Decreases in Circulating Concentrations of Short-Chain Acylcarnitines are Associated with Systolic Function Improvement After Decompensated Heart Failure

Wei-Siang Chen, MD, Min-Hui Liu, MSN, Mei-Ling Cheng, PhD and Chao-Hung Wang, MD

Summary

Impaired fatty acid metabolism is associated with heart failure (HF) prognosis. However, specific changes in acylcarnitine profiles and their potential clinical value have not been well explored in patients recovering from acute decompensation.

This study recruited 79 HF patients hospitalized because of acute decompensation with a left ventricular ejection fraction (LVEF) of < 40% and 51 normal controls. Patients were dichotomized into two groups, namely, the “improved (IMP)” and the “non-improved (NIMP)” groups, as defined by the changes in LVEF from baseline to 12 months after discharge. Mass spectrometry was used to quantify the acylcarnitine concentrations at baseline and 6 and 12 months after discharge. The IMP and NIMP groups contained 42 and 37 patients, respectively. At baseline, HF patients had higher plasma concentrations of specific long-, medium-, and short-chain acylcarnitines compared to normal controls. From baseline to 12 months post-discharge, the IMP group showed significant decreases in long- and short-chain acylcarnitine concentrations, but significant increases in medium-chain acylcarnitines. In the NIMP group, none of the acylcarnitines significantly decreased, and significant increases were noted in long-, medium-, and short-chain acylcarnitines. Generalized estimating equations demonstrated that nine acylcarnitines could discriminate the IMP group from the NIMP group, including three long-chain (C18:1, C16, and C16:1) and six short-chain acylcarnitines (C5, C5-OH, C4, C4:1-DC, C3, and C2). After adjusting for age, the six short-chain acylcarnitines remained significant. Changes in short-chain acylcarnitine profiles are independently associated with the improvement in cardiac systolic function after acute decompensation.

Key words: Fatty acid, Lipidomics, Metabolism

Fatty acids provide the major fuel for maintaining the energy requirements of a normal heart, with glucose providing the remainder. However, in heart failure (HF), substrate uptake, oxidative phosphorylation, and energy transfer are deranged. In addition, the pathophysiological processes caused by the failing heart include metabolic disturbances—not only in the myocardium but also in the skeletal muscle and multiple organs, as well as the gut microbiota.

Metabolomics studies have been gradually utilized in the study of cardiovascular diseases. Previously, we investigated the metabolic profile in peripheral blood as a surrogate for metabolic disturbance in HF and demonstrated that increased concentrations of specific acylcarnitines were associated with HF and therefore provided prognostic value. Ahmad, et al. further showed that the increased concentrations of some long-chain acylcarnitines predicted outcomes among HF patients and significantly decreased after implantation of left ventricular assist devices in patients with end-stage HF. However, changes in fatty acid metabolism and their clinical value have not been well explored in patients recovering from acute decompensated HF, who are encountered in clinical settings worldwide.

Increased plasma concentrations of long-chain acylcarnitines represent dysfunctional beta-oxidation of fatty acids in the mitochondria. However, the increased medium- and short-chain acylcarnitines in HF may be derived from amino acids and gut microbiota as well. Investigating the changes in the full spectrum of acylcarnitines may further elucidate patterns of abnormal energy utilization from the acute to chronic phases of HF.
The aims of this study included the following: (1) to investigate changes in acylcarnitine levels from acute decompensated HF to one year after discharge, (2) to investigate the relationship between improved cardiac systolic function and changes in long-, medium-, and short-chain acylcarnitines at different time points after acute HF; and (3) to develop acylcarnitine profiles that can discriminate patients with improved cardiac systolic function from those without improvement.

Methods

Patients and study design: From January 2015 to November 2016, we enrolled patients at HF stage C, which was defined according to the American College of Cardiology and the American Heart Association HF classification system.53 Patients at stage C were those hospitalized because of acute or decompensated chronic HF and aged 20-85 years, with a left ventricular ejection fraction (LVEF) of < 40%. Meanwhile, normal controls were aged 20-85 years and had no significant systemic disease such as hypertension, diabetes mellitus, or coronary artery disease. They were not on any medications and had an LVEF of > 60%. Exclusion criteria included the following: (1) the presence of systemic diseases such as hypothyroidism, decompensated liver cirrhosis, and systemic lupus erythematosus, (2) the presence of disorders other than HF that might compromise survival within 6 months, (3) patients who were bedridden for > 3 months and/or unable to stand alone, (4) patients with a serum creatinine of > 3 mg/dL, (5) patients with severe coronary artery disease without complete revascularization therapy, (6) patients with moderate to severe valvular heart disease, and (7) patients who were re-hospitalized because of worsening HF or who died 12 months after enrollment. Informed consent was obtained from all patients. The study was designed and carried out in accordance with the principles of the Declaration of Helsinki and with approval from the Ethics Review Board of Chang Gung Memorial Hospital.

Patients were dichotomized into two groups, namely, the “improved (IMP)” and “non-improved (NIMP)” groups. The IMP group was defined by an LVEF of > 50% at 12 months or more than 30% increase of LVEF at 12 months compared to baseline.

Measure of acylcarnitines: Stable isotope dilution-multiple reaction monitoring mass spectrometry was used for acylcarnitine quantification. The metabolomics analyses were carried out using the AbsoluteIDQ® p180 kit (Biocrates Life Science AG, Innsbruck, Austria). The kit enables us to identify and quantify 40 acylcarnitines. All reagents used in this analysis were of LC-MS grade. Ten μL aliquot of each plasma sample was mixed with isotopically labeled internal standards in a multiliter plate and dried under nitrogen. Three hundred μL of extraction solvent (5 mM ammonium acetate in methanol) was added, and after 30 minutes of incubation, it was centrifuged for 2 minutes. Subsequently, 150 μL of filtrate was mixed with 400 μL of running solvent for flow injection analysis coupled with tandem mass spectrometric analysis (FIA-MS/MS). The analysis was performed in positive and negative electrospray ionization modes. Multiple reaction monitoring (MRM) coupled with stable isotope dilution mass spectrometry (SID-MS) using a triple quadrupole mass spectrometer was used for quantitative measurement of target compounds. Identification and quantification were achieved by MRM. It was standardized by spiking in of isotopically labeled standards. LC-MS analysis was performed with Waters Zevo TQ coupled to UPLC (Waters Corp., Milford, USA). Metabolites were separated on a reverse phase column (2.1 mm × 50 mm, BEH C18, Waters Corp., Milford, USA) using a mobile phase, which was composed of a gradient mixture of solvent A (0.2% formic acid in water) and solvent B (0.2% formic acid in acetonitrile) (0 minutes, 0% B; 3.5 minutes, 60% B; 3.8 minutes, 0% B; and 3.9 minutes, 0% B). Elution was performed at a flow rate of 900 μL/minute. The column temperature was maintained at 50°C. For FIA, an isocratic method was used (100% organic running solvent) with varying flow conditions (0 minutes, 30 μL/minute; 1.6 minutes, 30 μL/minute; 2.4 minutes, 200 μL/minute; 2.8 minutes, 200 μL/minute; and 3 minutes, 30 μL/minute). The corresponding MS settings were as follows: dwell time of 0.019-0.025 seconds, capillary voltage of 3.92 KV for positive mode, capillary voltage of 1.5 KV for negative mode, nitrogen as a collision gas medium, and source temperature of 150°C.

Echocardiography: Echocardiographic images were obtained with the patients in the left lateral decubitus position at end-expiration at 2.5 MHz (two-dimensional) (HP Sonos 7500 machine). The LVEF was calculated using the Simpson method. We assessed the left ventricular end diastolic and systolic dimensions and other associated anatomical abnormalities, such as valvar lesions, using the criteria suggested by the American Society of Echocardiography.

Statistical analyses: Results are expressed as the mean ± SD for continuous variables and as the number (percentage) for categorical variables. Data were compared by two-sample or paired t-tests with Bonferroni correction and chi-square, when appropriate. Generalized estimating equations (GEEs) were used to compare the changes of metabolites from baseline to 6 and 12 months between groups. Metabolomics analysis was performed with several software. Data import and pre-processing steps for targeted MS data were done using TargetLynx (Waters, MA, USA). The integrated MetIDQ software (Biocrates, Innsbruck, Austria) was used to streamline data analysis by automated calculation of metabolite concentrations. All statistical analyses were two-sided and performed using SPSS software (version 15.0, SPSS, Chicago, IL, USA). A P value of < 0.05 was considered significant.

Results

Baseline characteristics: This study analyzed a total of 79 HF patients and 51 normal controls. The IMP and NIMP groups contained 42 and 37 patients, respectively. The baseline characteristics and laboratory data for the two groups are shown in Table I. Compared to the NIMP group, patients in the IMP group were younger. Comparison of the two groups revealed no significant differences in sex, LVEF, body mass index, blood pressure, etiology...
Acylcarnitines in HF patients and normal controls: eGFR, alanine aminotransferase, hemoglobin, albumin, and (ischemia or valve), incidence of comorbidities, guideline-based medications, BNP, lipid profiles, total bilirubin, alanine aminotransferase, hemoglobin, albumin, and eGFR.

**Acylcarnitines in HF patients and normal controls:** We measured acylcarnitines in normal controls and in HF patients at baseline and 6 and 12 months after discharge. At baseline, HF patients had higher plasma concentrations of certain long-chain acylcarnitines (C18:1, C18:2, C16, C16:1, and C16:1-OH), medium-chain acylcarnitines (C12:1, C9, C8, and C7-DC), and short-chain acylcarnitines (C6:1, C6-OH, C5, C5:1, C5:1-DC, C4, C4:1, C4:1-DC, C3:1, and C3-OH) when compared to normal controls (Table I).

In the following studies, we investigated the serial changes in each acylcarnitine from baseline to 12 months. Significant increases were noted at 12 months, but significant decreases in the circulating levels of C18:1, C18:2, C16:1-OH, after stabilization: C14:1-OH, C14:2, C10, C10:1, C8, and C3 at 6 months and C14:1-OH, C12, C10, C10:1, C9, C8, C4, C4:1-DC, C3, and C2 at 12 months. During the serial follow-ups, although there was a trend toward a decrease of long-chain acylcarnitines, such as C18:1, C18:2, C16:1, and C16:1-OH, the changes were not significant (Table II).

### Table I. Baseline Characteristics of Normal Controls and Patients with and without Improvement in Left Ventricular Systolic Function

| Variables                  | Normal | All | Improved | Not improved | P value |
|----------------------------|--------|-----|----------|--------------|---------|
| Age (years)                | 55.2 ± 4.4 | 61.5 ± 13.0 | 57.6 ± 12.6 | 65.9 ± 12.3 | 0.004   |
| Male (%)                   | 19 (37.3) | 31 (64.6) | 30 (71.4) | 21 (56.8) | 0.174   |
| LVEF (%)                   | 72.2 ± 8.1 | 29.7 ± 7.5 | 28.2 ± 7.6 | 31.3 ± 7.1 | 0.062   |
| NYHA functional class ≥ III (%) | 0 (0) | 34 (43) | 16 (38.1) | 18 (48.6) | 0.344   |
| Body mass index (kg/m²)    | 24.4 ± 3.28 | 25.3 ± 4.95 | 25.9 ± 5.31 | 24.6 ± 4.47 | 0.228   |
| Blood pressure (mm Hg)     | 125 ± 15.6 | 125 ± 18.4 | 121 ± 17.2 | 128 ± 19.1 | 0.065   |
| Heart rate (beats/minute)  | 72.1 ± 11.2 | 94.3 ± 21.9 | 97.9 ± 22.3 | 90.1 ± 21.2 | 0.115   |
| Comorbidity                |         |       |          |              |         |
| Diabetes mellitus (%)      | 0 (0)   | 34 (43) | 16 (38.1) | 18 (48.6) | 0.344   |
| Hypertension (%)           | 0 (0)   | 58 (73.4) | 29 (69) | 29 (78.4) | 0.349   |
| Atrial fibrillation (%)    | 0 (0)   | 20 (25.3) | 11 (26.2) | 9 (24.3) | 0.849   |
| COPD (%)                   | 0 (0)   | 14 (17.7) | 6 (14.3) | 8 (21.6) | 0.394   |
| Enology                    |         |       |          |              |         |
| Ischemia (%)               | 0 (0)   | 41 (51.9) | 19 (45.2) | 24 (64.9) | 0.074   |
| Valve (%)                  | 0 (0)   | 7 (8.9) | 2 (4.8) | 5 (13.5) | 0.243   |
| Medication                 |         |       |          |              |         |
| ACEI or ARB (%)            | 0 (0)   | 72 (91.1) | 40 (95.2) | 32 (86.5) | 0.172   |
| β-Blocker (%)              | 0 (0)   | 48 (60.8) | 27 (64.3) | 21 (56.8) | 0.494   |
| Diuretic (%)               | 0 (0)   | 57 (72.2) | 32 (76.2) | 25 (67.6) | 0.394   |
| Laboratory data            |         |       |          |              |         |
| BNP (log)                  | 0.88 ± 0.23 | 2.64 ± 0.51 | 2.61 ± 0.50 | 2.67 ± 0.52 | 0.552   |
| Cholesterol (mg/dL)        | 214 ± 35.5 | 175 ± 57.7 | 164 ± 41.4 | 1871 ± 70.4 | 0.070   |
| Triglyceride (mg/dL)       | 99.2 ± 55.9 | 135 ± 106 | 134 ± 119 | 136 ± 89.9 | 0.950   |
| Bilirubin (total) (mg/dL)  | 0.82 ± 0.31 | 1.20 ± 0.78 | 1.28 ± 0.84 | 1.10 ± 0.69 | 0.299   |
| ALT (U/L)                  | 26.6 ± 14.0 | 37.3 ± 18.4 | 36.8 ± 16.0 | 37.7 ± 21.0 | 0.831   |
| Serum sodium (mEq/L)       | 140 ± 1.35 | 140 ± 2.73 | 140 ± 2.86 | 140 ± 2.60 | 0.813   |
| Hemoglobin (g/dL)          | 13.8 ± 1.23 | 13.2 ± 2.17 | 13.6 ± 2.36 | 12.7 ± 1.85 | 0.067   |
| Albumin (g/dL)             | 4.39 ± 0.24 | 3.54 ± 0.57 | 3.54 ± 0.59 | 3.54 ± 0.55 | 0.967   |
| eGFR (mL/minute/1.73 m²)   | 99.1 ± 19.2 | 60.0 ± 30.3 | 65.0 ± 29.9 | 54.4 ± 30.0 | 0.121   |

Data are presented as mean ± standard deviation. ACEI indicates angiotensin-converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; and NYHA, New York Heart Association.
We used a GEE model to delineate changes in each acylcarnitine from baseline to 6 and 12 months (Table III). The analysis found nine acylcarnitines with the ability to discriminate the IMP group from the NIMP group (Table III), including C18:1, C16, C16:1, C5, C5-OH, C4, C4:1-DC, C3, and C2. Significantly lower levels of these acylcarnitines were evident in the IMP group at 12 months compared to the NIMP group.

After adjusting for age, only five acylcarnitines remained significant, including C5, C4, C4:1-DC, C3, and C2 (Table IV).
At the acute phase, the most abundantly accumulated metabolites were long- and short-chain acylcarnitines, rather than medium-chain ones. These findings suggest that the source of short-chain acylcarnitines might not be classical beta-oxidation of long-chain fatty acids. The short-chain acylcarnitines with odd numbers of carbons, namely, C3 and C5, are actually degraded branched chain amino acids that may derive from muscular breakdown or undigested dietary branched chain amino acids metabolized by gut microbiota.8,9) C4 contains two isotypes, namely, butyryl- and isobutyrylcarnitines, which are derived from fat and valine degradation, respectively.8-11) The elevated plasma levels of C4 and C2 may derive from gut microbiota as well. On the other hand, increased formation of clusters of dicarboxyl- and hydroxyl-acylcarnitines were suggested via \( \omega \)- and \( \omega-1 \) fatty acid oxidation by microsomal cytochrome p450 enzymes in peroxisomes, which is a rescue pathway in response to impaired mitochondrial function (Figure 1).13) In patients with recovered cardiac function, the decreased levels of these short-chain acylcarnitines suggest less protein breakdown, less utilization of branched chain amino acids for energy production, and improvement in the profile of the gut microbiota.

Interactions between the gut microbiota and host metabolism have received increased attention recently.11,14) HF is associated with congestion in the intestines, leading to bowel wall edema and impaired intestinal food digestion and barrier function. Gut microbiota are causally influenced by undigested dietary carbohydrates and protein. These conditions probably cause bacterial translocation and the presence of bacterial metabolites in the circulation. Our previous study demonstrated increased circulating concentrations of gut-derived metabolites such as p-
cresol sulfate and indoxyl sulfate in HF patients.\(^{15}\) Short-chain fatty acids are major fermentation products that are rapidly absorbed and utilized by the host, providing energy to the epithelium of intestines.\(^{16,17}\) Therefore, assessing acetylcarnitine, propionylcarnitine, and butyrylcarnitines, along with p-cresol sulfate and indoxyl sulfate, might be useful for evaluating host metabolism of gut fermentation end-products.

In patients without adequate recovery of cardiac function, these short-chain acylcarnitines paradoxically increased, suggesting increased protein breakdown, inappropriate overuse of amino acids for energy production, and an unfavorable gut microbiota profile. Further studies need to explore the changes in the gut microbiota from the acute to chronic phases of HF and unravel the underlying mechanisms associated with HF-related dysregulation of short-chain acylcarnitine metabolism. These pathophysiological insights should provide a basis for developing urgently needed clinical interventions that use nutrition and manipulate gut flora, since excess accumulation

---

**Table III.** Acylcarnitines in Patients with and without Improvement in Left Ventricular Ejection Fraction from Baseline to 12 Months After Discharge

| Acylcarnitine (pM) | Improved (n = 42) | Not improved (n = 37) | P value* | P value † |
|--------------------|------------------|----------------------|----------|--------|
| **Baseline**       | 6 months         | 12 months            |          |        |
| C18                | 65.3 ± 8.76      | 59.9 ± 6.31          |          |        |
| C18:1              | 193 ± 20.9       | 168 ± 14.9           |          |        |
| C18:1-OH           | 3.2 ± 0.94       | 3.8 ± 0.90           |          |        |
| C18:2              | 115 ± 12.8       | 95.2 ± 11.4          |          |        |
| C16                | 143 ± 16.7       | 130 ± 11.0           |          |        |
| C16-OH             | 4.1 ± 1.13       | 2.1 ± 0.66           |          |        |
| C16:1              | 43.0 ± 5.36      | 35.9 ± 4.17          |          |        |
| C16:1-OH           | 10.8 ± 2.04      | 13.4 ± 2.57          |          |        |
| C16:2              | 7.4 ± 2.09       | 8.5 ± 1.99           |          |        |
| C16:2-OH           | 2.6 ± 0.79       | 4.1 ± 1.00           |          |        |
| C14                | 70.9 ± 5.38      | 68.6 ± 5.81          |          |        |
| C14:1              | 115 ± 7.38       | 130 ± 8.42           |          |        |
| C14:1-OH           | 10.4 ± 2.18      | 14.9 ± 2.76          |          |        |
| C14:2              | 99.8 ± 14.1      | 131 ± 26.9           |          |        |
| C14:2-OH           | 6.8 ± 1.58       | 5.7 ± 1.52           |          |        |
| C12                | 74.1 ± 6.99      | 81.8 ± 5.52          |          |        |
| C12-DC             | 20.5 ± 2.23      | 18.3 ± 2.36          |          |        |
| C12                | 159 ± 9.74       | 160 ± 13.5           |          |        |
| C10                | 222 ± 10.5       | 288 ± 17.4           |          |        |
| C10:1              | 343 ± 17.2       | 396 ± 25.5           |          |        |
| C10:2              | 370 ± 29.5       | 384 ± 30.0           |          |        |
| C9                 | 24.5 ± 2.35      | 24.7 ± 2.99          |          |        |
| C8                 | 175 ± 7.96       | 211 ± 12.5           |          |        |
| C7-DC              | 45.7 ± 4.08      | 54.3 ± 3.60          |          |        |
| C6-1               | 20.6 ± 2.18      | 22.4 ± 2.15          |          |        |
| C6-OH              | 16.6 ± 1.85      | 13.7 ± 1.55          |          |        |
| C5                 | 175 ± 22.9       | 170 ± 10.5           |          |        |
| C5-M-DC            | 22.3 ± 1.92      | 21.4 ± 1.84          |          |        |
| C5-OH              | 20.2 ± 2.27      | 18.0 ± 2.50          |          |        |
| C5-1               | 31.7 ± 2.82      | 29.2 ± 3.11          |          |        |
| C5-1-DC            | 19.2 ± 1.83      | 15.5 ± 2.01          |          |        |
| C4                 | 315 ± 20.3       | 301 ± 20.6           |          |        |
| C4-1               | 110 ± 1.47       | 133 ± 1.95           |          |        |
| C4-1-DC            | 130 ± 8.71       | 135 ± 9.40           |          |        |
| C3                 | 427 ± 24.6       | 441 ± 29.3           |          |        |
| C3-OH              | 9.1 ± 1.23       | 6.6 ± 1.31           |          |        |
| C3-1               | 25.8 ± 1.76      | 24.3 ± 2.05          |          |        |
| C2                 | 6930 ± 357       | 7251 ± 445           |          |        |

Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, compared to the baseline in patients with improvement. \(^{1}P < 0.05, \(^{2}P < 0.01, \)compared to baseline. * †, compare the changes between groups from baseline to 6 and 12 months, respectively, by generalized estimating equations (GEE) model. C2 indicates acetyl carnitine; C3, propionylcarnitine; C3-OH, hydroxypropionylcarnitine; C4, butyrylcarnitine/isobutyrylcarnitine; C4:1, butyrylcarnitine; C4:1-DC, hexanoylcarnitine; C5, valerylcarnitine; C5:1, i-glycerylcarnitine; C5:1-DC, glutonylcarnitine; C5:1-M-DC, methygluloylcarnitine; C5:1-OH (C5:1-DC-M), methylmonoylcarnitine; C6, hexanoylcarnitine; C6-OH, glutarylcaritnine; C7-D, palmitoylcarnitine; C8, octanoylcarnitine; C9, nonanoylcarnitine; C10, decanoylcarnitine; C10:1, decenocarnitine; C10:2, deacylarnitnine; C12, dodecanoylcarnitine; C14:1, tetradecanoylcarnitine; C14:1-1, tetradecenylylcarnitnine; C14:1-OH, hydroxytetradecenylylcarnitnine; C14:2, tetradecenedioylcarnitnine; C14:2-OH, hydroxytetradecenedioylcarnitnine; C16, hexadecanoylcarnitnine; C16:1, hexadecenylylcarnitnine; C16:1-OH, hydroxyhexadecenylylcarnitnine; C16:2, hexadecenedioylcarnitnine; C16:OH, hydroxyhexadecenedioylcarnitnine; C18, octadecanoylcarnitnine; C18:1, octadecenylylcarnitnine; and C18:2, octadecenedioylcarnitnine.
of intracellular and circulating acylcarnitines may exacerbate metabolic disturbance and lead to cytotoxicity in the mitochondria and the kidneys.\(^9,10\)

**Limitations:** The conclusions of this study are based on changes in plasma acylcarnitines, with the assumption that intracellular metabolism is disturbed in HF patients. Identification of the source of the elevated metabolites requires a multi-omics study design that includes metabolomics, gut microbiota, and amino acid measurement. The advantages and disadvantages of the increases in short-chain acylcarnitines at the acute stage also need further investigation so that we can design specific, corresponding interventions.

### Table IV. Acylcarnitines in Discriminating Patients with and without Recovery of Left Ventricular Systolic Function After Adjusting for Age (Based on the GEE Model)

| Acylcarnitines | \(\beta\) | 95% CI          | \(P\) value |
|---------------|--------|----------------|------------|
| C18:1         |        |                |            |
| Baseline (reference) |      |                |            |
| 6 months      | -0.007 | -0.051−0.015  | 0.677      |
| 12 months     | -0.018 | -0.042−0.027  | 0.291      |
| C16           |        |                |            |
| Baseline (reference) |      |                |            |
| 6 months      | -0.001 | -0.026−0.024  | 0.921      |
| 12 months     | -0.001 | -0.026−0.024  | 0.958      |
| C16:1         |        |                |            |
| Baseline (reference) |      |                |            |
| 6 months      | -0.004 | -0.011−0.007  | 0.426      |
| 12 months     | -0.004 | -0.013−0.006  | 0.647      |
| C5            |        |                |            |
| Baseline (reference) |      |                |            |
| 6 months      | 0.009  | -0.010−0.027  | 0.349      |
| 12 months     | 0.018  | 0.002−0.038   | 0.033      |
| C5-OH         |        |                |            |
| Baseline (reference) |      |                |            |
| 6 months      | -0.002 | -0.006−0.002  | 0.297      |
| 12 months     | 0.002  | -0.003−0.007  | 0.453      |
| C4            |        |                |            |
| Baseline (reference) |      |                |            |
| 6 months      | 0.028  | -0.011−0.067  | 0.154      |
| 12 months     | 0.081  | 0.027−0.136   | 0.004      |
| C4:1-DC       |        |                |            |
| Baseline (reference) |      |                |            |
| 6 months      | 0.009  | -0.003−0.031  | 0.109      |
| 12 months     | 0.01   | 0.004−0.043   | 0.017      |
| C3            |        |                |            |
| Baseline (reference) |      |                |            |
| 6 months      | 0.067  | 0.019−0.114   | 0.006      |
| 12 months     | 0.097  | 0.035−0.159   | 0.002      |
| C2            |        |                |            |
| Baseline (reference) |      |                |            |
| 6 months      | 0.805  | 0.023−1.678   | 0.044      |
| 12 months     | 1.704  | 0.694−2.731   | 0.001      |

Generalized estimating equations (GEEs) are used for age adjustment. C2 indicates acetylcarnitine; C3, propionylcarnitine; C4, butyrylcarnitine; C4:1, butyrylcarnitine; C4:1-DC, hexanoylcarnitine; C5, valerylcarnitine; C5-OH, methylmalonylcarnitine; C16, hexadecanoylcarnitine; C16:1, hexadecenoylcarnitine; and C18:1, octadecanoylcarnitine.

### Conclusions

Changes in short-chain acylcarnitine profiles are independently associated with the improvement in cardiac systolic function after acute decompensation. Although the patients in the NIMP group did not experience events as evidence of clinical worsening, the increase in plasma acylcarnitine concentrations suggested that their metabolism was worsening. Specifically, patients’ HF status is significantly correlated with short-chain acylcarnitine metabolism, which is very likely related to the profile of the gut microbiota at different disease statuses. Whether ameliorating short-chain acylcarnitine metabolism can improve clinical outcomes warrants further investigation.

### Disclosure

**Conflicts of interest:** None declared.

### References

1. Zhou B, Tian R. Mitochondrial dysfunction in pathophysiology of heart failure. J Clin Invest 2018; 128: 3716-26.
2. Bu J, Wang Z. Cross-Talk between gut microbiota and heart via the routes of metabolite and immunity. Gastroenterol Res Pract 2018; 2018: 6458094.
3. Albert CL, Tang WHW. Metabolic biomarkers in heart failure. Heart Fail Clin 2018; 14: 109-18.
4. Cheng ML, Wang CH, Shiao MS, et al. Metabolic disturbances identified in plasma are associated with outcomes in patients with heart failure: diagnostic and prognostic value of metabolomics. J Am Coll Cardiol 2015; 65: 1509-20.
5. Fu H, Zhu K, Zhou D, Guan Y, Li W, Xu S. Identification and validation of plasma metabolomics reveal potential biomarkers for coronary heart disease. Int Heart J 2019; 60: 1387-97.
6. Wang CH, Cheng ML, Liu MH, Fu TC. Amino acid-based metabolic profile provides functional assessment and prognostic value for heart failure patients. Dis Markers 2019; 2019: 8632726.
7. Ahmad T, Kelly JP, McGarrah RW, et al. Prognostic implications of long-chain acylcarnitines in heart failure and reversibility with mechanical circulatory support. J Am Coll Cardiol 2016; 67: 291-9.
8. Schooneman MG, Vaz FM, Houten SM, Soeters MR. Acylcarnitines: reflecting or inflicting insulin resistance? Diabetes 2013; 62: 1-8.
9. Liu JJ, Ghosh S, Kovalik JP, et al. Profiling of plasma metabolites suggests altered mitochondrial fuel usage and remodeling of sphingolipid metabolism in individuals with type 2 diabetes and kidney disease. Kidney Int Rep 2017; 2: 470-80.
10. Xu J, Verbrugghe A, Lourencos M, et al. The response of canine faecal microbiota to increased dietary protein is influenced by body condition. BMC Vet Res 2017; 13: 374.
11. Ridaaru VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013; 341: 1241214.
12. Hunt SA, Abraham WT, Chin MH, et al. 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): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. Circulation 2005; 112: e154-235.
13. Wanders RJ, Komen J, Kemp S. Fatty acid omega-oxidation as a rescue pathway for fatty acid oxidation disorders in humans. FEBS J 2011; 278: 182-94.
14. Schwiertz A, Taras D, Schafer K, et al. Microbiota and SCFA in lean and overweight healthy subjects Obesity (Silver Spring). 2010; 18: 190-5.
15. Wang CH, Cheng ML, Liu MH, et al. Increased p-cresyl sulfate level is independently associated with poor outcomes in patients with heart failure. Heart Vessels 2016; 31: 1100-8.
16. Verbrugghe A, Janssens GP, Meininger E, et al. Intestinal fermentation modulates postprandial acylcarnitine profile and nitrogen metabolism in a true carnivore: the domestic cat (Felis catus). Br J Nutr 2010; 104: 972-9.
17. Rochus K, Janssens GP, Van de, Velde H, et al. Highly viscous guar gum shifts dietary amino acids from metabolic use to fermentation substrate in domestic cats. Br J Nutr 2013; 109: 1022-30.
18. McCain CS, Knotts TA, Adams SH. Acylcarnitines—old actors auditioning for new roles in metabolic physiology. Nat Rev Endocrinol 2015; 11: 617-25.