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Increased serum 2-oxoglutarate associated with high myocardial energy expenditure and poor prognosis in chronic heart failure patients

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ABSTRACT

Myocardial energy expenditure (MEE) and 2-oxoglutarate are elevated in chronic heart failure (CHF) patients compared with healthy controls. To explore whether 2-oxoglutarate could reflect the levels of MEE and predict the prognosis of CHF, 219 CHF patients and 66 healthy controls were enrolled. 2-Oxoglutarate was assayed with Liquid Chromatography–Mass Spectrometry/Mass Spectrometry (LC/MS/MS). CHF patients were divided into 4 groups according to interquartile range of MEE and followed for death or recurrent hospital admission due to CHF for the mean follow-up time 6.64 ± 0.16 months. 2-Oxoglutarate was increased in CHF patients compared with controls ($P < 0.01$) and correlated with estimated glomerular filtration rate ($r = 0.142$, $P = 0.036$), age ($r = -0.269$, $P < 0.01$) and MEE levels ($r = 0.307$, $P < 0.01$) in a multiple linear correlation analysis in CHF patients. Furthermore, 2-oxoglutarate (OR = 3.470, 95% CI = 1.557 to 7.730, $P = 0.002$), N-terminal pro-B-type natriuretic peptide (OR = 4.013, 95% CI = 1.553 to 10.365, $P = 0.004$), age (OR = 1.611, 95% CI = 1.136 to 2.283, $P = 0.007$) and left ventricular ejection fraction (OR = 7.272, 95% CI = 3.110 to 17.000, $P < 0.001$) were independently associated with MEE on multiple logistic regression analysis. Kaplan–Meier event curves showed that high 2-oxoglutarate levels were associated with adverse outcomes (Log Rank, $Chi^2 = 4.026$, $P = 0.045$). This study showed that serum 2-oxoglutarate is associated with MEE levels, which can be used as potential biomarkers for MEE, and it can reflect the clinical severity and short-term outcome of CHF.

1. Introduction

Heart failure (HF) is a complex syndrome characterized by mechanical dysfunction of the myocardium, abnormal metabolism and excessive, continuous neurohormonal activation [1]. Several myocardial metabolic abnormalities occur in chronic heart failure (CHF), including altered substrate utilization and decreased high energy phosphate content [2]. Despite recent great progress, the knowledge of metabolic abnormalities in CHF is still limited. Whether and how they alter according to etiology and the severity of CHF remain poorly understood.

Recently, studies showed that there were significant metabolic differences in serum [3], urine [4] and exhaled breath [5–7] samples between CHF patients and the healthy subjects. These findings suggested that some metabolites may associate with CHF and can reflect the state of cardiac energy metabolism. Of these, 2-oxoglutarate [3], a major intermediate metabolite of the tricarboxylic acid cycle, is a promising one due to its important roles in regulating myocardial energy metabolism.

Myocardial energy expenditure (MEE) is an important indicator reflecting myocardial energy metabolism. Different ways to estimate MEE in failing heart were provided in recent years, including positron emission tomography [8,9], nuclear magnetic resonance [10,11] and Doppler echocardiography [12,13]. It has been reported that elevated MEE is associated with decreased left ventricular ejection fraction (LVEF) and can be used as an independent predictor of cardiovascular mortality [12]. Recently, our preliminary results showed that in patients with CHF, elevation of MEE was associated with significant changes in serum metabolomic profiles by ^{1}H-NMR-based metabolic analysis and

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suggested that these compounds could be used as potential serum biomarkers to explore myocardial energy mechanism in CHF patients [14].

The purpose of this study was to evaluate the relationship between 2-oxoglutarate and MEE in CHF patients. Our goals were: 1) to test the hypotheses that whether the serum concentration of 2-oxoglutarate is associated with MEE levels and can reflect the severity of CHF; and 2) to assess the predictive value of 2-oxoglutarate for the prognosis of CHF.

2. Materials and methods

2.1. Study population

219 patients with CHF were consecutively enrolled after obtaining informed consent in 2 participating centers (Nanfang Hospital and Guangzhou First People's Hospital, China). Patients with acute coronary syndrome, diabetes mellitus and other metabolic diseases, estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2, sepsis, malignancy, autoimmune disease or severe hepatic disease were excluded. The underlying causes of CHF were classified as hypertension, ischemic heart disease, valvular heart disease and dilated cardiomyopathy on the basis of the patients' history, cardiac morphology and coronary angiography. Consensus of 2 experienced clinical cardiologists was required for the classification of New York Heart Association (NYHA) functional classes. The severity of CHF was evaluated by NYHA classification and MEE. Follow-up events, including all-cause mortality and recurrent hospital admission due to CHF, were ascertained via hospital database, medical records and contact with patients and their family members. Sixty-six age-matched control subjects with normal cardiac function were recruited from the health management center and outpatient department in Guangzhou First People's Hospital. The study complied with the Declaration of Helsinki and was approved by the institutional ethics committee of Nanfang Hospital and Guangzhou First People's Hospital, China. All subjects were provided with a hard copy of informed consent before recruitment.

2.2. Biochemistry detection

Antecubital venous blood was drawn into pyrogen-free tubes with or without EDTA as anticoagulant respectively on the same day of MEE measurement. After centrifugation at 3000 *g* at 4 °C for 10 min, all serum or platelet-poor plasma samples were stored at −80 °C. Serum 2-oxoglutarate was assayed with Agilent 6460 LC/MS/MS (USA). Chromatographic separations of prepared samples were achieved using an Eclipse Plus C 18 column (3.5 μm, 2.1 mm × 100 mm). The mass spectrometer was operated in the positive ion ESI mode with MRM for the analytes. The following optimized ESI parameters were applied: drying gas flow rate, 10 L/min; drying gas temperature, 350 °C; nebulizing gas pressure 30 psi; capillary voltage 4000 V; and fragmentor voltage 50 V. Free thyroxine, free triiodothyronine and thyroid stimulating hormone (TSH) were measured with a direct chemiluminescence immunoassay (Siemens Healthcare Diagnostics Inc., USA). N-terminal pro-B-type natriuretic peptide (NT-proBNP) was analyzed with the Elecsys NT-proBNP immunoassay (Roche Diagnostics). Estimated glomerular filtration rate (eGFR) was calculated based on MDRD formula. All subjects underwent oral glucose tolerance test (OGTT) with 75 g of oral anhydrous glucose as described previously [15].

2.3. MEE measurement

MEE was measured with a Siemens Sequoia 512 Encompass ultrasound system, using the method described previously [12,16]. Systolic blood pressure (SBP), left ventricular internal diameter at systole (LVIDs), left ventricular posterior wall end-systolic thickness (PWTs), left ventricular ejection time (LVET), LVEF and left ventricular stroke volume (LVSV) were measured. Finally, MEE was calculated as [12,16]: MEE (cal/min) = left ventricular circumferential end-systolic wall stress (cESS) × LVET × LVSV × heart rate × 4.2×10^{-4}.

$$\text{cESS} = \frac{\text{SBP} \times (\text{LVID}_s/2)^2 \times \left\{1 + \frac{(\text{LVID}_s/2 + \text{PWT}_s)^2}{(\text{LVID}_s/2 + \text{PWT}_s/2)^2}\right\}}{(\text{LVID}_s/2 + \text{PWT}_s)^2 - (\text{LVID}_s/2)^2}$$

2.4. Statistical analysis

The continuous normal variables were expressed as mean ± SD, and medians were presented with the 25th to 75th percentiles for skewed continuous variables. CHF patients were divided into quartiles on the basis of the levels of MEE. Categorical variables were compared with Pearson's χ^2 test. Differences between mean or median values for continuous variables were evaluated with Kruskall–Wallis test or 1-way ANOVA with S–N–K analysis, as appropriate. Pearson correlation for the normal and logarithmically transformed skewed variables was used to assess associations between study parameters. Multicollinearity (strong correlations among independent variables) was examined by collinearity diagnostic statistics. Variance inflation factor (VIF) values > 4.0 or tolerance < 0.25 may indicate a concern for multicollinearity in multivariate regression models [17]. The concentrations of 2-oxoglutarate were skewed and thus, were logarithmically transformed (Log 2-oxoglutarate) for calculation of associations with biochemical parameters (fasting blood glucose, postprandial blood glucose, hemoglobin A1c, alanine aminotransferase, aspartate aminotransferase) and other clinical parameters (NT-proBNP, eGFR, NYHA classification, LVEF, age, sex and MEE) in Pearson correlation and multiple linear regression analysis. Multivariable logistic regression analysis was used to investigate associations between MEE levels (dependent variable) and other parameters (independent variables) including fasting blood glucose, postprandial blood glucose, hemoglobin A1c, age, sex, creatinine, eGFR, 2-oxoglutarate, NYHA classification, free triiodothyronine, free thyroxine, thyroid-stimulating hormone, body mass index, LVEF and NT-proBNP. Events of recurrent hospital admission due to CHF or death in 8 months were investigated with Kaplan–Meier analysis by Log rank test. *P* values were two-sided and considered significant when <0.05. Statistical analyses were carried out using the software package SPSS version 17.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Baseline characteristics

Serum 2-oxoglutarate was higher in CHF patients compared with controls (median, 13.02 μg/mL [IQR 6.14 to 26.89] versus 10.58 μg/mL [IQR 7.69 to 13.42], $P < 0.01$). Patients with CHF were divided into 4 groups according to the interquartile range of MEE. Those with a MEE < 59.51 cal/min were included in the MEE 1 group; 59.51 cal/min ≤ MEE < 99.94 cal/min in the MEE 2 group; 99.94 cal/min ≤ MEE < 184.18 cal/min in the MEE 3 group; and MEE ≥ 184.18 cal/min in the MEE 4 group. Basic characteristics of 4 groups were presented in Table 1. Clinical parameters in 4 groups were similar except for higher NT-proBNP, left ventricular mass index (LVMI), Tei index, NYHA classes, as well as lower free triiodothyronine and high-density lipoprotein in the MEE 4 group compared with the MEE 1 group. Patients belonging to NYHA classes III and IV were with significantly higher MEE values than class I and II patients. NYHA classes were higher with greater MEE (Fig. 1).

Serum 2-oxoglutarate levels were lower in the MEE 1 group than those in the MEE 3 and 4 groups (both $P < 0.01$). Compared with the MEE 3 and 4 groups, the similar results were found in the MEE 2 group (both $P < 0.05$). However, there were no significant differences

Table 1
Baseline characteristics between 4 groups of patients with CHF according to MEE.

Characteristics	Control (n = 66)	MEE 1 (n = 55)	MEE 2 (n = 55)	MEE 3 (n = 54)	MEE4 (n = 55)	*P* value
Age, years	60.64 ± 10.62	64.02 ± 12.39	61.87 ± 9.98	64.93 ± 10.03	62.95 ± 12.30	0.239
Female, n (%)	29 (43.94%)	29 (52.73%)	27 (49.09%)	19 (35.19%)	17 (30.91%)	0.108
Causes of CHF						
Hypertension, n (%)	–	28 (50.91%)	29 (52.73%)	23 (42.59%)	22 (40.00%)	0.466
Ischemic heart disease, n (%)	–	25 (45.45%)	25 (45.45%)	28 (51.85%)	29 (52.73%)	0.793
Valvular heart diseases, n (%)	–	9 (16.36%)	6 (10.91%)	6 (11.11%)	6 (10.91%)	0.775
Dilated cardiomyopathy, n (%)	–	2 (3.64%)	3 (5.45%)	8 (14.81%)	7 (12.73%)	0.119
Medication use						
ACE inhibitors or ARBs, n (%)	–	26 (47.27%)	26 (47.27%)	22 (40.74%)	40 (72.73%)	<0.010
Aldosterone antagonists, n (%)	–	19 (34.55%)	20 (36.36%)	31 (57.41%)	43 (78.18%)	<0.010
Aspirin, n (%)	–	24 (43.64%)	27 (49.09%)	31 (57.41%)	31 (56.36%)	0.429
Beta–blockers, n (%)	–	23 (41.82%)	22 (40.00%)	26 (48.15%)	32 (58.18%)	0.216
Diuretics, n (%)	–	32 (58.18%)	27 (49.09%)	35 (64.81%)	45 (81.82%)	<0.010
Digitalis, n (%)	–	12 (21.82%)	10 (18.18%)	15 (27.78%)	21 (38.18%)	0.091
Statin, n (%)	–	24 (43.64%)	22 (40.00%)	27 (50.00%)	26 (47.27%)	0.890
Clinical measures						
NYHA class I, n (%)	66 (100%)	16 (29.09%)	22 (40.00%)	4 (7.41%)	0	<0.010
NYHA class II, n (%)	0	14 (25.45%)	10 (18.18%)	18 (33.33%)	7 (12.73%)	0.033
NYHA class III, n (%)	0	13 (23.64%)	14 (25.45%)	17 (31.48%)	26 (47.27%)	0.033
NYHA class IV, n (%)	0	12 (21.82%)	9 (16.36%)	15 (27.78%)	22 (40.00%)	0.071
LVEF < 45%, n (%)	0	4 (7.27%)	9 (16.36%)	22 (40.74%)	38 (69.09%)	<0.010
LVEF ≥ 45%, n (%)	66 (100%)	51 (92.73%)	46 (83.64%)	32 (59.26%)	17 (30.91%)	<0.010
Body mass index, kg/m^2	23.17 ± 2.86	22.08 ± 2.71	23.49 ± 3.12	23.21 ± 2.77	22.88 ± 2.89	0.797
Creatinine, μmol/L	101.94 ± 17.22	107.31 ± 43.27	107.49 ± 53.17	117.04 ± 39.49	124.92 ± 63.55	0.053
eGFR, mL/min/1.73 m^2	69.04 ± 16.62	61.47 ± 22.54	65.06 ± 24.06	58.77 ± 19.89	59.89 ± 23.08	0.053
Free triiodothyronine, pmol/L	–	3.73 ± 0.97	3.86 ± 1.27	3.50 ± 0.99	3.15 ± 0.92†	<0.010
Free thyroxine, pmol/L	–	15.67 ± 3.89	15.82 ± 3.76	15.81 ± 3.53	15.28 ± 3.64	0.860
TSH, uIU/L	–	2.21 ± 1.53	1.93 ± 1.69	2.18 ± 1.86	2.50 ± 2.63	0.506
Fasting blood glucose, mmol/L	5.20 ± 0.65	5.23 ± 1.09	5.39 ± 1.27	5.29 ± 1.14	5.31 ± 1.57	0.923
2 h PBG, mmol/L	6.87 ± 0.80	7.66 ± 2.61*	7.84 ± 2.34*	8.18 ± 2.60*	8.06 ± 2.66*	0.013
Hemoglobin A1c, %	5.80 (5.50–6.00)	5.80 (5.50–6.20)	5.70 (5.40–6.20)	5.95 (5.68–6.23)	5.90 (5.60–6.40)	0.056
Total cholesterol, mmol/L	4.99 ± 0.38	4.17 ± 0.94*	4.26 ± 1.16*	4.07 ± 1.06*	4.43 ± 1.16*	<0.010
LDL, mmol/L	2.74 ± 0.51	2.43 ± 0.63	2.48 ± 0.92	2.55 ± 0.83	2.82 ± 0.91	0.059
Triglyceride, mmol/L	1.33 ± 0.32	1.24 ± 0.67	1.61 ± 1.19	1.27 ± 0.94	1.30 ± 0.56	0.095
HDL, mmol/L	1.05 ± 0.18	1.12 ± 0.46	1.02 ± 0.29	0.94 ± 0.25$^{*,\#}$	0.96 ± 0.27$^{*,\#}$	0.010
Aspartate aminotransferase, U/L	26 (20–38)	26 (19–37)	26 (19–38)	26 (18–43)	27 (21–37)	0.298
Alanine aminotransferase, U/L	19 (12–28)	16 (11–23)	17 (10–25)	20 (12–36)	19 (12–31)	0.055
Hematoglobin, g/L	122.62 ± 6.79	124.72 ± 16.97	123.20 ± 18.29	128.67 ± 21.38	126.85 ± 20.49	0.122
LVMI, g/m^2	79 (70–98)	81 (71–106)	97 (80–135)$^{*,\#}$	123 (103–152)$^{*,\#,\dagger}$	165 (132–205)$^{*,\#,\dagger}$	<0.010
Tei index	0.49 (0.40–0.56)	0.63 (0.58–0.84)*	0.60 (0.53–0.75)*	0.83 (0.67–0.94)*,†	0.92 (0.72–1.14)*,†	<0.010
LVEF, %	58 (55–61)	57 (52–61)	58 (52–62)	47 (35–56)$^{*,\#}$	38 (29–51)$^{*,\#}$	<0.010
MEE, cal/min	46 (36–57)	41 (34–52)	80 (69–92)$^{*,\#}$	128 (112–153)$^{*,\#,\dagger}$	276 (225–397)$^{*,\#,\dagger}$	<0.010
NT-proBNP, pg/mL	78 (57–106)	1393 (187–3946)*	1212 (175–4110)*	4603 (1834–10033)$^{*,\#,\dagger}$	7662 (4128–21671)$^{*,\#,\dagger}$	<0.010
2-Oxoglutarate, μg/mL	10.58 (7.69–13.42)	8.92 (4.81–16.46)	11.43 (5.65–18.42)	16.75 (9.44–28.56)$^{*,\#,\dagger}$	26.25 (12.42–30.45)$^{*,\#,\dagger}$	<0.010

Data are mean ± SD, or median (interquartile range). Abbreviations: CHF, chronic heart failure; MEE, myocardial energy expenditure; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; TSH, thyroid-stimulating hormone; PBG, postprandial blood glucose; LDL, low density lipoprotein; HDL, high density lipoprotein; LVMI, left ventricular mass index; NT-proBNP, N-terminal pro-B-type natriuretic peptide.
* Vs control group, $P < 0.05$.
$^{\#}$ Vs MEE 1 group, $P < 0.05$.
† Vs MEE 2 group, $P < 0.05$.

between the MEE 1 and 2 groups, neither the MEE 3 and 4 groups (Table 1).

3.2. Associations between clinical factors and 2-oxoglutarate

Logarithmically transformed 2-oxoglutarate (Log 2-oxoglutarate) levels were used for calculation of associations with biochemical parameters and other clinical parameters in Pearson correlation and multiple linear regression analysis. Pearson correlation showed that Log 2-oxoglutarate was significantly correlated with Log NT-proBNP ($r = 0.283$, $P < 0.01$), eGFR ($r = 0.142$, $P = 0.036$), NYHA classification ($r = 0.284$, $P < 0.01$) and Log MEE ($r = 0.307$, $P < 0.01$), inversely correlated with age ($r = -0.269$, $P < 0.01$) and Log LVEF ($r = -0.192$, $P < 0.01$). Multiple linear regression found that concentrations of 2-oxoglutarate were significantly correlated with age ($B = -1.035$, $P = 0.001$), eGFR ($B = 0.002$, $P = 0.040$), and MEE ($B = 0.275$, $P = 0.002$) (Table 2). Additionally, collinearity statistics were >0.25 for tolerance and <3.5 for VIF, suggesting that multicollinearity was not a concern among the independent variables.

3.3. Relationship between MEE and other clinical parameters

To identify the determinant factors of MEE, a multivariate logistic regression analysis was performed and showed that 2-oxoglutarate (OR = 3.470, 95% CI = 1.557 to 7.730, $P = 0.002$), N-terminal pro-B-type natriuretic peptide (OR = 4.013, 95% CI = 1.553 to 10.365, $P = 0.004$), age (OR = 1.611, 95% CI = 1.136 to 2.283, $P = 0.007$) and left ventricular ejection fraction (OR = 7.272, 95% CI = 3.110 to 17.000, $P < 0.001$) were associated with increased MEE (Table 3). There were no significant associations with sex, NYHA classes, fasting blood glucose, postprandial blood glucose, hemoglobin A1c, creatinine, eGFR, body mass index, free triiodothyronine, free thyroxine or thyroid-stimulating hormone.

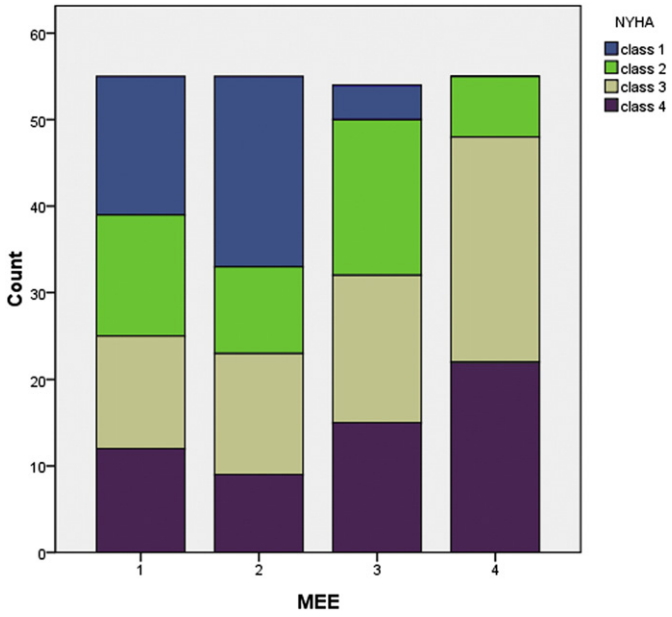

Fig. 1. Distribution of NYHA classification in different MEE groups.

3.4. Association of 2-oxoglutarate and short-term prognosis in CHF

208 patients had clinical follow up data at 8 months, the mean follow-up duration was 6.64 ± 0.16 months. There were 7 deaths, 52 recurrent hospital admissions due to CHF. According to the median level of 2-oxoglutarate, Kaplan–Meier event curves for CHF with low (<13.03 μg/mL) or high (≥13.03 μg/mL) 2-oxoglutarate levels, showed a significant association between high 2-oxoglutarate levels and increased short-term adverse outcomes in CHF (Log Rank, $Chi^2 = 4.026$, $P = 0.045$, Fig. 2).

4. Discussion

In the present study, we found that the levels of serum 2-oxoglutarate were elevated in patients with CHF compared with healthy age-matched controls. Importantly, 2-oxoglutarate levels increased in CHF patients were closely associated with the elevation of MEE, independent of NT-proBNP and NYHA classes. Furthermore, high levels of 2-oxoglutarate were correlated with adverse short-term events in patients with CHF. These findings suggest that serum 2-oxoglutarate could be a potential biomarker of MEE in CHF patients, and may be involved in the prognosis of CHF.

Table 2
Multiple linear regression analysis for serum 2-oxoglutarate.

	B (95% CI)	SE	t	*P* value
Age	−1.035 (−1.642 to −0.428)	0.308	−3.361	0.001
Log NT-proBNP	0.089 (−0.013 to 0.190)	0.052	1.715	0.088
Log LVEF	0.165 (−0.272 to 0.603)	0.222	0.745	0.457
eGFR	0.002 (0.000 to 0.005)	0.001	2.061	0.040
Log LVMI	−0.215 (−0.609 to 0.179)	0.200	−1.074	0.284
NYHA class	0.060 (−0.012 to 0.132)	0.036	1.650	0.100
Log MEE	0.275 (0.103 to 0.448)	0.088	3.143	0.002

NT-proBNP, N-terminal pro-B-type natriuretic peptide; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; LVMI, left ventricular mass index; NYHA, New York Heart Association; MEE, myocardial energy expenditure.

Table 3
Multivariate logistic regression analysis for the level of MEE.

	OR (95% CI)	B	*P* value
Sex (female vs male)	1.725 (0.382–3.576)	0.545	0.142
Age (per 10 years)	1.611 (1.136–2.283)	0.477	0.007
NYHA classes (per class)	0.918 (0.574–1.468)	−0.085	0.722
2-Oxoglutarate (<13.0 μg/mL vs ≥13.0 μg/mL)	3.470 (1.557–7.730)	1.244	0.002
NT-proBNP (<2000 pg/mL vs ≥2000 pg/mL)	4.013 (1.553–10.365)	1.689	0.004
LVEF (≤45% vs >45%)	7.272 (3.110–17.000)	1.984	<0.001
FBG (<5.6 mmol/L vs ≥5.6 mmol/L)	0.658 (0.303–1.427)	−0.419	0.289
PBG (<7.8 mmol/L vs ≥7.8 mmol/L)	0.978 (0.473–2.025)	−0.022	0.953
HbA1C (<5.6% vs ≥5.6 %)	1.573 (0.764–3.242)	0.453	0.219
Creatinine (<120 μmol/L vs ≥120 μmol/L)	1.446 (0.514–4.068)	0.369	0.485
eGFR (per 30 mL/min/1.73 m^2)	1.235 (0.664–2.298)	0.211	0.504
BMI (<24 kg/m^2 vs ≥24 kg/m^2)	1.087 (0.578–2.046)	0.084	0.796
Free thyroxine	1.021 (0.907–1.150)	0.021	0.731
Free triiodothyronine	0.824 (0.541–1.256)	−0.194	0.368
TSH	1.073 (0.880–1.309)	0.071	0.487

NYHA, New York Heart Association; NT-proBNP, N-terminal pro-B-type natriuretic peptide; LVEF, left ventricular ejection fraction; FBG, fasting blood glucose; PBG, postprandial blood glucose; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate; BMI, body mass index.; TSH, thyroid-stimulating hormone.

4.1. Alterations of MEE in CHF

MEE is a major indicator of myocardial energy metabolism, which is abnormal in failing heart. A previous study identified that MEE was an effective parameter of myocardial bioenergetics and significantly correlated with cardiac function in patients with CHF, particularly with reduced LVEF [16,18]. More importantly, elevated MEE was thought to be more effective in predicting cardiac death than LVEF [12]. Recently, our group found that higher doses of perindopril can improve left ventricular systolic function and decrease MEE further than lower doses in patients with HF after myocardial infarction [19]. In the present study, we also showed that most patients with high MEE belonged to NYHA class III or IV. These data supported alterations of MEE in CHF and suggested that it could be a good indicator for CHF.

4.2. The cause of elevation of 2-oxoglutarate in CHF and the relationship between MEE and 2-oxoglutarate

It has been reported that a metabolic shift from free fatty acid to glucose as a preferred substrate in CHF existed [20,21]. When the failing heart suffers from insufficient oxidative metabolism and favors glucose

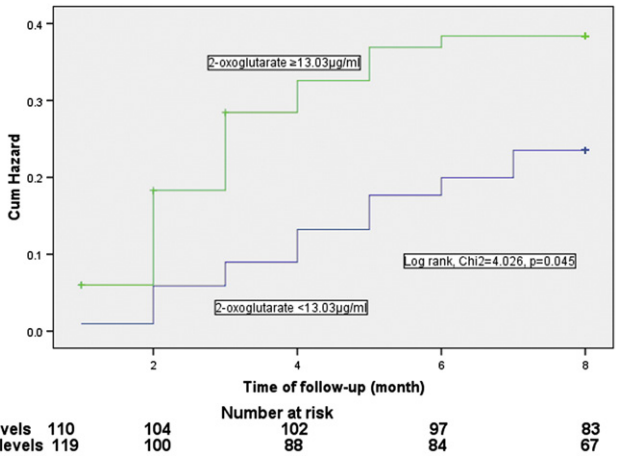

Fig. 2. Kaplan–Meier event curves for CHF with low (<13.03 μg/mL) or high (≥13.03 μg/mL) 2-oxoglutarate Levels.

utilization at the expense of fatty acid, some intermediate metabolites of the tricarboxylic acid cycle may decrease flux through the tricarboxylic acid cycle and overflow into the circulation [3,22]. 2-Oxoglutarate is a major intermediate metabolite of the tricarboxylic acid cycle, as well as a classical product of overflow in intermediate metabolite [23,24]. The amount of this overflow was increased when CHF occurred, which caused the elevation of 2-oxoglutarate. The question as to whether the elevation of 2-oxoglutarate is maladaptive or adaptive is still unsolved and whether 2-oxoglutarate is the reporter of the metabolic switch from fatty acid to glucose is also not known, but the significant association between 2-oxoglutarate and MEE observed in our study showed that 2-oxoglutarate can reflect MEE in spite of the origin of 2-oxoglutarate.

In the current study, our results were consistent with a prior report, which showed that 2-oxoglutarate was significantly increased in CHF patients [3]. More importantly, we highlight that the increase of 2-oxoglutarate was correlated with disturbed cardiac energy metabolism; patients with higher MEE values had significantly elevated 2-oxoglutarate levels. In particular, we found that the levels of 2-oxoglutarate varied significantly in different MEE groups, especially in the MEE 4 group, which was increased by about 2 fold of the mean levels of all CHF patients. This may be due to the fact that, at the mild stage of CHF, the substrate was provided mainly by the free fatty acid and mitochondrial function might be preserved despite the metabolic alterations in substrate oxidation [25], so the amounts of 2-oxoglutarate overflow into the circulation increased slightly. With the deterioration of CHF and the elevation of MEE, associated with mitochondrial abnormalities and difficulties in ATP transport, both fatty acid and glucose oxidation reduced and oxygen could be partially used to generate superoxide anion [21], which may cause significant alterations in the permeability of cardiomyocyte membranes, resulting in a dramatic increase of 2-oxoglutarate overflowing into the circulation. According to the definition of biomarkers made by the National Institute of Health [26] and three criteria for the appraisal of novel biomarkers [27], 2-oxoglutarate added some new information to CHF and energy metabolism and it can help the clinician to manage patients and be measured easily. In this view, 2-oxoglutarate could be a potential indicator of MEE and an effective marker assessing the severity of CHF objectively.

4.3. Predictive roles of 2-oxoglutarate on prognosis of CHF

Furthermore, we found that all cause mortality and recurrent hospital admission due to CHF were associated with higher 2-oxoglutarate levels. These findings suggested that 2-oxoglutarate may be useful in identifying high risk outpatients with CHF. To our knowledge, no evidence was provided to prove whether the significant elevation of 2-oxoglutarate in serious CHF was maladaptive or adaptive, our results showed that high 2-oxoglutarate levels meant the elevation of MEE and the adverse prognosis. Considering that 2-oxoglutarate may be the biomarker of MEE, this was consistent with the previous results that patients with elevated MEE had high cardiovascular mortality [12]. He W et al. [28] had demonstrated that 2-oxoglutarate was a ligand of the GPR99 G-protein coupled receptor that may regulate the renin–angiotensin system and it is also involved in angiogenesis and growth by changing the expression of VEGF receptor-1 and placental growth factor [29]. In this view, 2-oxoglutarate may also be an important player in the pathogenesis of CHF.

Hepatic congestion and abnormal glucose metabolism were often accompanied with CHF [30]. So markers of liver dysfunction and glucose metabolism may affect the levels of 2-oxoglutarate in CHF patients. However, our results showed that 2-oxoglutarate was not associated with fasting blood glucose, postprandial blood glucose, hemoglobin A1c, alanine aminotransferase and aspartate aminotransferase. This indicated that the increase of 2-oxoglutarate in CHF patients was not a result of liver dysfunction or abnormal glucose metabolism.

4.4. Limitations

Several limitations of our study should be discussed. First, because 2-oxoglutarate is also abundant in the kidney [31], we do not know whether 2-oxoglutarate can still reflect the levels of MEE and the severity of CHF in patients with renal dysfunction. Second, according to our study, 2-oxoglutarate can reflect the severity of CHF, but different causes of CHF exist in our study and whether they affect the levels of 2-oxoglutarate is unclear. Assessment between 2-oxoglutarate and the causes of CHF would help to define their associations and effects on cardiac metabolism in the future. Third, our current results were from a relative short-term follow-up duration. The study of 2-oxoglutarate for long-term prognosis in CHF patients is still undergoing and it is necessary to validate this work.

5. Conclusion

Increased serum 2-oxoglutarate levels are associated with higher MEE and adverse outcomes in CHF patients. These results suggest that 2-oxoglutarate could be a good biomarker of MEE and can reflect clinical severity and short-term outcome of CHF.

Conflict of interest

The authors have declared that there is no conflict of interest.

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References

[1] H. Ardehali, H.N. Sabbah, M.A. Burke, S. Sarma, P.P. Liu, J.G. Cleland, A. Maggioni, G.C. Fonarow, E.D. Abel, U. Campia, M. Gheorghiade, Targeting myocardial substrate metabolism in heart failure: potential for new therapies, Eur. J. Heart Fail. 14 (2012) 120–129.
[2] M. van Bilsen, P.J. Smeets, A.J. Gilde, G.J. van der Vusse, Metabolic remodelling of the failing heart: the cardiac burn-out syndrome? Cardiovasc. Res. 61 (2004) 218–226.
[3] W.B. Dunn, D.I. Broadhurst, S.M. Deepak, M.H. Buch, G. McDowell, I. Spasic, D.I. Ellis, N. Brooks, D.B. Kell, L. Neyses, Serum metabolomics reveals many novel metabolic markers of heart failure, including pseudouridine and 2-oxoglutarate, Metabolomics 3 (2007) 413–426.
[4] S.M. Kang, J.C. Park, M.J. Shin, H. Lee, J. Oh, H. Ryu do, G.S. Hwang, J.H. Chung, (1)H nuclear magnetic resonance based metabolic urinary profiling of patients with ischemic heart failure, Clin. Biochem. 44 (2011) 293–299.
[5] J.H. Chung, J.S. Kim, O.Y. Kim, S.M. Kang, G.S. Hwang, M.J. Shin, Urinary ketone is associated with the heart failure severity, Clin. Biochem. 45 (2012) 1697–1699.
[6] F.G. Marcondes-Braga, I.G. Gutz, G.L. Batista, P.H. Saldiva, S.M. Ayub-Ferreira, V.S. Issa, S. Mangini, E.A. Bocchi, F. Bacal, Exhaled acetone as a new biomarker of heart failure severity, Chest 142 (2012) 457–466.
[7] M.A. Samara, W.H. Tang, F. Cikach Jr., Z. Gul, L. Tranchito, K.M. Paschke, J. Viterna, Y. Wu, D. Laskowski, R.A. Dweik, Single exhaled breath metabolomic analysis identifies unique breathprint in patients with acute decompensated heart failure, J. Am. Coll. Cardiol. 61 (2013) 1463–1464.
[8] R. Brunken, M. Schwaiger, M. Grover-Mckay, M.E. Phelps, J. Tillisch, H.R. Schelbert, Positron emission tomography detects tissue metabolic activity in myocardial segments with persistent thallium perfusion defects, J. Am. Coll. Cardiol. 10 (1987) 557–567.
[9] J. vom Dahl, W.H. Herman, R.J. Hicks, F.J. Ortiz-Alonso, K.S. Lee, K.C. Allman, E.R. Wolfe Jr., V. Kalff, M. Schwaiger, Myocardial glucose uptake in patients with insulin-dependent diabetes mellitus assessed quantitatively by dynamic positron emission tomography, Circulation 88 (1993) 395–404.
[10] D.G. Allen, P.G. Morris, C.H. Orchard, J.S. Pirolo, A nuclear magnetic resonance study of metabolism in the ferret heart during hypoxia and inhibition of glycolysis, J. Physiol. 361 (1985) 185–204.

[11] L. Kaufman, L. Crooks, P. Sheldon, H. Hricak, R. Herfkens, W. Bank, The potential impact of nuclear magnetic resonance imaging on cardiovascular diagnosis, Circulation 67 (1983) 251–257.
[12] V. Palmieri, M.J. Roman, J.N. Bella, J.E. Liu, L.G. Best, E.T. Lee, B.V. Howard, R.B. Devereux, Prognostic implications of relations of left ventricular systolic dysfunction with body composition and myocardial energy expenditure: the Strong Heart Study, J. Am. Soc. Echocardiogr. 21 (2008) 66–71.
[13] S.H. Shah, W.E. Kraus, C.B. Newgard, Metabolomic profiling for the identification of novel biomarkers and mechanisms related to common cardiovascular diseases: form and function, Circulation 126 (2012) 1110–1120.
[14] Z. Du, A. Shen, Y. Huang, L. Su, W. Lai, P. Wang, Z. Xie, Z. Xie, Q. Zeng, H. Ren, D. Xu, 1H-NMR-based metabolic analysis of human serum reveals novel markers of myocardial energy expenditure in heart failure patients, PLoS ONE 9 (2014) e88102.
[15] C. Bianchi, R. Miccoli, R.C. Bonadonna, F. Giorgino, S. Frontoni, E. Faloia, G. Marchesini, M.A. Dolci, F. Cavalot, G.M. Cavallo, F. Leonetti, S. Del Prato, GENFIEV investigators. Pathogenetic mechanisms and cardiovascular risk: difference between HbA1c and oral glucose tolerance test for the diagnosis of glucose tolerance, Diabetes Care 35 (2012) 2607–2612.
[16] V. Palmieri, J.N. Bella, D.K. Arnett, A. Oberman, D.W. Kitzman, P.N. Hopkins, D.C. Rao, M.J. Roman, R.B. Devereux, Hypertension Genetic Epidemiology Network study. Associations of aortic and mitral regurgitation with body composition and myocardial energy expenditure in adults with hypertension: the hypertension genetic epidemiology network study, Am. Heart J. 145 (2003) 1071–1077.
[17] J. Pallant, SPSS Survival Manual: A Step by Step Guide to Data Analysis Using SPSS for Windows (Version 10), Open University Press, 2001.
[18] R. Aquilani, C. Opasich, M. Verri, F. Boschi, O. Febo, E. Pasini, O. Pastoris, Is nutritional intake adequate in chronic heart failure patients? J. Am. Coll. Cardiol. 42 (2003) 1218–1223.
[19] J. Liang, S. Bai, D. Xu, Z. Cheng, Effect of different doses of perindopril on myocardial energy expenditure in patients with heart failure following myocardial infarction, Nan Fang Yi Ke Da Xue Xue Bao 32 (2012) (1816–1819) 1832.
[20] J.S. Ingwall, Energy metabolism in heart failure and remodelling, Cardiovasc. Res. 81 (2009) 412–419.
[21] P.S. Azevedo, M.F. Minicucci, P.P. Santos, S.A. Paiva, L.A. Zornoff, Energy metabolism in cardiac remodeling and heart failure, Cardiol. Rev. 21 (2013) 135–140.
[22] Z. Du, Q. Zeng, A. Shen, W. Lai, Z. Xie, H. Ren, D. Xu, Characterization of serum metabolites in a rat heart failure model by gas chromatography/mass spectroscopy, Exp. Clin. Cardiol. 20 (2014) 517–546.
[23] O.M. Neijssel, D.W. Tempest, The role of energy-spilling reactions in the growth of *Klebsiella aerogenes* NCTC 418 in aerobic chemostat culture, Arch. Microbiol. 110 (1976) 305–311.
[24] K.L. Olszewski, M.W. Mather, J.M. Morrisey, B.A. Garcia, A.B. Vaidya, J.D. Rabinowitz, M. Llinás, Branched tricarboxylic acid metabolism in plasmodium falciparum, Nature 466 (2010) 774–778.
[25] V. Lionetti, W.C. Stanley, F.A. Recchia, Modulating fatty acid oxidation in heart failure, Cardiovasc. Res. 90 (2011) 202–209.
[26] Biomarkers Definitions Working Group, Biomarkers and surrogate endpoints: preferred definitions and conceptual framework, Clin. Pharmacol. Ther. 69 (2001) 89–95.
[27] D.A. Morrow, J.A. de Lemos, Benchmarks for the assessment of novel cardiovascular biomarkers, Circulation 115 (2007) 949–952.
[28] W. He, F.J. Miao, D.C. Lin, R.T. Schwandner, Z. Wang, J. Gao, J.L. Chen, H. Tian, L. Ling, Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors, Nature 429 (2004) 188–193.
[29] T. Nikolaidou, M. Mamas, D. Oceandy, L. Neyses, Biological action of alpha-ketoglutarate in the heart and kidney — a metabolite identified through a metabolomic search in patients with heart failure, Eur. J. Heart Fail. (Suppl. 9) (2010) S268.
[30] D.E. Høfsten, B.B. Løgstrup, J.E. Møller, P.A. Pellikka, K. Egstrup, Abnormal glucose metabolism in acute myocardial infarction influence on left ventricular function and prognosis, JACC Cardiovasc. Imaging 2 (2009) 592–599.
[31] J.R. Welborn, S. Shpun, W.H. Dantzler, S.H. Wright, Effect of alpha-ketoglutarate on organic anion transport in single rabbit renal proximal tubules, Am. J. Physiol. 274 (1998) F165–F174.