%, and 58 (12%) had a decrease of more than 10% (*P*<.001). Overall, 42% of participants had a further LVEF reduction within the next two-years.The Kaplan-Meier curves showed a significant difference in survival by the extend of LVEF decrease (*P*<.001; **Figure 3**). Patients with a decrease of >10% had the worst survival.

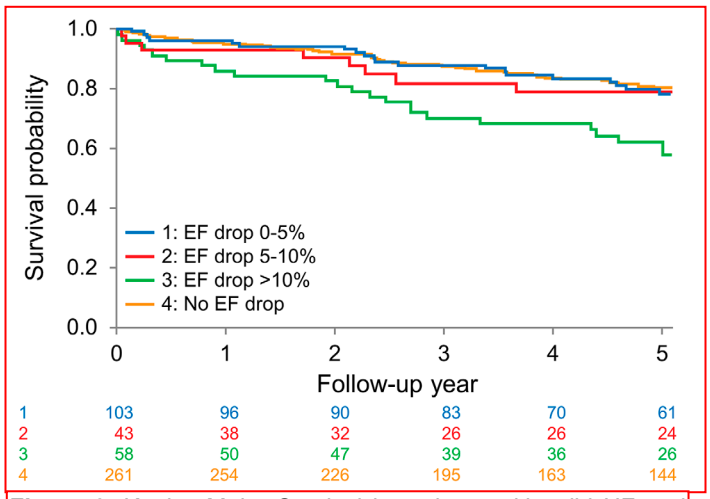

Figure 3. Kaplan-Meier Survival in patients with mild HF and LBBB according to LVEF changes at follow-up.

Metabolomic profiles in HF and CRT

Baseline transcardiac metabolomics and acute response to CRT. We studied the metabolomic profiles of 18 patients with advanced HF who underwent CRT-D implantation and 7 controls who underwent catheter ablation for supraventricular tachycardia. Blood samples were collected from the peripheral vein, femoral artery, and coronary sinus at baseline. Metabolomic profiling of plasma samples was performed using gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy (NMR).[52] Compared with controls, the HF patients had an increased release of myocardial succinate, which is a biomarker of mitochondrial dysfunction, and increased uptake of fatty acids and ketone bodies (2-HB) indicating alterations in substrate metabolism and/or deposition (**Figure 4**). After acute biventricular pacing for 15 minutes, the repeat transcardiac

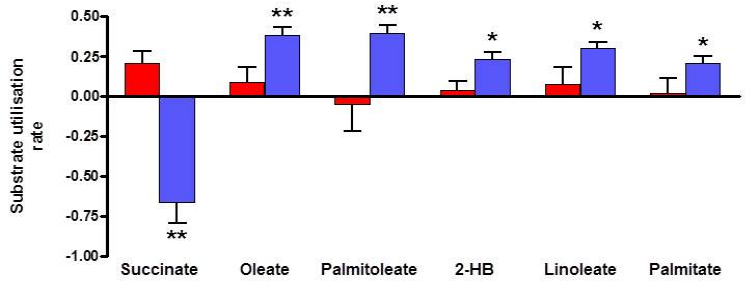

Figure 4. Transcardiac metabolomics changes (peripheral vs coronary sinus) in HF patients (blue) compared with controls (red). 2-HD -2-hydroxybutyrate; * *P*<.05, ***P*<.01. HF, heart failure

metabolomic profile shifted to a different state, with increased myocardial uptake and metabolism of free fatty acids and decreased release of creatinine.

Metabolomic profile in severe HF and chronic response to CRT. In our prospective study of the effect of chronic CRT on plasma metabolomic profiles,[52] we analyzed 24 patients who had CRT implantation for advanced HF (LVEF≤35%) and 10 controls who underwent catheter ablation for supraventricular tachycardia. Blood samples were collected before and 3 months after the procedure. We performed a metabolomic profiling, using GC-MS and NMR. The plasma metabolomic profiles changed significantly 3 months after CRT (**Figure 5A**). For each patient with HF, scores of the integral metabolomic profile after CRT clustered separately from the original state on the orthogonal partial least squares–discriminant analysis (PLS-DA) plot, which indicates a transition to a new metabolic state. An integrated panel of the most important metabolites for discrimination of the CRT group included isoleucine, glutamine, and glycerol-1-phosphate (**Figure 5B**). The pattern of metabolite changes (decrease in succinate and increase in glutamate) indicated an improved mitochondrial substrate metabolism after CRT (**Figure 5C**). Among the most improved metabolic pathways were amino acid and lipid metabolism (**Figure 5D**).

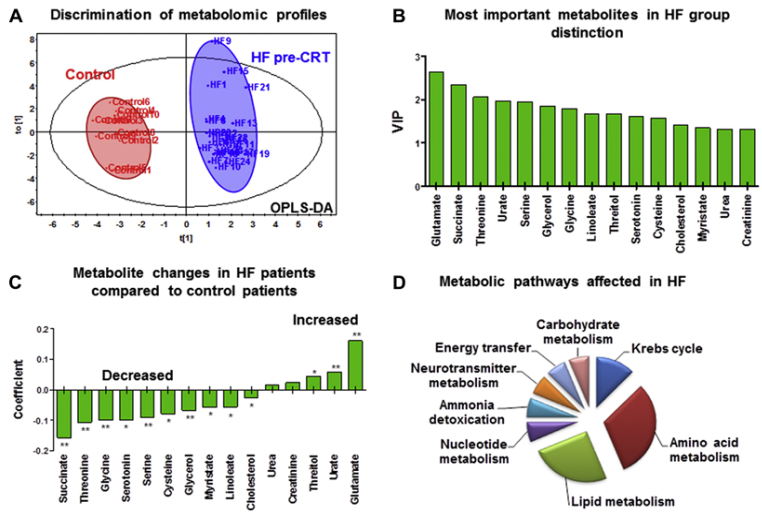

Figure 5. Metabolomic signature of heart failure (HF). (A) Changes in plasma metabolomic profile of HF patients and controls by orthogonal partial least squares discriminant analysis (OPLS-DA). CRT, cardiac resynchronization therapy. (B) Most important metabolites in the metabolomic signature of HF by variable importance in the projection (VIP). (C) Pattern of plasma metabolite changes in HF patients (n = 24; *P < .05; **P < .001). (D) Metabolic pathways most affected in HF as deduced from an altered plasma metabolomic profile.

Metabolomic profile in mild HF. We prospectively studied metabolomic profiles in subjects with a normal LVEF of >55% and LBBB (control, n=9), in patients with LVEF of 36%-50% and LBBB (HF mild, n=8), LVEF of ≤35% and LBBB before (HF severe, n=9) and after CRT (HF-CRT). **Figure 6** illustrates the power of metabolomics in different HF stages separating from the control group. The most important metabolites in the discrimination of HF severity were amino acids (asparagine, lysine, glutamine), branched-chain amino acids (isoleucine, leucine, valine) and products of catabolism, carnitine and nucleotide degradation. The finding suggests an early alteration and progression in the amino acid, fatty acid and nucleotide metabolism in mild HF with LBBB in parallel to the advancement of HF.

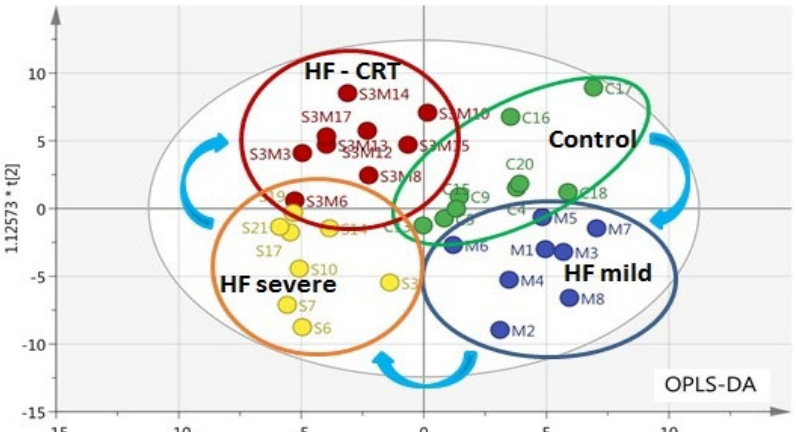

Figure 6. Transitions in metabolomics states from control to mild heart failure (HF mild), to severe heart failure (HF severe) and 3-month after CRT in severe heart failure (HF-CRT). Principal component (OPLS-DA) analysis of metabolomics profiles.

To further specify the metabolite biomarker changes, we measured the carnitine and acylcarnitine levels using liquid chromatography-tandem mass spectrometry (LCMS), and discovered a significant elevation of plasma carnitine, acetylcarnitine and long-chain oleoylcarnitine in mild HF patients compared to controls (**Figure 7).** Such changes indicate that impaired tissue free fatty acid oxidation in mitochondria is associated with an increased release of metabolites into plasma and deficient overall lipid metabolism.[44]

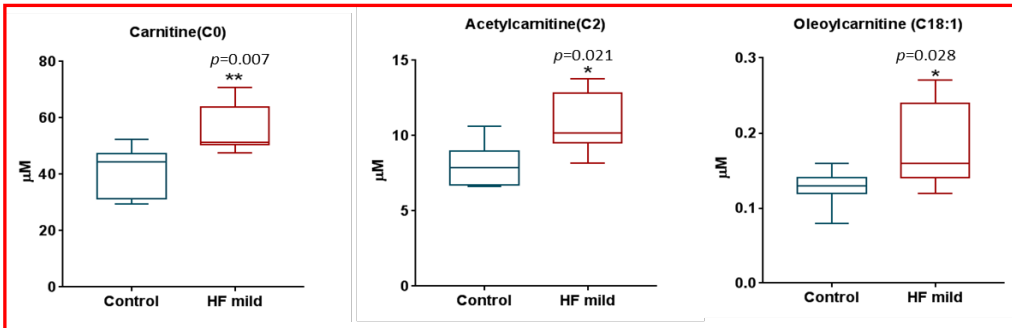

Figure 7. Changes in plasma carnitine and acylcarnitine levels in mild HF. n=7 in each group.

Metabolomic alteration in LBBB. To further understand whether LBBB itself impacts the metabolomics profile, we compared metabolic measurements in subjects, who had an ECG diagnosis of LBBB with a normal LVEF of >55%, to the control group who had a normal QRS and LVEF, and the group who had mild HF (LVEF 36%-50%) and LBBB. Notably, the metabolomics profile in LBBB with normal LV function **(Figure 8)** is different from the normal QRS controls and is further shifted in patients with mild HF and LBBB. The principal components for group separation are lactate, glucose, carnitine, glutamine and succinate, indicating altered pathways of carbohydrate, amino acid and nucleotide metabolism by LBBB alone without the presence of HF.

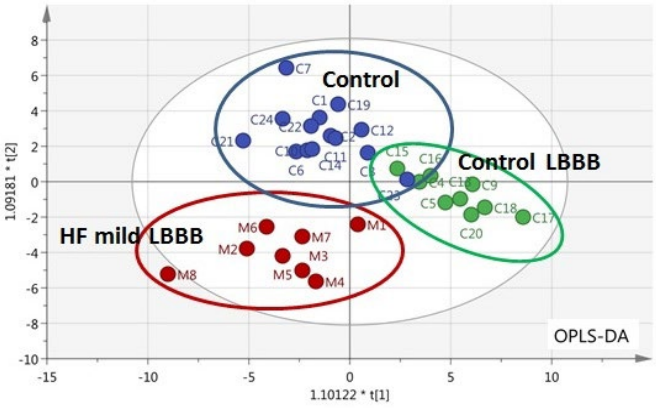

Figure 8. Patients with LBBB (Control LBBB) have different metabolic state which is further exacerbated by mild heart failure (HF mild LBBB) as compare to control with no LBBB (Control). Principal component (OPLS-DA) analysis of metabolomics profiles. n=8-14.

Metabolomic profiles in ischemic and nonischemic cardiomyopathy. In our previous study, patients with NICM (n=14) and ICM (n=10) had different basal plasma metabolomics profiles (**Figure 9**). After CRT, metabolomic profiles shifted differently, with NICM showing better improvement in the plasma metabolomics. Compared with ICM, patients with NICM, had higher levels of amino acids, lactate, and acetate after CRT associated with more active amino acid, carbohydrate and fatty acid metabolism.

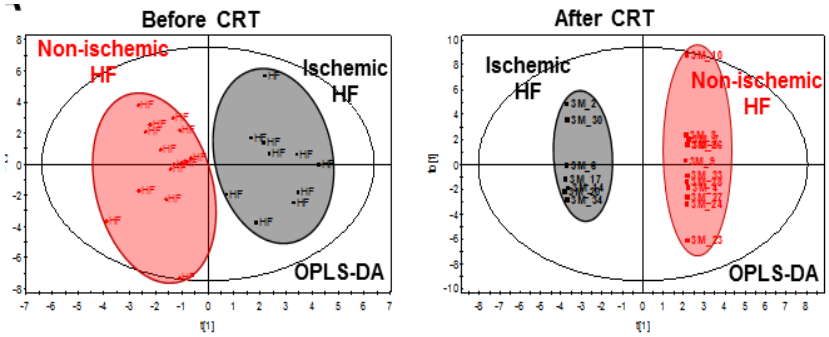

Figure 9. Different Metabolomic Profiles in Patients With Ischemic and Nonischemic HF Before and After CRT by orthogonal PLS-DA.

Metabolomic profile predicts improvement in LVEF after CRT. Of the 24 patients with HF who received CRT, 10 (41%) had an improvement in LVEF by >5% and a decrease of 1 or greater in the New York Heart Association (NYHA) functional class; they were considered to be CRT responders. This group of patients differed in their pre-CRT baseline plasma metabolomic profile from non-responders in the orthogonal PLS-DA plot analysis (**Figure 10A**). The panel of the most important metabolites allowing distinction between responders and non-responders to CRT is shown (**Figure 10B**). Patients who responded to CRT had higher levels of branched-chain amino acids (isoleucine, leucine, and valine), essential for protein synthesis and metabolic signaling. (**Figure 10C**; *P*<.05). It indicates branched-chain amino acids are catabolized to metabolic intermediates as important substrates for energy production in the failing heart.

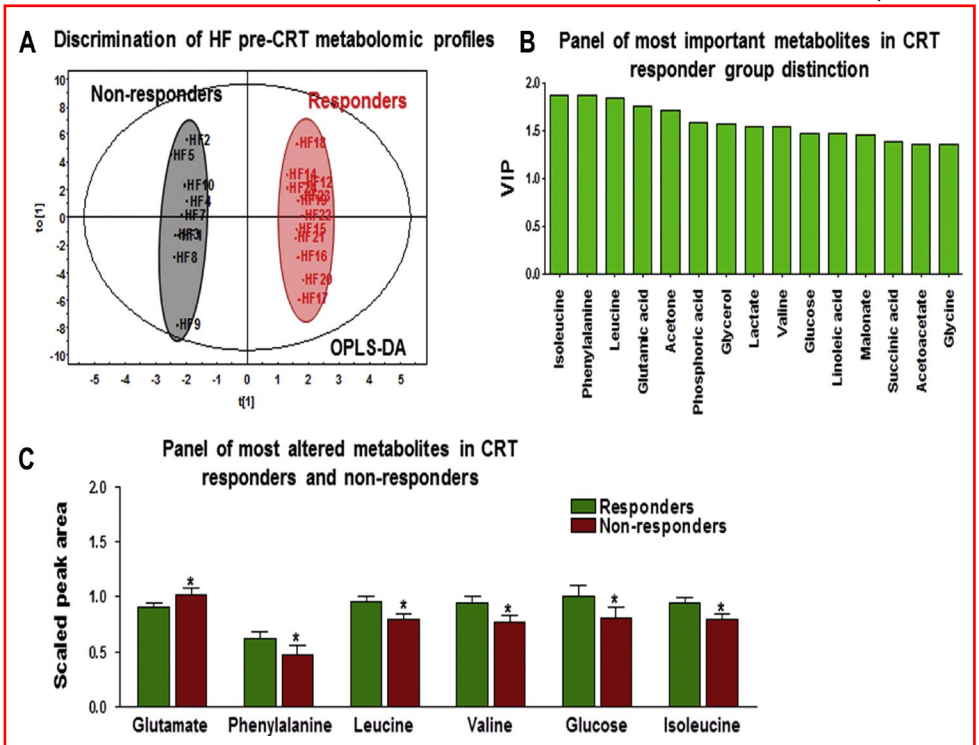

Figure 10. Metabolomic profiles of responders and nonresponders to cardiac resynchronization therapy (CRT). (A) Distinction of basal plasma metabolomic profiles of responders and nonresponders to CRT by orthogonal partial least squares discriminant analysis (OPLS-DA). (B) An integral panel of the most important metabolites permitting prediction of responders by variable importance in the projection (VIP). (C) A panel of the most important metabolite differences between responders and nonresponders (*P < .05).

CRT and gender difference

Women have a greater response to CRT than men. We conducted a retrospective study of 728 consecutive CRT recipients at our institution who met the guidelines for CRT-D device placement. The outcomes of CRT were compared between women (n=166, 22.8%) and men (n=562, 77.2%). Women were found to more often have NICM (68% vs 36%, *P*<.01). After CRT, as compared to men, women had a greater improvement in the NYHA class and a trend greater improvement in LVEF and reverse LV remodeling.[62]

Women and men with HF have different metabolomic profiles. The metabolomic profile was prospectively studied in 38 patients (7 women, 18%) who had advanced HF with LVEF ≤35%. Male and female patients had different plasma metabolomic profiles (**Figure 11A**). Women had higher plasma levels of linoleic acid, palmitoleic acid, glycolic acid and glycine, which indicated a better lipid and amino acid metabolism than men (**Figure 11B**). A distinct feature of the female metabolome was a higher

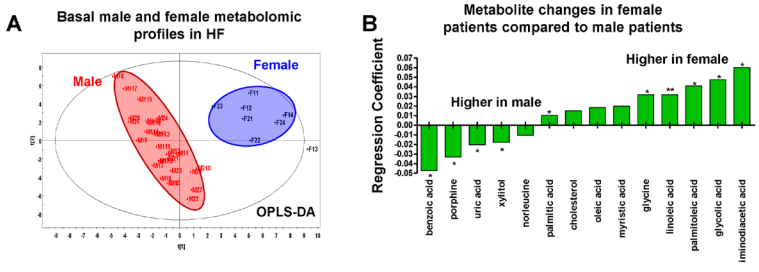

Figure 11. Plasma metabolomics profiles of male and female HF patients (A); Metabolite differences in female and male HF patients (B). [n = 31 (male) and 7 (female); *P < .05; **P < .001].

glycerol/glycerol-1-phosphate ratio (mean [SD], 2.79 [1.30] than men, 1.19 [0.18]; *P*<.05) indicating lower triglyceride biosynthesis in women. The NMR data indicates that male metabolome had higher levels of N-methylhistidine and creatinine, which is associated with higher protein degradation and energetic stress.

Experimental protocol

Aim: To determine early alterations in metabolic pathways and metabolomic biomarkers in patients with mild HF and LBBB, and their role in the prediction of outcomes by early CRT.

Rationale

Reversal of myocardial dysfunction in the early stage is of great importance to prevent the progression of HF. Here we will investigate the novel use of CRT in patients who have mild HF and LBBB. This patient population is at high risk for progression from mild to severe HF. However, the metabolomics signature of mild HF with LBBB and metabolic pathways affected have not been determined.

Study Patients

Subject inclusion criteria. LVEF 35%-50%, NYHA class I-II, QRS duration of ≥120ms[63], LBBB[64], and on optimal and stable medical therapy (ACE inhibitor or AT1 blocker, beta blocker over the last 6-month), able and willing to sign informed consent.

Subject exclusion criteria. Advanced comorbid conditions with life expectancy <1 year, unwilling or unable to return for required follow-up visits, <18 of years of age, existing CRT device, female patients who is pregnant or not on a reliable form of birth control. Women of childbearing potential are required to have negative pregnancy test within 7days prior to device implantation.

Our preliminary studies have indicated that among 1,436 patients with LVEF of 36% to 50% and LBBB, 78 reside in Olmsted and surrounding counties, 256 reside in Minnesota, and 422 reside in nearby states and are living candidates for enrollment. In addition, as a referral center, Mayo Clinic is estimated to have approximately 80 to 100 new patients eligible for study enrollment each year.

Study design and randomization

We propose a single-center, randomized cross-over study design. The study will be approved by the Mayo Clinic Institutional Review Board, and a consent form will be signed by each participant. We will enroll 100 patients at Mayo Clinic, Rochester, Minnesota, who have LVEF of 35% to 50%. Of these patients, 30 will be women. Participants will undergo placement of a CRT-pacemaker (CRT-P) after the completion of the baseline assessment if all inclusion and none of the exclusion criteria are met. Participants who have an ICD in place, or who meet clinical indication for an ICD, will undergo placement of a CRT-defibrillator (CRT-D). Defibrillation will not be added for patients who do not have an existing ICD system or do not meet clinical indications for an ICD. Participants who have a successful CRT implantation will be randomly assigned 1:1 to have the CRT programmed on (CRT-ON, treatment group) or off (CRT-OFF, control group) within 7 days of completing the implant procedure. Participants will be randomly assigned using an allocation method defined by a biostatistician. Investigators managing HF in study participants will be blinded to the randomization method to minimize potential bias. A washout period after the device implantation will not be used in this study.[19] At 6-month follow-up, the CRT function will be turned on in the CRT-OFF group and the CRT function will be turned off in the CRT-ON group in a cross-over design. All participants will cross-over at the 6-month follow-up visit. At 12-month follow-up, when each patient completes his/her cross-over 6-month therapy, the CRT function will be programmed on to provide chronic benefit from CRT for a battery life of 8.6 years. The subjects will be blinded from whether biventricular pacing is on or off. For participants who receive a CRT-D, the defibrillation function will be programmed on for the duration of the study.

Study device

A United States Food and Drug Administration (FDA)-approved CRT device will be used for this study. Because the clinical application of CRT in patients with an LVEF of 35% to 50% has not been approved by the FDA and current AHA/ACC/HRS guidelines,[16] we have obtained the approval of an Investigational Device Exemption (IDE) from the FDA for this study aim (FDA-approval letter is attached). BIOTRONIK SE & Co. KG has agreed to donate the FDA-approved CRT device, models Edora HF-T QP or Ilivia HFT-QP DF4, along with Setrox S, Solia S, Plexa S, Plexa DF-1 S, and Sentus QP leads (agreement letter is attached) for the study.

Preprocedure Evaluation

Clinical evaluation. Patient baseline information including age, comorbid conditions such as hypertension, coronary artery disease, diabetes mellitus, and etiology of cardiomyopathy (ICM vs NICM) will be collected. The HF symptoms (measured by Kansas City Cardiomyopathy Questionnaire: KCCQ), physical signs, NYHA class, ECG, echocardiography (may include 3D echo images), and 6-minute walk test will be assessed.

12-Lead ECG. The ECG results will be reviewed by electrophysiologists who are blinded to the patients' clinical status. Intrinsic QRS duration and morphology will be measured and validated.

Six-minute walk test. The 6-minute walk test will be conducted according to a standardized protocol. Patients will perform the test using a 100-foot internal hallway,[66, 67] and will be asked to walk the hallway at their own pace for 6 minutes. The patient will be supervised by a nurse during the test, and blood pressure and pulse rate will be monitored during the study.

Echocardiography Two-dimensional echocardiography will be performed to assess changes in LV dimension, volumes, and LVEF. Volumes will be estimated by averaging those derived from the 2-chamber and -chamber views according to the Simpson method, and the LVEF will be calculated in the usual fashion (derived from 2-dimensional measurements of diastolic and systolic LV dimension or volumetric analysis using the method of disks).[17,19] Two echocardiograms are required to confirm a stable reduced LVEF prior to randomization (one echo must be within 12 months of enrollment). Three-dimensional (3D) echo offers a reproducible assessment of left ventricular (LV) structure, function, volumes and LV mechanical dyssynchrony. It has been shown that the LV mechanical dyssynchrony quantification by 3D echo is reproducible and, systolic dyssynchrony index (SDI) is an excellent predictor of response to CRT. For the purpose of the study patients will undergo an additional 3D echocardiographic examination. Datasets will be obtaiined by commercially available echocardiographic systems equipped with 3D probes. Full-volume 6-beat LV 3D echo data sets will be acquired from the apical approach during patient breath hold.

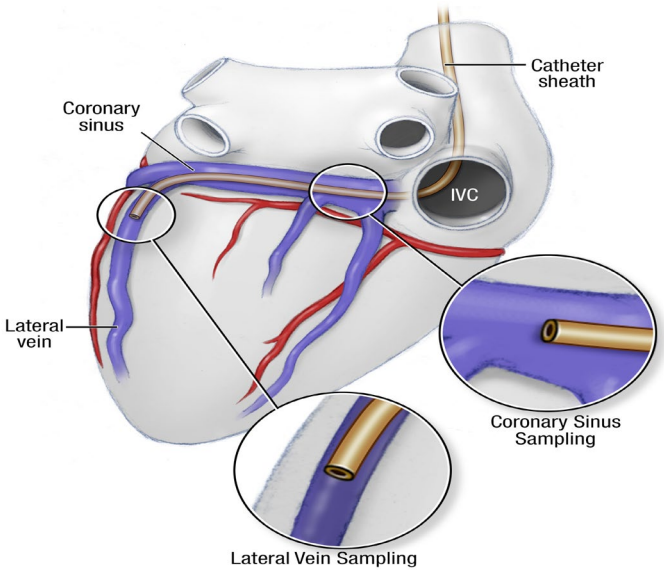

Figure 12. Shows coronary sinus blood sampling locations. The circles indicate blood exampling from the coronary sinus ostium and the proximal lateral vein from the LV lateral wall (the site of latest activation) that reflect the local metabolism will be collected and compared.

The LV-3D echocardiographic images will be exported in VolDICOM format to a separate workstation equipped with dedicated vendor-independent software packages for 3D echo (4D LV-Analysis 3.1, TomTec Imaging Systems, Unterschleissheim, Germany).

LV end-diastolic (EDV) and end-systolic volumes (ESV), LV ejection fraction (EF), strain including global longitudinal strain (GLS) and global circumferential strain (GCS) and, systolic dyssynchrony index (SDI) will be calculated offline.

CRT implantation

The FDA-approved, commercially available CRT devices and leads will be used. The transvenous LV lead will be placed into coronary lateral vein to correct delayed lateral wall activation in LBBB, if possible.[68,69] After the blood sample is taken, the LV lead will be advanced to the distal tributary of this vein. A QRS-to–local ventricular activation time (QRS-LV time) will be measured to assess the conduction delay.[70-72]

Patients will be observed in the hospital for 24 hours, and devices will be interrogated before hospital dismissal.

Blood sample collection for metabolomic measurement

Peripheral venous blood samples will be collected before CRT, at 6-month and 12-month follow-up (either CRT-ON for 6-month or CRT-OFF for 6-month). At implant, blood samples from femoral artery and coronary sinus will be collected to measure transcardiac metabolomic gradient. More often, the LV lead deliver sheath for coronary sinus cannulation is able to be advanced into the beginning of lateral vein to facilitate the LV lead placement (70%-80%), where blood sample will be collected to determine the regional metabolic profile as shown in **Figure 12**. The rational for collecting the regional blood is to determine 1) whether there is a differential metabolisms between ICM (discrete scar vs no scar accessed by echocardiography) vs NICM (diffused fibrosis), and 2) metabolic feature at the most delayed conduction area at the LV lateral wall in coping with QRS-LV time.

Muscular samples collection for metabolomics measurement

When the patient is undergoing a CRT device implant, a pre-pectoral pocket is made routinely to house the generator. The skin and subcutaneous planes are dissected to expose the fascia of the pectoralis major. A small piece (0.5-1.0g) of the chest muscle (Pectoralis major) biopsy will be taken for metabolomics analysis, and mitochondrial physiology. Approximately 100mg of muscle tissue will be immediately used to evaluate mitochondrial capacity and bioenergetic efficiency using high-resolution respirometry and spectrofluorometry, as we have described previously [sup 1,2]. The remainder of the muscle tissue will be immediately blotted, frozen in liquid nitrogen, and stored at -80□C. Muscle mitochondrial content will be evaluated by laboratory-based enzymatic assays, protein expression, and mRNA abundance [sup 3]. Mass spectrometry and NMR spectroscopy will be used to detect and quantitate metabolites in muscle tissue lysates [sup 4]. Muscle biopsy may cause bleeding. We will use readily available cautery to stop the bleeding.

Device programming and follow-up

Device pacing settings will be programmed in the DDDR mode at 60 to 150 beats/minutes. The CRT-ON or CRT-OFF will be programmed according to the randomization. Patients will be called at 1-month to evaluate for potential pocket discomfort and procedure-related complications. Patients will return for follow-up at 6-month (initial program CRT-ON or CRT-OFF) and 12-month (cross over program CRT-ON or CRT-OFF). Peripheral blood will be drawn for metabolomic study, and echocardiography, 6-minute walk, KCCQ, and NYHA class will be assessed at each follow-up. Patients will undergo standard clinical care to maximize CRT response. Device function will be closely monitored on a daily basis via a remote monitoring system. Patients will continue a stable medical therapy through entire study to minimize confounding due to drug changes during the study. Any adjustment in medications, if clinically needed, will be carefully recorded and we will take into consideration the confounding drug effect in analysis. After patients complete the study, they will be followed annually by phone to assess adverse events.

Schedule of Events

	Screening (within 90 days of implant)	Device Implant (Day 0 ± 4 days)	Post-Implant (Day 0 ± 4 days)	1 Month Phone Call (± 7 days)	6 Month (±30 days)	7 month (± 7 days)	12 Month (± 30 days)	24, 36, & 48 Month Phone Calls (± 30 days)
Echocardiogram (may include 3D echo imaging)	X[2]				X		X	
Physical exam	X				X		X	
12-Lead ECG	X		X					

Six-minute walk test	X				X		X	
Kansas City Cardiomyopathy Questionnaire (KCCQ)	X				X		X	
Chest X-ray			X					
Pregnancy test (if applicable)	X[1]							
Blood collection For biomarker analysis	X	X			X		X	
Device programming/ interrogation			X		X		X	
SAE/AE Assessment (heart failure symptoms, pocket pain, device function, etc.)			X	X	X	X	X	X
Medication Reconciliation	X		X	X	X	X	X	

1. Females of child-bearing potential must have a negative pregnancy test within 7 days of device implant.
2. Imaging can be done within 12 months of implant.

Metabolomic analyses

To obtain comprehensive and confident information about the metabolome and metabolic pathways, a combination of different techniques are necessary because each technique covers different classes, masses, and concentration ranges of metabolites. Comparing quantitative ^{1}H and ^{31}P, NMR with semiquantitave mass spectrometric technologies permits better assessment of metabolite levels and dynamics. Selected metabolomics profiling will be done using LC/MS, GC/MS and ^{1}H NMR technologies available at our Metabolomics Resources Core. The Core is staffed with experienced analytical and statistical/bioinformatics staff. In addition, we will employ novel whole blood ^{18}O-stable isotope labeling technique – ^{18}O-assisted GC/MS and ^{31}P NMR, to detect subtle alterations in metabolite dynamics and metabolic pathways.[42,73]

In addition to NT BNP, subject samples will be tested for several other specific metabolomics biomarkers. In HF, the plasma long-chain acylcarnitines are substantially elevated due to impaired mitochondrial oxidative capacity. Simply a signal of mitochondrial dysfunction, the long-chain acylcarnitines is considered as a prognostic biomarker in end-stage HF and as an early detectable biomarker in mild LV dysfunction. Amino acids are important substrates for energy production in the heart. The branched-chain amino acids, specifically valine, leucine and isoleucine are elevated in HF. The amnio acids valine, leucine and isoleucine will be determined as biomarkers. Using the new technology of whole blood 18O metabolic labeling, we will measure 2,3-BPG[18O] and G-3-P[18^{O}] turnover rates that are reduced, while inorganic phosphate (Pi[18O]) and γ-ATP[18O] are elevated in mild LV dysfunction.

LC/MS and GC/MS. The LC-MS analyses will be performed using a 6500 Series Accurate-Mass Quadrupole Time-of-Flight LC-MS (Agilent Technologies) coupled with an Acquity UPLC System (Waters). Metabolomic profiling data are acquired under both positive and negative electrospray ionization conditions. Metabolite separation is achieved using 2 columns of differing polarity. The analysis is run against the

METLIN metabolite database to give putative molecular identifications. Components assigned putative identification are further verified by comparison to purchased reference standards and MS-MS data. Acyl-carnitines were measured by LCMS as previously described.[74] The samples and calibration standards were analyzed on Thermo TSQ Quantiva mass spectrometer (West Palm Beach, FL). Data acquisition was done using selective ion monitoring (SRM). Data files from GC-MS analyses will be deconvoluted using AMDIS software; SpectConnect will be used to list and track metabolite peaks.[42,75] The Agilent Fiehn RTL Library will be used for metabolite identifications.

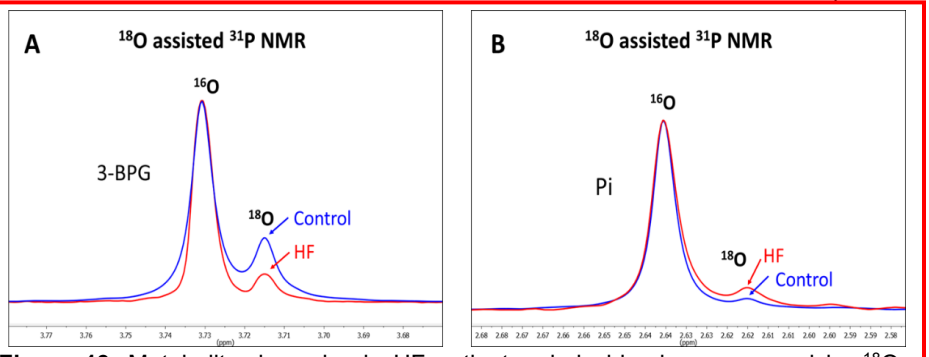

Figure 13. Metabolite dynamics in HF patients whole blood as assessed by ^{18}O-assisted ^{31}P NMR. HF patient had lower labeling and turnover rate of 2,3-bisphosphoglycerate (2,3-BPG[^{18}O]), which regulates hemoglobin affinity to O_2 and blood oxygen transport capacity. At the same time labeling, the turnover rate of inorganic phosphate (Pi[^{18}O]) was increased, signifying a higher ATP hydrolysis and degradation rate (ATP+$H_2{}^{18}O$→ADP+Pi[^{18}O]).

Human whole blood ^{18}O metabolic labeling. This novel technology was developed by Dr. Petras Dzeja at Mayo Clinic. Blood plasma and cells carry a wealth of metabolic information about health status and disease biomarkers.[25,76,77] Labeling of fresh whole blood will be performed by supplementing with 30% $H_2[^{18}O]$ in 0.9% NaCl and labeling for 5-10 min, extracting metabolites and analyzing by ^{18}O-assisted GC/MS and ^{31}P NMR.[42] Preliminary studies indicate that abnormal G-3-P[^{18}O] turnover indicates defects in α-glycerophosphate shuttle and substrate supply to mitochondria.[72] **Figure 13** shows an example of dysregulation of 2,3-BPG[^{18}O] and Pi[^{18}O] turnover rates, which normally regulates nucleotide metabolism and hemoglobin affinity to O_2 and blood oxygen transport capacity in a severe HF patient.

^{18}O-assisted ^{31}P NMR and ^{1}H NMR. This technology is unique to Mayo Clinic permiting detection dynamics of phosphorylated metabolites in human blood.[42] High-resolution ^{1}H NMR spectra will be acquired on a Bruker Avance III 600 spectrometer (Avance) while ^{31}P spectra are acquired with 500 MHz on a Bruker 11 T spectrometer (Avance). Percentages of ^{16}O, $^{18}O_1$, $^{18}O_2$ and $^{18}O_3$ phosphoryl species in phosphometabolites are proportional to the integrals of their respective peaks in the ^{31}P NMR spectrum. Spectra peaks will be identified according to Chenomx NMR Suite 6.1 software as previously described.[78,79]

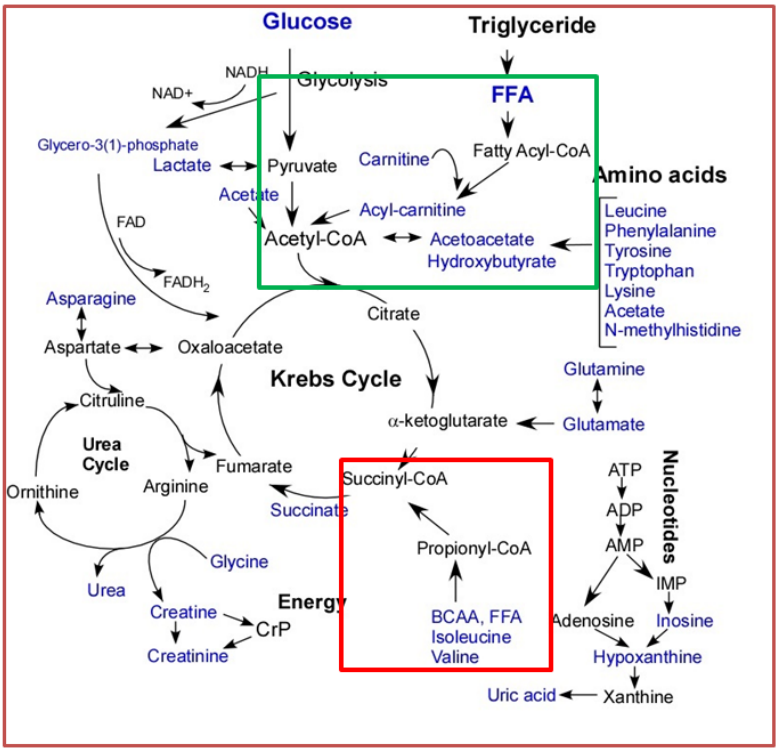

Figure 14. Metabolic pathways and metabolites involved in metabolic remodeling of myocardium in heart failure. Marked in blue are metabolites altered in plasma.

Objectives of metabolomic measurements

1) We will perform anatomical metabolomic mapping in correlation with the electrical conduction delay in the LV lateral wall. The blood samples from the coronary sinus that reflect the overall myocardial metabolism and from the LV lateral wall (the site of latest activation) that reflect the local metabolism will be collected and compared. A differential feature of regional metabolism will provide insight into fundamental metabolomic regulation in ventricular dyssynchrony. QRS duration is a clinical predictor of CRT response. We will correlate the metabolic predictive value of CRT outcomes with QRS duration.

2) The metabolomic mapping will characterize regional metabolism in ICM vs NICM.

3) We will gain insight in early perturbation of myocardial metabolomic profile in patients with mildly reduced LVEF. Specific focus will be paid to the following metabolic pathways or markers.

a. Myocardium primarily meets its requirements for energy through the oxidation of long-chain fatty acids (LCFA), where carnitine plays a key role as a carrier. The "carnitine shuttle" facilitates transfer of LCFA to cross mitochondrial membrane for β-oxidation.[80] In HF, the plasma long-chain acylcarnitines (LCAC) are substantially elevated due to impaired mitochondrial oxidative capacity (Figure 7 and 14, green box).[55] The LCAC has adverse effects on HF including an increase in cytoloic calcium loading, myocardial inflammation, and bioenergetics deficiency.[50] Simply a signal of mitochondrial dysfunction, LCAC is considered as a prognostic biomarker in end-stage HF.[51] With a cross-over study design, the role of LCAC will be examined as an early detectable biomarker in relation to LV dysfuction.

b. Amino acids are important substrates for energy production in the heart. Through a series of reactions, branched-chain amino acids (BCAAs) can be converted to metabolic intermediates and fed into the tricarboxylic acid (TCA) cycle as potential sources of anaplerotic input (Figure 14, red box). Due to the down regulation of gene expression related to the BCAA degradation and utilization, specifically, valine, leucine and isoleucine are accumulated in HF.[53,81,82] Our preliminary data (shown in Figure 6 and 10), showed that the level of these BCAAs were disturbed even in mild HF and was significantly lower in CRT non-responders than in CRT responders with severe HF. Further, amino acids are not only cell building blocks, energy source and signaling molecules but are also regulators of gene expression and protein synthesis. Taken together, the BCAAs are critical metabolite markers in HF.

c. Using the new technology of whole blood ^{18}O metabolic labeling, we will test the hypothesis that the 2,3-BPG[^{18}O] and G-3-P[^{18}O] turnover rates are reduced, while inorganic phosphate (Pi[^{18}O]) and γ-ATP[^{18}O] are elevated in mild LV dysfunction in which the impaired O2 and substrate transport and ATP synthesis may precede the HF symptoms (Figure 13).

d. We will develop strong metabolic biomarkers as proposed in above to determine the prognostication with clinical outcomes in patients with mild HF.

Primary clinical efficacy objective

The study primary endpoint is a reduction in the LV end-systolic volume index (LVESVI, reverse LV remodeling) in the CRT-ON group as compared to CRT-OFF group.

Secondary clinical efficacy objectives (composite events)

1) Hospital admission for HF; 2) Increase in NT pro-BNP level by 30% and its predictive value of response to CRT; 3) Sustained ventricular tachycardia or fibrillation greater than 30 seconds; 4) Decrease in LVEF to ≤35%; and 5) death.

The analytical imprecision of natriuretic peptides depends on the assay but for NTprpoBNP it is roughly 1.6%. Inter and intradividual variation is roughly 30% (American Journal of Cardiology. 92(5):628-31, 2003). Thus values above 30% have been suggested to define significant changes in natriuretic peptides (Journal of Investigative Medicine. 61(6):950-5, 2013 Aug,). This value was shown to have prognostic significance in the VALHEFT trial (Journal of the American College of Cardiology. 52(12):997-1003, 2008 Sep 16) and thus will provide an end point in our proposed analysis.

Primary safety objectives

Freedom from system-related complications in randomized participants will be calculated at 1, 6, and 12 months post-implant to determine the safety profile, including the device pocket hematoma, pneumothorax, myocardial perforation, lead dislodgment, lead revision, and device-related infection. This safety profile will be balanced against efficacy to quantify the risk-benefit ratio. This analysis will occur when all subjects have completed study procedures.

Sample Size Considerations and Statistical analysis

The timing of primary endpoint analysis will occur when all subjects have completed study procedures. The primary endpoint for Aim 1 is the change in LVESVI. We propose a paired t-test, which combines both

randomized groups together (n=50 + 50 = 100), and compares the CRT-ON condition to the CRT-OFF condition. The power can be estimated based on REVERSE (LVEF ≤40%) showing a mean change of -18.4 +/- 29.5 ml/m2. Let us assume that in the current mildly impaired EF group, the reduction will be, not -18, but -12. Assuming the SD is 26, the power for the one-sample paired t-test is over 99%. If we assume an 80% attrition (n=40 + 40), the detectable difference with 80% power is 8.3 ml/m2, less than half of that observed in REVERSE. A secondary analysis will be a generalized linear model in which baseline, period 1, and period 2 values of LVESVI will be analyzed together, with the possibility of an order effect. If no order effect is detected, then the analysis will amount to the paired t-test already described. Data may be missing due to the occurrence of interim events. This would mean that the data are not missing at random. We propose to accommodate this by a rank-based procedure, in which the ON-OFF differences are ranked in both groups together. As a secondary endpoint, we will analyze the composite clinical endpoint described above. This endpoint will be analyzed as the time to the first of any of these component events. The crossover design can be accommodated by fitting the treatment variable ON-OFF as a time dependent binary variable that is 1 (ON) or 0 (OFF). Order effects can be accommodated by including interactions with time-period in the model.

Metabolomics data analysis. The timing of metabolomics data analysis will occur when all subjects have completed study procedures. Data sets will be mean-centered and pareto-scaled to adjust the importance of high- and low-abundance metabolites to an equal level before carrying out statistical analyses.[42,52,77-79,84-86] The SIMCA-P+ 12.0 software package (Umetrics, Umea, Sweden) will be used for pattern recognition multivariate analysis, including unsupervised principal component analysis (PCA), supervised partial least squares discriminant analysis (PLS-DA), and orthogonal partial least-squares discriminant analysis (OPLS-DA). The data will be examined with PCA scatter plot of the first two score vectors (t1-t2) in order to reveal the homogeneity of the data, any groupings, outliers, and trends. Then PLS-DA is applied to increase the class separation, simplify interpretation, and find potential biomarkers. The OPLS-DA will be used to identify the significantly different metabolites between groups. For validation, R2 (the fraction of variance explained by a component) and Q2 (the fraction of the total variation predicted by a component) values will be considered as measures of goodness of model and the model robustness, respectively. The value of Q2 ranges from 0 to 1 and typically a Q2 value of greater than 0.4 is considered a good model. Most important metabolites will be selected according to the variable importance in the projection (VIP) values and the variables with VIP>1 will be considered for significantly changed metabolites. Metabolic pathway analysis will be performed using MetaboAnalyst 3.0 software.[83] In addition, we will explore the new multivariate metabolomics data (80 variables on 80 subjects), and construct principal components agnostically using SAS PROC FACTOR. Preliminary data suggest that 5 to 10 factors will represent 60% or more of the joint variation among the 80 metabolites. The 5-10 factors will then be calculated on each subject, and used to predict group differences (using a two-sample t-test), and response to CRT therapy. In particular, we will then relate these principal components (PC's) to response to CRT using multiple linear regression (for the LVESVI endpoint) and Cox Regression (for the Clinical endpoint).

Safety and Adverse Events

All adverse events occurring related to the procedure or protocol required testing during the study, including those not meeting the criteria of an Unanticipated Adverse Device Effect (UADE) will be recorded on the appropriate case report form. Records of these events will be maintained and reports submitted to the FDA and IRB according to the regulatory requirements. Expected clinical adverse events and nonsignificant (not serious) clinical adverse events will not be reported. Expected clinical adverse events and anticipated adverse device effects are those listed below.

The safety and clinical performance of market-released CRT systems have been demonstrated through previous clinical studies. All products used within this study are FDA approved and market-released. Therefore, it is not anticipated that subjects enrolled in this study will be exposed to any risks beyond those normally associated with CRT systems, transvenous lead systems, or their implant procedure. Because only patients who have existing ICD systems or who meet clinical indications for ICD implant will receive

CRT-D implant, there is no additional risk for these patients aside from the CRT risks described here for all patients in this study. With the exception of the risks associated with the device and implant procedure implant, the risks associated with metabolomics blood draw are considered minimal, Therefore, the risk analysis in this section is specific to the implantation, management and therapy from the implanted CRT system. The subject will remain in the study for intention to treat analysis, but the investigator will avoid any procedures that may be determined harmful.Study subjects will receive a CRT device with the associated three leads (one right atrial, one right ventricular and one left ventricular lead).

The risks normally associated with the implant of the CRT system include, but are not limited to the followings: pain, swelling and/or bruising around the device, collection of blood (hematoma) or fluid (seroma) in the tissues, infection, allergy to the device material, movement of the device from its original location, or generator erosion through the skin over time. The risks normally related to the use of transvenous lead systems include, but are not limited to the following: cardiac perforation, cardiac tamponade, pneumothorax, pericardial effusion, pericarditis, valve damage, thromboembolism, endocarditis, ventricular fibrillation or other arrhythmias, non-cardiac muscle and/or nerve stimulation, myopotential sensing, hemothorax, thrombosis, heart failure, tissue necrosis or infection. The pacemaker, software, or programmer may malfunction and fail to detect and/or treat arrhythmia leading to death. Other potential adverse events related to the leads include, but are not limited to, the following: lead insulation failure, lead breakage difficulty removing the lead, poor connection to the device, elevated thresholds, lead damage lead disloged from its original location that may require that the lead be replaced or repositioned. Additional risks associated with the CRT system include, but are not limited to:

- The placement of the LV lead requires the use of a dye to conduct the venogram. With the venogram there is a risk of an allergic reaction to this x-ray dye that Could result in serious injury (such as shock or death). There is also a chance that the dye may damage the kidneys.
- Exposure to an increased amount of radiation through fluoroscopy and x-rays.
- Vein wall rupture, dissection and perforation of the coronary sinus, cardiac vein,
- Myocardium or pericardium from the left ventricular pacing lead or implant tool.
- Due to the nature of the coronary sinus anatomy, a chronically implanted coronary sinus lead may be more difficult to remove than a traditional endocardial lead.
- There may be risks associated with worsening of the subject's heart failure.
- Complications during surgery include, but are not limited to: stroke, heart attack, blood clots, bleeding, and death.
- The Cardiac Resynchronization Therapy (CRT) is considered investigational and/or outside current medical guidelines in qualifying patients and may be no more effective or less effective than optimal medical therapy.
- Study-related blood tests (e.g. BNP) might cause mild discomfort or irritation or some discomfort following blood draw.
- Contrast agent allergy is usually infrequent and symptoms are shortness of breath, hives, itching, back pain, chest pain, or rash; allergic and potentially life threatening hypersensitivity reactions may occur rarely, including anaphylactoid and/or anaphylactic reactions, shock, bronchospasm, tongue and/or throat swelling, decreased oxygen saturation, and loss of consciousness.

Risk Minimization

The potential risks associated with the commercially available CRT implant were identified and have been successfully mitigated from clinical practice. Any potential risks associated with this study are further minimized by selecting qualified investigators. A Data Monitoring Committee will be formed to review safety issues as part of the study. In addition, investigators will be actively involved in the implantation and follow-up of the subjects implanted with the CRT systems. Risks will be minimized by careful assessment of each subject prior to, during, and after implant of the CRT. Prior to implant, it is recommended subjects undergo a complete cardiac evaluation. After implantation, subjects will be followed at regular intervals to monitor the condition of the implanted system and the battery. At each protocol required follow-up, , the investigator must interrogate the CRT to verify appropriate CRT function and to evaluate pacing and sensing characteristics and to assess any adverse events.

Recording and reporting of adverse event

Unanticipated Adverse Device Effect (UADE): A UADE is any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device if that effect, problem or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or IDE application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Adverse Effect (Event): Any untoward medical occurrence in a subject involved in clinical study of an investigational device; regardless of the causal relationship of the problem with the device or, if applicable, other study related treatment(s).

Associated with the investigational device: There is a reasonable possibility that the adverse effect may have been caused by the investigational device.

Life-threatening adverse effect: Any adverse effect that places the subject, in the view of either the investigator or the sponsor, at immediate risk of death from the effect **as it occurred**. It does not include a reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse effect: An adverse effect is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- death
- a life-threatening AE
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect.

Unanticipated adverse effect: Any adverse effect, the nature, specificity, severity, or frequency of which is not consistent with the risk information in the clinical study protocol or elsewhere in the current IDE application.

General Physical Examination Findings: At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as an unanticipated adverse device effect unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.
Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the local investigator should instruct each subject to report, to the local investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The local investigator should notify the study regulatory sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the local investigator should become aware of the development of problems, cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)

Any unanticipated problem or adverse event that meets all of the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, AND
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Study subjects will be routinely questioned about adverse effects at study visits. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF) or in a separate adverse event worksheet. All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

All adverse events related to the procedure or testing required by the protocol occurring during the study period must be recorded. All observed or volunteered adverse effects (serious or non-serious) and abnormal test findings, regardless of the treatment group if applicable or suspected causal relationship to the investigational device or if applicable other study treatment or diagnostic product(s) will be recorded in the subjects' case history. For all adverse effects sufficient information will be pursued and or obtained as to permit; an adequate determination of the outcome, an assessment of the casual relationship between the adverse effect and the investigational device or, if applicable other study treatment or diagnostic product. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final

outcome. Any serious adverse event that occurs after the study period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

Reporting of Unanticipated Adverse Device Effects and Unanticipated Problems
When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required. The sponsor-investigator will promptly review documented Unanticipated Adverse Device Effects and as necessary shall report the results of such evaluation to FDA within 10 working days and Mayo IRB within 5 working days of initial notice of the effect. Thereafter the sponsor-investigator will submit such additional reports concerning the effect as requested. A copy of this completed form will be provided to the DSMB and all participating sub-investigators.The completed FDA Form 3500A will be submitted to the FDA as soon as possible and, in no event, later than 10 working days after the sponsor-investigator first receives notice of the adverse effect.

Medical Monitoring
It is the responsibility of the sponsor-investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see Section 10 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

Data and Safety Monitoring Board
A Data Safety Monitoring Board (DSMB) has been established for this study.

Data Handling and Record Keeping

Confidentiality
Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

Source Documents
Source data comprise all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical

departments involved in the clinical trial. When applicable, information recorded on the CRF shall match the Source Data recorded on the Source Documents.

Case Report Forms

Case Report Forms (CRFs) will be completed for each subject enrolled into the clinical study.

Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents.

The sponsor-investigator will retain the specified records and reports for:

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified.

   OR

2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" http://mayocontent.mayo.edu/research-policy/MSS_669717, whichever is longer.

Study Monitoring, Auditing, and Inspecting

Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

Auditing and Inspecting

The sponsor-investigator will permit study-related monitoring, audits, and inspections by the IRB, the monitor, and government regulatory agencies, of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data etc.). The sponsor-investigator will ensure the capability for inspections of applicable study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.).

Participation as a sponsor-investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed and dated by the subject and the individual obtaining the informed consent.

Potential Benefits

The potential benefits of having the CRT include reduction in heart failure symptoms, reduced rate of sudden cardiac arrest, mortality and improved ventricular function.The information gained from this study could result in the improved management of other patients with congestive heart failure. Additionally, information collected from this study may assist in the design of new therapies. The arrhythmia monitoring features of CRT, if programmed on as determined by the investigator, may offer benefit in the event that the patient experiences any arrhythmia. Electronic capture of the specific arrhythmia may aid in the diagnosis and treatment selection for these arrhythmias. Subjects will have devices with approximately 8 years of remaining battery life to continue heart failure therapy.

Risk-to-Benefit Analysis

The cohort of patients defined for inclusion in this study is a result of their heart failure. The CRT offers the opportunity to reduce these symptoms, improve heart function, and potentially extend quality of life. These benefits have been proven in patients with severe heart failure. CRT has a two decade-long track record of published safety and efficacy for heart failure.

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