***Study Patients***

This single-center prospective study consisted of 24 consecutive patients who received CRT-D (defibrillation) for advanced HF at Mayo Clinic, Rochester, Minnesota, from March 1 through November 30, 2010. All patients were clinically recommended for device implantation according to current guidelines for CRT [32](#_ENREF_32" \o "Tracy, 2012 #43). The Mayo Clinic Institutional Review Board approved this study, and all patients provided signed consent for the study. Ten age-matched control patients underwent catheter ablation for supraventricular arrhythmia with a normal left ventricular ejection fraction (LVEF) of greater than 55%.

***Baseline Evaluation***

All HF patients underwent a baseline evaluation before CRT, including assessment of New York Heart Association (NYHA) functional class, concomitant cardiovascular conditions (e.g., hypertension, coronary artery disease, and diabetes mellitus), electrocardiographic QRS duration and morphologic characteristics, and transthoracic echocardiography. Echocardiographic parameters included LV end-systolic and diastolic dimensions, LVEF, and pulmonary artery systolic pressure. **Medication use was recorded and confirmed that patients were taking optimal medication dose for HF. Patients continued on stable medication dosage during the study.**

***CRT Implantation and Blood Sample Collection***

All patients underwent conscious sedation throughout the procedure for CRT implantation. The right femoral artery was cannulated for continuous monitoring during the procedure in both HF and control patients. Blood samples were taken from a peripheral vein before the device was implanted. These blood samples (5 mL) were collected into EDTA tubes containing 100 μL of injected stop solution (dipyrimidole,10 μM; EHNA, 5 μM; iodotubercidin, 2 μM; AOPCP, 50 μM, in 0.9% NaCl) to reduce blood nucleotide metabolism; stored on ice; and then centrifuged at 3,200 rpm for 10 minutes at 4°C. Plasma aliquots were stored at −80°C until analyses.

***Patient Follow-up***

The HF patients returned for a clinical follow-up 3 months after CRT. The NYHA functional class was reassessed, and echocardiography was repeated. Peripheral venous blood samples were collected for post-CRT metabolic measurements. Metabolomic profile was successfully determined in 19 patients. **Improvement in LVEF by more than 5% and reduction of NYHA ≥ 1 class were considered as CRT responders.**

***Metabolomic Analyses***

*Sample Treatment and Instrumental Conditions for GC-MS Metabolomic Analysis*

For gas chromatography–mass spectrometry (GC-MS), plasma (100 μL) was extracted using a 900-μL methanol:water (8:1, v/v) mixture containing 5 μg internal standard, myristic-d27 acid, at ambient temperature [33](#_ENREF_33). Supernatant (900 μL) was transferred and completely dried in a vacuum concentrator. Subsequently, the tubes were methoximated and derivatized and then analyzed using an Agilent 6890 GC oven with Agilent 5973 MS [34](#_ENREF_34).

*Sample Treatment and Instrumental Conditions for 1H NMR Metabolomic Analysis*

For nuclear magnetic resonance (NMR) imaging, plasma (60 μL) was diluted 140 μL with 0.2M phosphate buffer (pH 7.4):D2O containing a mixture of 16 mM formate and 4 mM TSP (1:1, v/v). After filtering (0.22 μm), the samples were transferred into a 3-mm-diameter NMR tube. High-resolution 1H NMR spectra were acquired at 600 MHz on a Bruker Avance III 600 spectrometer. Spectra peaks were identified according to Chenomx NMR Suite 6.1 software and data in the literature [35](#_ENREF_35), [36](#_ENREF_36).

*Metabolomic Data Analysis*

The data files from GC-MS analyses were deconvoluted using AMDIS software [37](#_ENREF_37), and then SpectConnect (http://spectconnect.mit.edu/index.php) was used to list and track metabolite peaks [38](#_ENREF_38). Quality control-based signal correction and integration of data were used to minimize intra- and inter-day variations [29](#_ENREF_29). The Agilent Fiehn GC/MS Metabolomics RTL Library was used for metabolite identification. Data analysis of 1H NMR spectra was done with the MestRenova (Mestrelab). The identified peaks were integrated and normalized according to an internal standard (fumarate). **Analysis of the 1H NMR spectra and GC-MS chromatogram permitted detection over 400 metabolite peaks, major of which are presented in Supplemental Table 1.** Orthogonal partial least squares discriminant analysis (OPLS-DA) was performed using Umetrics SIMCA-P+ version 12.0. The variable importance in the projection values was calculated to identify a panel of the most important metabolites, and regression coefficients were used to validate group separation.

***Statistical Analysis***

All data are expressed as mean ± standard error of the mean. The Student unpaired *t* test was used, and differences were considered statistically significant at *P*<.05. One-way analysis of variance was used to test differencesamong groups.