

# Associations between acylcarnitine to free carnitine ratio and adverse prognosis in heart failure patients with reduced or preserved ejection fraction

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## Abstract

**Aims** The failing heart is accompanied by disturbed energy metabolism with mitochondrial dysfunction. Carnitine transports fatty acids into mitochondria for  $\beta$ -oxidation. Decreased myocardial carnitine levels accompanied by increased plasma carnitine levels in heart failure (HF) have been reported. The plasma acylcarnitine to free carnitine ratio (AC/FC) is recognized as a marker of carnitine deficiency. We aimed to investigate the impact of the AC/FC on HF prognosis, taking into consideration differences between HF patients with preserved ejection fraction (HFpEF) and those with reduced ejection fraction (HFrEF).

**Methods and results** Consecutive 168 HF patients were divided into three groups based on their AC/FC: first to third tertiles ( $n = 56$ , respectively). We followed up all patients for cardiac events including cardiac death and/or worsening HF. During the follow-up period (1004 days), there were 23 cardiac deaths and 28 worsening HF. In the Kaplan–Meier analysis, the cardiac event rate of the third group was highest among the three groups ( $P = 0.022$ ). In the Cox proportional hazard analysis, AC/FC was a predictor of cardiac events ( $P = 0.007$ ). When HFpEF ( $n = 79$ ) and HFrEF ( $n = 89$ ) were analysed separately, the cardiac event rate of the third group was highest with regard to HFpEF ( $P = 0.008$ ), but not HFrEF ( $P = 0.321$ ). In the Cox proportional hazard analysis, AC/FC was a predictor of cardiac events with regard to HFpEF ( $P = 0.031$ ), but not HFrEF ( $P = 0.095$ ). Therefore, the impact of the AC/FC on cardiac events was different between HFpEF and HFrEF ( $P = 0.042$  for interaction).

**Conclusions** The AC/FC can identify high risk HF patients, especially in HFpEF.

**Keywords** Heart failure; Carnitine deficiency; Acylcarnitine; Preserved ejection fraction; Prognosis

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## Background

Heart failure (HF) is the leading cause of death in many countries and is accompanied by disturbed energy metabolism with mitochondrial dysfunction.<sup>1</sup> Carnitine transports long-chain fatty acids into the mitochondria for  $\beta$ -oxidation and protects the myocardium against ischaemic injury, the occurrence of angina, diastolic dysfunction, and HF.<sup>2,3</sup> Carnitine deficiency results in impaired mitochondrial  $\beta$ -oxidation, decreased glucose oxidation, and accelerated cellular apoptosis.<sup>4,5</sup> In HF patients, decreased myocardial carnitine levels accompanied by increased plasma carnitine levels, potentially reflecting increased

leakage of carnitine through damaged cardiomyocyte membranes, have been reported.<sup>1,6</sup> The plasma acylcarnitine to free carnitine ratio (AC/FC) is recognized as a marker of carnitine deficiency in the myocardium.<sup>7,8</sup>

## Aims

The aim of the present study was to investigate the impact of the AC/FC on HF prognosis, taking into consideration the differences between HF patients with preserved ejection fraction (HFpEF) and those with reduced ejection fraction (HFrEF).

**Table 1** Comparisons of clinical features among acylcarnitine to free carnitine ratio classification ( $N = 168$ )

	AC/FC first tertile (AC/FC < 0.19, $n = 56$ )	AC/FC second tertile ( $0.19 \leq$ AC/FC < 0.27, $n = 56$ )	AC/FC third tertile (AC/FC $\geq$ 0.27, $n = 56$ )	<i>P</i> -value
AC/FC ratio	0.15 $\pm$ 0.02	0.23 $\pm$ 0.03**	0.38 $\pm$ 0.10**††	<0.001
Total carnitine ( $\mu$ mol/L)	67.6 $\pm$ 16.7	64.8 $\pm$ 15.1	71.1 $\pm$ 20.8	0.172
Acylcarnitine ( $\mu$ mol/L)	9.1 $\pm$ 2.7	11.9 $\pm$ 3.1**	19.3 $\pm$ 7.3**††	<0.001
Free carnitine ( $\mu$ mol/L)	58.5 $\pm$ 14.2	52.8 $\pm$ 12.2	51.7 $\pm$ 14.9*	0.021
Age (years)	59.9 $\pm$ 13.1	62.6 $\pm$ 14.0	62.7 $\pm$ 14.4	0.493
Male gender ( $n$ , %)	42 (75.0)	43 (76.8)	36 (64.3)	0.281
Body mass index (kg/cm <sup>2</sup> )	24.5 $\pm$ 4.5	24.1 $\pm$ 3.5	25.2 $\pm$ 4.8	0.405
NYHA class III/IV ( $n$ , %)	5 (8.9)	3 (5.4)	6 (10.7)	0.580
Ischaemic aetiology ( $n$ , %)	20 (35.7)	23 (41.1)	22 (39.3)	0.839
Reduced LVEF ( $n$ , %)	29 (51.8)	28 (50.0)	32 (57.1)	0.733
LVEF (%)	46.9 $\pm$ 16.1	48.1 $\pm$ 14.1	46.7 $\pm$ 15.7	0.893
Co-morbidity				
Hypertension ( $n$ , %)	47 (83.9)	46 (82.1)	46 (82.1)	0.959
Diabetes ( $n$ , %)	19 (33.9)	25 (44.6)	28 (50.0)	0.216
Dyslipidemia ( $n$ , %)	47 (83.9)	46 (82.1)	50 (89.3)	0.543
Atrial fibrillation ( $n$ , %)	17 (30.4)	18 (32.1)	19 (33.9)	0.921
CKD ( $n$ , %)	19 (33.9)	21 (37.5)	36 (64.3)	0.002
Anaemia ( $n$ , %)	26 (46.4)	30 (53.6)	25 (44.6)	0.606
Medications				
RAS inhibitors ( $n$ , %)	47 (83.9)	45 (80.4)	47 (83.9)	0.846
$\beta$ -blockers ( $n$ , %)	49 (87.5)	45 (80.4)	50 (89.3)	0.360
Diuretics ( $n$ , %)	31 (55.4)	25 (44.6)	34 (60.7)	0.221
Inotropic agents ( $n$ , %)	6 (10.7)	7 (12.5)	9 (16.1)	0.693
Laboratory data				
Log BNP	2.1 $\pm$ 0.6	2.0 $\pm$ 0.7	2.5 $\pm$ 0.8 **††	0.003
Log CRP	-0.8 $\pm$ 0.6	-0.9 $\pm$ 0.6	-0.7 $\pm$ 0.8	0.218
Total protein (g/dL)	7.2 $\pm$ 0.6	7.3 $\pm$ 0.7	7.1 $\pm$ 0.7	0.401
Sodium (mEq/L)	139.2 $\pm$ 3.6	139.6 $\pm$ 3.6	139.9 $\pm$ 3.5	0.607

AC/FC, acylcarnitine/free carnitine; BNP, B-type natriuretic peptide; CKD, chronic kidney disease; CRP, C-reactive protein; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; RAS, renin-angiotensin-aldosterone system.

\* $P < 0.05$ ; \*\* $P < 0.01$  vs. first tertile, †† $P < 0.01$  vs. second tertile.

## Methods

### Subjects and study protocol

Consecutive 168 patients admitted for the treatment of decompensated HF at Fukushima Medical University between 2010 and 2011 were divided into three groups based on their AC/FC: first (AC/FC < 0.19,  $n = 56$ ); second ( $0.19 \leq$  AC/FC < 0.27,  $n = 56$ ); and third tertile (AC/FC  $\geq$  0.27,  $n = 56$ ). Diagnosis of decompensated HF was made by several cardiologists based on the Framingham criteria.<sup>9</sup> Patients with acute coronary syndrome ( $n = 8$ ) and on dialysis ( $n = 7$ ) were excluded. Plasma carnitine profiles (acylcarnitine, free carnitine, and total carnitine) were determined at discharge by an enzymatic cycling method with carnitine dehydrogenase (Kinos Co., Tokyo, Japan). HFpEF was defined as left ventricular ejection fraction (LVEF)  $\geq$ 50%, whereas HFrEF was defined as LVEF <50%. We compared clinical features among the three groups. The patients were followed up for cardiac events, which were composite end points of cardiac death and/or worsening HF, until 2016. The endpoint classification committee, comprising two

experienced cardiologists who were not study investigators, reviewed the data and, if any problems were encountered, asked the primary physician to confirm the endpoint. We could follow up all the patients. Informed consent was obtained from each subject, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a prior approval by the institution's human research committee.

### Statistical analysis

Normally distributed data are presented as mean  $\pm$  SD, and non-normally distributed data are log transformed. A  $\chi^2$  test was used for comparisons of categorical variables. Analysis of variance followed by Tukey's post hoc test was used for comparisons of continuous variables. The Kaplan–Meier method was used for presenting the cardiac event rates, and a log-rank test was used for initial comparisons. Cox proportional hazard analysis was used to analyse predictors of cardiac events. A value of  $P < 0.05$  was considered significant for all comparisons.

All analyses were performed using a statistical software package (SPSS ver.21.0, IBM, Armonk, NY, USA).

## Results

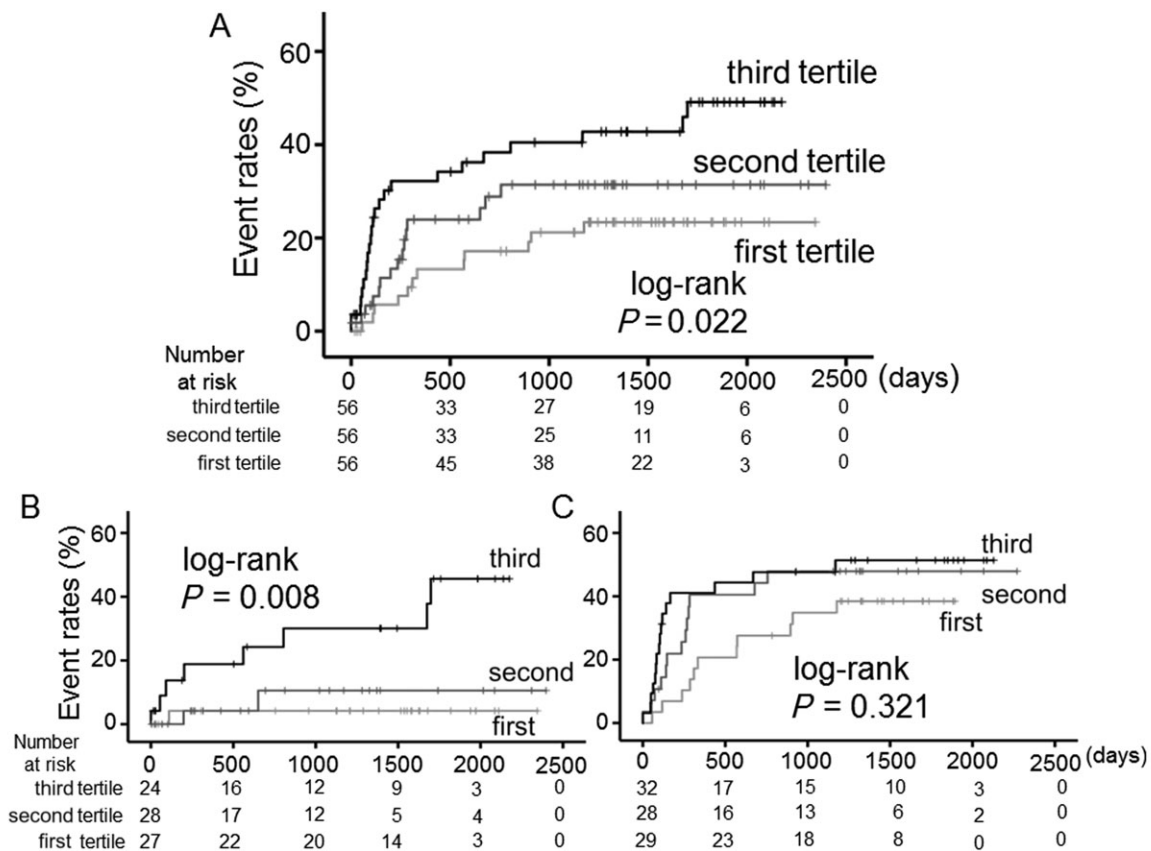
The AC/FC in all patients was  $0.25 \pm 0.11$  (range 0.10–0.68). There was no significant difference in AC/FC between the HFpEF and HFREF groups ( $0.24 \pm 0.09$  vs.  $0.26 \pm 0.13$ ,  $P = 0.194$ ). Median B-type natriuretic peptide (BNP) was higher in the HFREF than in the HFpEF (409.9 vs. 186.3 pg/mL,  $P < 0.01$ ). As shown in Table 1, the third group had highest levels of acylcarnitine and BNP, lower levels of free carnitine, and the highest prevalence of chronic kidney disease (CKD). In contrast, age, gender, body mass index, New York Heart Association class III or IV, ischaemic aetiology, LVEF, other co-morbidities except CKD, medications, C-reactive protein, total protein, and sodium did not differ among the three groups. In the multiple regression analysis to associate the AC/FC, among considerable clinical variables

such as age, sex, body mass index, hypertension, diabetes, dyslipidemia, atrial fibrillation, CKD, anaemia, BNP, C-reactive protein, and LVEF, CKD and BNP were independent predictors of the AC/FC (CKD,  $\beta = 0.215$ ,  $P = 0.021$ ; BNP,  $\beta = 0.195$ ,  $P = 0.036$ ).

During the follow-up period (mean 1004 days), there were 23 cardiac deaths and 28 cases of worsening HF. As shown in Figure 1A, the cardiac event rate of the third group was the highest among the three groups ( $P = 0.022$ ). In the Cox proportional hazard analysis, the AC/FC was a predictor of cardiac events (unadjusted HR 1.029, 95% CI 1.008–1.051,  $P = 0.007$ ; adjusted HR for CKD and BNP, 1.021, 95% CI 1.001–1.042,  $P = 0.048$ ).

When HFpEF ( $n = 79$ ) and HFREF ( $n = 89$ ) were analysed separately (Figure 1B,C), the Kaplan–Meier analysis revealed that the cardiac event rate of the third group was the highest among the three groups in HFpEF ( $P = 0.008$ ), but not in HFREF ( $P = 0.321$ ). In the Cox proportional hazard analysis, the AC/FC was a predictor of cardiac events in HFpEF (HR 1.056, 95% CI 1.005–1.110,  $P = 0.031$ ), but not in HFREF (HR 1.020, 95% CI 0.997–1.043,  $P = 0.095$ ). Therefore, impact of

**Figure 1** Comparison of cardiac event rates according to the acylcarnitine to free carnitine ratio. Kaplan–Meier analysis for cardiac event rates. (A) All heart failure patients ( $n = 168$ ); (B) heart failure with preserved ejection fraction patients ( $n = 79$ ); and (C) heart failure with reduced ejection fraction patients ( $n = 89$ ).



the AC/FC on cardiac events was different between HFpEF and HFrEF ( $P = 0.042$  for interaction).

## Discussion

In the present study, we firstly demonstrated that the AC/FC predicts adverse prognosis of HF, especially in HFpEF.

Carnitine insufficiency in the myocardium may result from a leakage of carnitine from the myocardium into the blood through damaged cardiomyocyte membranes secondary to persistent inflammation and a defect in myocardial uptake via specific carrier protein.<sup>10</sup> It has been reported that plasma levels of long-chain acylcarnitines predict cardiovascular mortality in dialysis patients,<sup>11</sup> and palmitoyl-carnitine is associated with adverse events in HF patients, such as all-cause mortality and heart transplantation.<sup>1</sup>

Regarding HFpEF, recent metabolomics have revealed that the serum levels of long-chain acylcarnitines are higher in HFpEF than those in HFrEF and control subjects,<sup>12</sup> suggesting that an increase in acylcarnitine may imply inefficient  $\beta$ -oxidation, especially in HFpEF patients.<sup>12,13</sup> On the other hand, carnitine supplementation attenuates cardiac fibrosis in HFpEF model rats<sup>14</sup> and improves echocardiographic diastolic parameters and symptoms in HFpEF patients.<sup>15</sup> Thus, the impact of the AC/FC on the prognosis of HFpEF may be greater than that of HFrEF.

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## Study limitations

There are several limitations in the present study. First, it is an observational study of a single institution, so the number of subjects was relatively small. Second, assessment was carried out using only variables on hospitalization, without consideration for changes after treatment. Third, we could not distinguish between long-chain acylcarnitines, short-chain acylcarnitines, and carnitine precursors.<sup>1,11</sup> Thus, the results of the present study should be viewed as preliminary, and further studies with larger populations are needed.

## Conclusions

The AC/FC, as a marker of cardiac carnitine deficiency, can identify high-risk HF patients, especially in those with HFpEF.

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## Conflict of interest

None declared.

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