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【 ORIGINAL ARTICLE 】

# The Relationship between Circulating Polyunsaturated Fatty Acid Levels and Exercise Responses of Patients with Non-ischemic Heart Failure

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**Abstract:**

**Objective** Polyunsaturated fatty acids (PUFAs) are associated with heart failure (HF) as well as coronary artery disease. However, little is known about the relationships between PUFAs and the exercise responses of patients with HF. We evaluated the relationships between PUFAs and the parameters of cardiopulmonary exercise tests (CPETs) in patients with non-ischemic HF.

**Methods** Fifty patients with stable non-ischemic HF underwent CPETs at our hospital. Data were analyzed to evaluate the relationships between PUFAs and echocardiographic findings as well as CPET and other test parameters.

**Results** Correlations were significant and negative between dihomo-γ-linolenic acid (DGLA) + arachidonic acid (AA) and minute ventilation versus carbon dioxide production ($VE/VCO_2$) slope, and positive between N-terminal pro-B-type natriuretic peptide (NT-proBNP) and $VE/VCO_2$ slope. A multivariate regression analysis selected DGLA+AA and AA as independent predictors of $VE/VCO_2$ slope. However, eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) were not significantly correlated with the CPET parameters.

**Conclusion** Low levels of circulating DGLA+AA and AA among PUFAs were associated with decreased exercise responses in patients with stable non-ischemic HF. These findings suggest that high levels of omega-6 PUFAs may improve the clinical outcomes of patients with non-ischemic HF via their effects on exercise responses.

**Key words:** cardiopulmonary exercise test, diet, nutrition

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## Introduction

Integrative exercise responses involving the pulmonary, cardiovascular, hematopoietic, neuropsychological, and skeletal muscle systems of patients with heart failure (HF) can be assessed using established cardiopulmonary exercise tests (CPETs) (1). Variables derived from CPETs, such as the ventilatory efficiency (minute ventilation versus carbon dioxide production; $VE/VCO_2$ slope), peak exercise oxygen consumption ($VO_2$), or percent predicted peak $VO_2$ (%pp$VO_2$), and exercise oscillatory ventilation are closely associated with the clinical outcomes among patients with HF (1-3).

Polyunsaturated fatty acids (PUFAs) play structural and functional roles as membrane components and precursors of many factors involved in inflammation and signaling (4). The most common PUFAs are n-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and n-6 dihomo-γ-linolenic acid (DGLA) and arachidonic acid (AA). Among

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these, n-3 PUFAs are particularly associated with the clinical outcomes of patients with ischemic heart disease (5-8).

Recent reports have indicated that lower PUFAs levels at the time of admission are significantly associated with worse clinical outcomes among patients with acute decompensated HF (9, 10). However, little is known about associations between serum levels of PUFAs and exercise tolerance in patients with HF, especially those without ischemic heart disease.

The present study aimed to determine exercise responses in patients with non-ischemic HF by investigating the relationships between n-3 and n-6 PUFA levels and CPET variables.

## Materials and Methods

The Ethics Committee at Nagasaki University Hospital approved the study protocol, which was conducted in accordance with the Declaration of Helsinki (2013 revision).

### Patient population

We reviewed raw exercise physiological data for 275 patients with congestive HF (CHF) who underwent CPETs at our hospital between July 2012 and June 2018. Patients were diagnosed with CHF based on a history of dyspnea and exercise intolerance with signs of pulmonary or peripheral edema. Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or being treated with anti-hypertensive medication. Diabetes mellitus was defined according to the treatment guide for diabetes published by the Japan Diabetes Society. Patients with the following were excluded: ischemic heart disease, age <18 or >80 years old, undergoing EPA or DHA therapy, neuromuscular diseases, chronic kidney disease, liver dysfunction, malignancy, or recent clinical instability.

Ultimately, data were analyzed from 50 patients (mean age, 51.5±11.8 years old; male, n=44) who met the inclusion criteria.

### CPETs

Exercise tests were performed using the ramp protocol in our cardiopulmonary exercise laboratory under room air. All patients underwent symptom-limited CPETs. Pharmacological therapy was continued before and throughout the exercise tests. The equipment was calibrated daily as recommended by the manufacturer. Values for $VO_2$, carbon dioxide production ($VCO_2$), and minute ventilation (VE) acquired breath-by-breath were averaged over 10-second intervals using a ventilatory expired gas analysis system (AE310; Minato Medical Science, Japan). Peak $VO_2$ was defined as the highest 10-second averaged $VO_2$ during the last stage of the symptom-limited exercise test. The %pp$VO_2$ was determined using the Wasserman formula, and the VE/$VCO_2$ slope was calculated from rest to gas exchange at peak exercise.

### Measurement of PUFAs

Blood samples were collected early in the morning after a 12-hour overnight fast. Serum levels of EPA, DHA, AA, and DGLA were measured using capillary gas chromatography (SRL, Tokyo, Japan).

### Echocardiography

Patients underwent echocardiography using standard ultrasound equipment. Cardiac chambers were quantified using two-dimensional echocardiography according to the guidelines of the American Society of Echocardiography (11).

### Statistical analyses

Data are expressed as the mean ± standard deviation or as the number (%). The associations between the CPET values (VE/$VCO_2$ slope, peak $VO_2$, or %pp$VO_2$) and variables were evaluated using a univariate linear regression analysis. Relationships between CPET values and variables and between PUFAs and other serum variables were analyzed using Spearman's rank correlation coefficients. Independent determinants associated with CPET values among factors with $p<0.05$ on a univariate analysis were determined using a multivariate regression analysis.

Values with $p<0.05$ were considered significant. Data were statistically analyzed using the JMP software program, ver. 10 (SAS Institute, Cary, USA).

## Results

### Patients' characteristics

Table 1 shows the characteristics of the patients. Ten (20%), 36 (72%), and 4 (8%) of the patients had New York Heart Association (NYHA) class I, II, and III HF; none had class IV HF. The etiologies of CHF were dilated cardiomyopathy, dilated phase-hypertrophic cardiomyopathy, and others in 82%, 12%, and 6%, respectively. At the time of the exercise tests, 98%, 96%, 90%, and 62% of the patients were medicated with β-blockers, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, diuretics, and spironolactone, respectively.

### Echocardiographic and CPET findings

Echocardiography findings showed that the average left ventricular ejection fraction was 29.6% ±9.8% (Table 2). Peak $VO_2$ was 17.0±3.9 mL/kg/min, %pp$VO_2$ was 65.0±14.2%, and VE/$VCO_2$ slope was 30.5±6.9 (Table 2).

### Associations between CPET parameters and variables, including PUFAs

The VE/$VCO_2$ slope was positively correlated with N-terminal pro-B-type natriuretic peptide (NT-proBNP) ($\rho$=0.44, p=0.0015) and the HbA1c ($\rho$=0.33, p=0.020) and negatively correlated with AA ($\rho$=−0.42, p=0.003), DGLA+AA ($\rho$=−0.41, p=0.0033), and albumin ($\rho$=−0.36, p=0.0099)

**Table 1. Patients' Characteristics.**

| | |
|---|---|
| Total | 50 |
| Age (years) | 51.5±11.8 (18-73) |
| Male (%) | 44 (88%) |
| SBP (mmHg) | 111.3±17.9 (78-149) |
| DBP (mmHg) | 70.6±12.4 (47-104) |
| HR (beats/min) | 66.3±10.0 (50-96) |
| BMI (kg/m$^2$) | 23.5±4.1 (15.5-39.5) |
| NYHA (I/II/III/IV) | 10 / 36 / 4 / 0 |
| Hypertension (%) | 14 (28) |
| Diabetes mellitus (%) | 11 (22) |
| Hyperlipidemia (%) | 27 (54) |
| Smoking (%) | 25 (50) |
| Medication | |
| Beta-blocker (%) | 49 (98) |
| ACE or ARB (%) | 48 (96) |
| Diuretics (%) | 45 (90) |
| Spironolactone (%) | 31 (62) |
| Statin (%) | 14 (28) |

Values presented as n (%) or means±SD. SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, BMI: body mass index, NYHA: New York Heart Association, DCM: dilated cardiomyopathy, DHCM: dilate-phase hypertrophic cardiomyopathy, ACE: angiotensin converting enzyme inhibitor, ARB: angiotensin II receptor blocker

(Table 3). Peak $VO_2$ was positively correlated with AA ($\rho$=0.30, p=0.032) and albumin ($\rho$=0.29, p=0.0038). The % pp$VO_2$ was positively correlated with albumin ($\rho$=0.29, p=0.041) and high density lipoprotein-cholesterol (HDL-C) ($\rho$=0.31, p=0.0263) and negatively correlated with C-reactive protein (CRP) ($\rho$=−0.40, p=0.0046) and NT-proBNP ($\rho$=−0.33, p=0.0204) (Table 3).

The univariate analysis showed that the VE/$VCO_2$ slope was negatively correlated with the serum values for DGLA+AA ($\beta$=−0.37, p=0.00097), AA ($\beta$=−0.35, p=0.0139), and albumin ($\beta$=−0.35, p=0.0139) and positively correlated with NT-proBNP ($\beta$=0.35, p=0.00146) (Table 4). The multivariate regression analysis including DGLA+AA, AA, albumin, and NT-proBNP (model 1) did not identify any independent factors associated with the VE/$VCO_2$ slope (Table 5). The multivariate regression analysis including DGLA+AA, albumin, and NT-proBNP (model 2) selected DGLA+AA as the only independent factor associated with the VE/$VCO_2$ slope (standard $\beta$=−0.28, $R^2$=0.22, p<0.05) (Table 5). The multivariate regression analysis with AA, albumin, and NT-proBNP (model 3) selected AA as the only independent factor associated with the VE/$VCO_2$ slope (standard $\beta$=−0.29, $R^2$=0.22, p<0.05; Table 5).

Furthermore, while EPA+DHA was negatively correlated with CRP ($\rho$=−0.39, p=0.0059), DGLA+AA was not significantly correlated with CRP, although a significant correla-

**Table 2. Laboratory, UCG and CPET Data.**

| Laboratory data | | UCG data | |
|---|---|---|---|
| DGLA (mg/mL) | 37.4±14.5 (13.6-79.1) | LVIVS (mm) | 10.1±2.1 (6-16) |
| AA (mg/mL) | 176.6±42.7 (96.9-269.1) | LVPW (mm) | 10.4±1.8 (7-15) |
| DGLA+AA (mg/mL) | 211.9±55.3 (72-323.2) | LVDD (mm) | 62.1±10.7 (43-92) |
| EPA (mg/mL) | 46.7±29.1 (8-155.7) | LVDS (mm) | 53.7±11.4 (55-81) |
| DHA (mg/mL) | 114.6±44.8 (10.3-208.9) | LVEF (%) | 29.6±9.8 (10-57) |
| EPA+DHA (mg/mL) | 161.3±69.9 (35.7-351.1) | | |
| EPA/AA | 0.27±0.16 (0.05-0.74) | CPET data | |
| (EPA+DHA/AA) | 1.00±0.69 (0.18-5.00) | Peak $VO_2$ (mL/kg/min) | 17.0±3.9 (8.1-29.1) |
| (EPA+DHA)/(DGLA+AA) | 0.79±0.43 (0.14-2.92) | %pp$VO_2$ (%) | 65.0±14.2 (31-90) |
| AA/DGLA | 5.14±1.90 (1.4-10.8) | VE/$VCO_2$ slope | 30.5±6.9 (19.2-50.9) |
| NT-pro BNP (pg/mL) | 1,185.8±999.2 (109.6-4,024) | | |
| Hb (g/dL) | 15.1±1.8 (10.4-18.8) | | |
| eGFR (mL/min/1.73m$^2$) | 64.3±16.2 (37.3-108.4) | | |
| TC (mg/dL) | 179.4±36.9 (101.4-265) | | |
| LDL-C (mg/dL) | 107.4±28.0 (61-197) | | |
| HDL-C (mg/dL) | 44.3±12.2 (12-85) | | |
| TG (mg/dL) | 138.4±76.8 (36-416) | | |
| HbA1c (%) | 6.2±1.0 (5.2-10.1) | | |
| Alb (g/dL) | 4.0±0.5 (2.3-4.7) | | |
| CRP (mg/dL) | 0.29±0.48 (0.02-2.23) | | |

Values presented as n (%) or means±SD. DGLA: dihomo-γ-linolenic acid, AA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, NT-pro BNP: N-terminal pro-brain natriuretic peptide, Hb: hemoglobin, eGFR: estimate glomerular filtration rate, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, HbA1c: hemoglobin A1c, Alb: albumen, CRP: C-reactive protein, UCG: ultrasonic echocardiography, IVS: interventricular septum, LVPW: left ventricular posterior wall, LVDD: left ventricular internal dimension in diastole, LVDS: left ventricular diameter at end systole, EF: ejection fraction, CPET: cardiopulmonary exercise test, peak $VO_2$: peak oxygen uptake, %pp$VO_2$: percent predicted peak $VO_2$, VE/$VCO_2$ slope: regression slope relating minute ventilation to carbon dioxide output

**Table 3. Significant Correlation between CPET Value and Variables.**

| CPET value | Variables | $\rho$ | p value |
|---|---|---|---|
| $VE/VCO_2$ slope | NT-proBNP | 0.44 | 0.0015 |
| | AA | -0.42 | 0.003 |
| | DGLA+AA | -0.41 | 0.0033 |
| | Alb | -0.36 | 0.0099 |
| | HbA1c | 0.33 | 0.020 |
| | DGLA | -0.22 | 0.13 |
| | EPA+DHA | -0.17 | 0.24 |
| | DHA | -0.17 | 0.24 |
| | EPA/AA | 0.12 | 0.42 |
| | EPA | -0.09 | 0.55 |
| | AA/DGLA | -0.07 | 0.61 |
| | (EPA+DHA)/AA | 0.07 | 0.63 |
| | (EPA+DHA)/(DGLA+AA) | 0.06 | 0.66 |
| Peak $VO_2$ | AA | 0.30 | 0.032 |
| | Alb | 0.29 | 0.038 |
| | DGLA+AA | 0.26 | 0.068 |
| | (EPA+DHA)/AA | 0.23 | 0.12 |
| | (EPA+DHA)/(DGLA+AA) | 0.21 | 0.13 |
| | EPA/AA | 0.17 | 0.23 |
| | DGLA | 0.16 | 0.27 |
| | EPA | 0.03 | 0.83 |
| | AA/DGLA | -0.03 | 0.84 |
| | DHA | -0.03 | 0.85 |
| | EPA+DHA | 0.02 | 0.89 |
| $\%ppVO_2$ | CRP | -0.40 | 0.0046 |
| | NT-pro BNP | -0.33 | 0.0204 |
| | HDL-C | 0.31 | 0.0263 |
| | Alb | 0.29 | 0.041 |
| | AA | 0.23 | 0.10 |
| | DGLA+AA | 0.23 | 0.11 |
| | EPA | 0.19 | 0.18 |
| | DGLA | 0.18 | 0.21 |
| | EPA+DHA | 0.17 | 0.24 |
| | DHA | 0.13 | 0.37 |
| | EPA/AA | 0.04 | 0.78 |
| | AA/DGLA | -0.04 | 0.80 |
| | (EPA+DHA)/AA | -0.02 | 0.88 |
| | (EPA+DHA)/(DGLA+AA) | -0.02 | 0.88 |

DGLA: dihomo-γ-linolenic acid, AA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, NT-pro BNP: N-terminal pro-brain natriuretic peptide, HDL-C: high-density lipoprotein cholesterol, HbA1c: hemoglobin A1c, Alb: albumen, CRP: C-reactive protein, CPET: cardiopulmonary exercise test, peak $VO_2$: peak oxygen uptake, $\%ppVO_2$: percent predicted peak $VO_2$, $VE/VCO_2$ slope: regression slope relating minute ventilation to carbon dioxide output

**Table 4. Univariable Liner Regression Analysis of CPET Values.**

| $VE/VCO_2$ slope | | | | |
|---|---|---|---|---|
| Variables | $R^2$ | Adjusted $R^2$ | F-value | Standardized $\beta$ |
| DGLA+AA | 0.13 | 0.12 | 7.26** | -0.37** |
| AA | 0.12 | 0.10 | 6.53* | -0.35* |
| DGLA | 0.08 | 0.06 | 3.83 | -0.27 |
| EPA+DHA | 0.006 | -0.01 | 0.29 | -0.07 |
| EPA | 0.01 | -0.01 | 0.47 | -0.10 |
| DHA | 0.003 | 0.02 | 0.16 | 0.69 |
| NT-proBNP | 0.12 | 0.10 | 6.44* | 0.35* |
| Alb | 0.08 | 0.06 | 4.20* | -0.29* |
| HbA1c | 0.03 | 0.01 | 1.62 | 0.18 |

| Peak $VO_2$ | | | | |
|---|---|---|---|---|
| Variables | $R^2$ | Adjusted $R^2$ | F-value | Standardized $\beta$ |
| Alb | 0.12 | 0.10 | 6.71* | 0.35* |
| AA | 0.084 | 0.06 | 4.40* | 0.29* |
| DGLA+AA | 0.06 | 0.04 | 3.19 | 0.25 |
| DGLA | 0.04 | 0.02 | 2.08 | 0.20 |
| EPA+DHA | 0.006 | -0.01 | 0.29 | 0.08 |
| EPA | 0.022 | 0.002 | 1.01 | 0.15 |
| DHA | 0.0006 | -0.02 | 0.03 | 0.02 |

*p<0.05, **p<0.01

DGLA: dihomo-γ-linolenic acid, AA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, NT-pro BNP: N-terminal pro-brain natriuretic peptide, HbA1c: hemoglobin A1c, Alb: albumen, CPET: cardiopulmonary exercise test, peak $VO_2$: peak oxygen uptake, $VE/VCO_2$ slope: regression slope relating minute ventilation to carbon dioxide output

tion was noted between DGLA+AA and other parameters (Table 6).

In summary, the $VE/VCO_2$ slope correlated positively with NT-proBNP and HbA1c and negatively with AA, DGLA+AA, and albumin. The univariate analysis showed that $VE/VCO_2$ was significantly correlated with serum DGLA+AA, serum AA, serum albumin, and NT-proBNP. The multivariate regression analysis selected DGLA+AA and serum AA as independent factors associated with the $VE/VCO_2$ slope, whereas EPA+DHA was not significantly correlate with any CPET values.

## Discussion

The relationships between circulating n-6 PUFAs and exercise responses in patients with stable non-ischemic HF undergoing conventional therapy have not been investigated. The present findings suggest that n-6 PUFAs play a pivotal role in the exercise responses of such patients.

Lower PUFA levels are associated with worse clinical outcomes in patients with acute decompensated HF (ADHF) (9, 10). Nagai et al. (9) reported that lower levels of n-6 (AA+DGLA) but not n-3 PUFAs on admission were significantly associated with worse clinical outcomes in patients with ADHF. In contrast, Ouchi et al. (10) reported that decreased levels of DHA, DGLA, and AA were independently associated with long-term mortality in patients with ADHF. These previous findings suggest that at least lower DGLA and AA values are independently associated with worse clinical outcomes, including long-term mortality (9, 10). Our findings are compatible with these results in that lower DGLA and AA values were associated with higher values for the $VE/VCO_2$ slope, which is a parameter associated with the prognosis among patients with HF.

One report from the USA found that n-3 PUFAs affect

**Table 5. Multivariable Liner Regression Analysis of CPET Values.**

| $VE/VCO_2$ slope | | | | |
|---|---|---|---|---|
| model 1 | | | | |
| Variables | $R^2$ | Adjusted $R^2$ | F-value | Standardized $\beta$ |
| DGLA+AA | 0.22 | 0.15 | 3.15* | -0.06 |
| AA | | | | -0.24 |
| Alb | | | | -0.09 |
| NT-proBNP | | | | 0.25 |
| model 2 | | | | |
| Variables | $R^2$ | Adjusted $R^2$ | F-value | Standardized $\beta$ |
| DGLA+AA | 0.22 | 0.16 | 4.12* | -0.28* |
| NT-proBNP | | | | 0.25 |
| Alb | | | | -0.09 |
| model 3 | | | | |
| Variables | $R^2$ | Adjusted $R^2$ | F-value | Standardized $\beta$ |
| AA | 0.22 | 0.17 | 4.27** | -0.29* |
| NT-proBNP | | | | 0.25 |
| Alb | | | | -0.12 |
| Peak $VO_2$ | | | | |
| model 1 | | | | |
| Variables | $R^2$ | Adjusted $R^2$ | F-value | Standardized $\beta$ |
| Alb | 0.16 | 0.12 | 4.48* | 0.29* |
| AA | | | | 0.20 |

*p<0.05, **p<0.01

DGLA: dihomo-γ-linolenic acid, AA: arachidonic acid, NT-pro BNP: N-terminal pro-brain natriuretic peptide, Alb: albumen, CPET: cardiopulmonary exercise test, peak $VO_2$: peak oxygen uptake, $VE/VCO_2$ slope: regression slope relating minute ventilation to carbon dioxide output

**Table 6. Significant Correlation of n-3 and n-6 PUFA.**

| DGLA+AA | | |
|---|---|---|
| Variables | $\rho$ | p value |
| $VE/VCO_2$ slope | -0.41 | 0.0033 |
| EPA | 0.31 | 0.0285 |
| Alb | 0.31 | 0.0272 |
| EPA+DHA | 0.40 | 0.0038 |
| DHA | 0.45 | 0.0012 |
| TG | 0.46 | 0.0009 |
| BMI | 0.46 | 0.0008 |
| LDL-C | 0.55 | <.0001 |
| TC | 0.61 | <.0001 |

| EPA+DHA | | |
|---|---|---|
| Variables | $\rho$ | p value |
| CRP | -0.39 | 0.0059 |
| LVPW | -0.32 | 0.0233 |
| Alb | 0.29 | 0.0437 |
| Age | 0.29 | 0.0413 |
| HDL-C | 0.33 | 0.021 |
| AA | 0.34 | 0.0147 |
| TC | 0.39 | 0.0057 |
| DGLA | 0.39 | 0.0055 |
| DGLA+AA | 0.40 | 0.0038 |

PUFA: polyunsaturated fatty acids, DGLA: dihomo-γ-linolenic acid, AA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, Alb: albumen, CRP: C-reactive protein, LVPW: left ventricular posterior wall, $VE/VCO_2$ slope: regression slope relating minute ventilation to carbon dioxide output

the peak $VO_2$ in patients with dilated cardiomyopathy (12), but the present study noted no correlation between n-3 PUFAs and CPET values. Fish oils are rich in n-3 PUFAs, whereas sunflower, safflower, and corn oils as well as farm animal meat are rich in n-6 PUFAs (13). The Japanese population in general consumes more fish oil than oils containing n-6 PUFAs, which might explain the more prominent effects of n-6 PUFAs than n-3 PUFAs in our study cohort.

The cross-sectional nature of the present study did not allow for the determination of how decreased n-6 PUFAs values were associated with a diminished exercise response. However, some mechanisms may explain this association. The benefits and harms of n-6 PUFAs remain controversial. Eicosanoids, such as prostaglandins (PGs), thromboxanes, and leukotrienes are oxygenated derivatives of PUFAs. Eicosanoids derived from n-6 PUFAs are generally regarded as pro-inflammatory, and an excess might contribute to the pathogenesis of heart disease. In contrast, some studies of humans have suggested that n-6 PUFAs might act as anti-inflammatory or antioxidant agents (14, 15). AA is converted to a series 2 PG, which helps maintain hemostasis at low levels but increases inflammation at high levels (16-18). The n-6 PUFA DGLA is converted to a series 1 beneficial PG that can inhibit platelet aggregation, reduce inflammation, and maintain homeostasis (19-21). The anti-inflammatory effects of DGLA have been attributed to both the anti-inflammatory properties of DGLA-derived metabolites and the ability of DGLA to compete with AA in the synthesis of pro-inflammatory AA products (21). Somewhat paradoxically from the perspective of inflammation, AA can also be synthesized from DGLA in a reaction catalyzed by an enzyme originally known as Δ-5 desaturase. The present study found that DGLA+AA was not correlated with the CRP level. Thus, the anti-inflammatory effect of DGLA+AA does not seem to explain its association with the exercise response.

Both DGLA and AA are sources of various vasoactive eicosanoids (22). Among them, the potent arteriolar vasodilator $PGE_1$ is formed from DGLA and confers beneficial effects on myocardial energetics and the cardiac function in

patients with severe ischemic HF (23). Prostacyclin ($PGI_2$) is a product of AA that may exert similar actions, as it is also a potent vasodilator with antiarrhythmic actions, like $PGE_1$ (24). These PGs enhance nitric oxide (NO) synthesis and release (25-27), suggesting that NO and PGs act in concert to modulate the cardiac function. Based on these findings, we propose that a deficiency of n-6 PUFAs, especially DGLA and AA, results in decreased $PGE_1$, $PGE_2$, and NO production, which subsequently diminishes the exercise responses in patients with HF.

We also cannot explain why $VE/VCO_2$ was significantly correlated with n-6 PUFAs whereas the peak $VO_2$ and % $ppVO_2$ were not similarly correlated. Others have shown that the $VE/VCO_2$ slope predicts mortality, hospitalization, or both more accurately than the peak $VO_2$ among patients with CHF (28-31). The peak $VO_2$ might have been underestimated because of a reduced patient motivation or the premature termination of exercise by the examiner. Furthermore, Kahler et al. (32) showed that DGLA and AA caused a relaxant effect on the tracheal smooth muscle. DGLA and AA may therefore be more closely related to the $VE/VCO_2$ than the peak $VO_2$ or $\%ppVO_2$ because the $VE/VCO_2$ is an index of the ventilator response to exercise.

In conclusion, low circulating levels of DGLA+AA and AA were associated with decreased exercise responses in patients with stable non-ischemic HF. This suggests that omega-6 PUFAs are important factors that are involved in the exercise responses of patients with non-ischemic HF.

### Study limitations

This study was conducted at a single facility in a relatively small population. We did not assess the effects of n-6 PUFAs on CPET values. Further studies are needed in order to elucidate the effects of n-6 PUFA intake so that exercise responses and the prognoses of patients with non-ischemic HF can be improved. Finally, we measured only EPA and DHA as n-3 PUFAs and only DGLA and AA as n-6 PUFAs. The effects of other PUFAs should be analyzed in a future study.

**The authors state that they have no Conflict of Interest (COI).**

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