

# Association of Decreased Docosahexaenoic Acid Level After Statin Therapy and Low Eicosapentaenoic Acid Level with In-Stent Restenosis in Patients with Acute Coronary Syndrome

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**Aim:** It is speculated that statin therapy modulates the synthesis of polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, the data available on the effects of statin therapy on the serum levels of PUFA and the subsequent impact on in-stent restenosis (ISR) in patients with acute coronary syndrome (ACS) are limited.

**Methods:** A total of 120 ACS patients who received emergent coronary stent implantation, follow-up coronary angiography to evaluate ISR, and new statin therapy were enrolled. We measured the serum levels of the PUFA and lipids at the onset of ACS and at the follow-up coronary angiography.

**Results:** The follow-up coronary angiography revealed 38 ISR cases. New statin therapy significantly reduced the serum levels of DHA and low-density lipoprotein cholesterol (LDL-C), while it did not affect EPA level. Single regression analysis revealed that a decreased serum level of LDL-C was associated with decreased DHA level. The multiple logistic regression analysis revealed that the decreased DHA level after statin therapy and low serum level of EPA on admission were determinants of prevalence of ISR.

**Conclusion:** Statin therapy decreased the serum level of DHA with a parallel reduction in LDL-C level in patients with ACS. Decreased DHA level after statin therapy and low EPA level on admission are risk factors for ISR, indicating that in patients with ACS, decreased serum levels of DHA may be a residual target for the prevention of ISR.

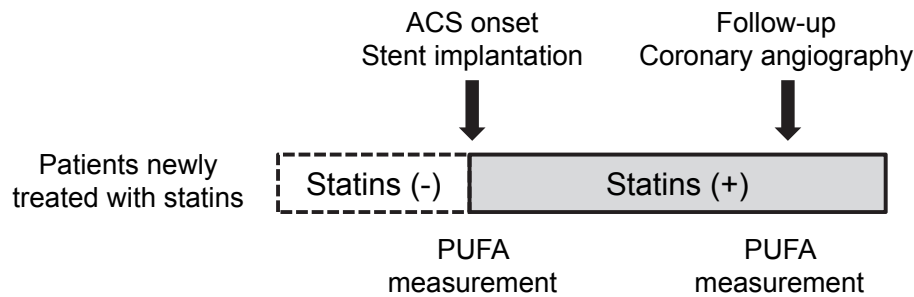
**Key words:** Polyunsaturated fatty acids, Eicosapentaenoic acid, Docosahexaenoic acid, In-stent restenosis

## Introduction

Primary percutaneous coronary intervention (PCI) with stent implantation improves outcomes in patients with acute coronary syndrome (ACS)<sup>1-4</sup>. However, the treatment of in-stent restenosis (ISR) remains a significant

clinical challenge, especially in patients with ACS.

Decreased serum levels of n-3 polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are reportedly associated with an increased incidence of cardiovascular (CV) events and mortality<sup>5, 6</sup>. In addition,



**Fig. 1.** Flowchart of the study

PUFA therapy and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) decrease CV events in patients with or without coronary artery diseases<sup>7,8</sup>. This may be attributed to the pleiotropic effects of PUFA including vascular anti-inflammatory effects and inhibitory effects on neointimal proliferation<sup>9-12</sup>, which suggests their possible role in the prevention of ISR. However, the data available on the correlation between PUFA therapy and the risk of developing ISR are limited<sup>13</sup>.

Statin therapy is established for the primary and secondary prevention of CV events<sup>14</sup>. In addition, they are speculated to modulate the activity of PUFA synthesis and to decrease the levels of EPA and DHA<sup>15</sup>. This study aimed to investigate the effects of statin therapy on the serum levels of PUFA and the subsequent impact on ISR in patients with ACS, because decreased serum levels of n-3 PUFA may be used as a target for the prevention of ISR following statin therapy and the data available on the impact of statins on PUFA and ISR in patients with ACS are limited.

## Material and Methods

We retrospectively reviewed data from Japanese patients with ACS who underwent successful emergent PCI with stents implantation for *de novo* lesions at the Department of Cardiovascular Medicine in Tokushima University Hospital between January 2009 and July 2017. A total of 120 patients with ACS who received emergent coronary stent implantation, follow-up scheduled coronary angiography to evaluate ISR at least 3 months following the emergent PCI, and statin therapy, including rosuvastatin, pitavastatin, atorvastatin, pravastatin, fluvastatin, and simvastatin were serially enrolled (**Fig. 1**). According to the drug informa-

tion for dyslipidemic Japanese patients, a regular-dose statin regimen is defined as 5 mg of rosuvastatin, 2 mg of pitavastatin, 10 mg of atorvastatin, 10 mg of pravastatin, or 30 mg of fluvastatin. A high dose is defined as a dose higher than the regular dose, and a low dose is defined as a dose lower than the regular dose.

Excluded were patients using fish oil supplements or n-3 PUFA-containing drugs on admission or those who had used fish oil supplements or received n-3 PUFA therapy after ACS onset. In addition, patients with symptomatic, active malignant diseases, liver dysfunction (aspartate aminotransferase levels >100 IU/L, alanine aminotransferase levels >100 IU/L), or severe renal dysfunction with hemodialysis were excluded.

ACS included acute myocardial infarction and unstable angina. Acute myocardial infarction was defined as the transient increase of the muscle and brain (MB) fraction of creatine kinase to a threshold of more than 3 times the 99th percentile of the upper reference limit (150 U/L) following PCI in patients with ischemic symptoms and/or typical electrocardiographic findings (ST elevation)<sup>7</sup>. Unstable angina was defined as angina at rest, accelerated exertional angina combined with typical electrocardiographic changes (ST depression), or an increase in the intensity of anti-ischemic therapy with a transient increase of the MB fraction of creatine kinase to a threshold of less than 3 times the 99th percentile of the upper reference limit, as described previously<sup>5</sup>.

ISR was defined<sup>16</sup> as binary angiographic restenosis when  $\geq 50$  percent of luminal narrowing at follow-up angiography was detected and as clinical restenosis when both binary angiographic restenosis and clinical symptoms or signs of ischemia (either at rest or with stress) were present OR when a  $\geq 70$  percent of luminal narrowing at follow-up angiography was de-

**Table 1.** Clinical characteristics of patients with/without in-stent restenosis at the onset of acute coronary syndrome

| Variables                            | All patients | ISR(-)      | ISR(+)      | P-value |
|--------------------------------------|--------------|-------------|-------------|---------|
| Number of patients                   | 120          | 82          | 38          |         |
| Age (years)                          | 67 ± 13      | 67 ± 12     | 68 ± 13     | 0.67    |
| Male gender, n (%)                   | 89 (74%)     | 59 (72%)    | 30 (79%)    | 0.26    |
| Body mass index (kg/m <sup>2</sup> ) | 24 ± 4       | 24 ± 4      | 23 ± 4      | 0.70    |
| HbA1c (%)                            | 6.3 ± 1.3    | 6.2 ± 1.1   | 6.5 ± 1.6   | 0.31    |
| eGFR (mL/min/1.73 m <sup>2</sup> )   | 70 ± 23      | 70 ± 24     | 71 ± 22     | 0.41    |
| TG (mg/dL)                           | 122 ± 64     | 125 ± 71    | 116 ± 43    | 0.46    |
| HDL-C (mg/dL)                        | 51 ± 14      | 50 ± 15     | 51 ± 13     | 0.84    |
| LDL-C (mg/dL)                        | 121 ± 33     | 120 ± 35    | 122 ± 27    | 0.75    |
| Fatty acid concentrations            |              |             |             |         |
| EPA (µg/mL)                          | 57 ± 33      | 62 ± 34     | 47 ± 29     | 0.02    |
| DHA (µg/mL)                          | 134 ± 44     | 139 ± 46    | 122 ± 37    | 0.06    |
| AA (µg/mL)                           | 174 ± 52     | 171 ± 48    | 181 ± 59    | 0.34    |
| EPA/AA                               | 0.37 ± 0.35  | 0.41 ± 0.39 | 0.28 ± 0.20 | 0.07    |
| DHA/AA                               | 0.84 ± 0.44  | 0.89 ± 0.48 | 0.74 ± 0.29 | 0.09    |
| Complications                        |              |             |             |         |
| Dyslipidemia, n (%)                  | 38 (32%)     | 27 (32%)    | 11 (29%)    | 0.66    |
| Hypertension, n (%)                  | 79 (66%)     | 54 (65%)    | 25 (65%)    | 0.99    |
| Diabetes mellitus, n (%)             | 39 (33%)     | 27 (33%)    | 12 (32%)    | 0.88    |
| Current smoking, n (%)               | 53 (44%)     | 37 (45%)    | 16 (42%)    | 0.76    |
| Culprit lesion, LAD/RCA/LCX, n       | 60/45/15     | 39/32/11    | 21/13/4     | 0.73    |
| Number of stents                     | 143          | 99          | 44          |         |
| Stent diameter, mm                   | 3.1 ± 0.4    | 3.1 ± 0.4   | 3.0 ± 0.5   | 0.46    |
| Stent length, mm                     | 18.4 ± 4.4   | 18.8 ± 4.5  | 17.5 ± 4.1  | 0.13    |
| Drug eluting stents, n (%)           | 31 (26%)     | 23 (28%)    | 8 (21%)     | 0.42    |

Abbreviations: ISR; in-stent restenosis; HbA1c, glycated hemoglobin; eGFR estimated glomerular filtration rate; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol, LDL-C, low-density lipoprotein cholesterol; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid LAD, left anterior descending artery; RCA, right coronary artery, LCX, left circumflex artery.

**Table 2.** Administered drugs during the follow-up period in patients newly treated with statins

| Variables                       | All patients | ISR(-)   | ISR(+)   | P-value |
|---------------------------------|--------------|----------|----------|---------|
| Number of patients              | 120          | 82       | 38       |         |
| Statins                         |              |          |          |         |
| Rosuvastatin                    | 58 (48%)     | 38 (32%) | 20 (17%) |         |
| Pitavastatin                    | 36 (30%)     | 24 (20%) | 12 (10%) |         |
| Atorvastatin                    | 15 (13%)     | 11 (9%)  | 4 (3%)   | 0.67    |
| Pravastatin                     | 7 (6%)       | 5 (4%)   | 2 (2%)   |         |
| Fluvastatin                     | 4 (3%)       | 4 (3%)   | 0 (0%)   |         |
| ACEI/ARB, n (%)                 | 101 (84%)    | 67 (82%) | 34 (89%) | 0.27    |
| β-blockers, n (%)               | 32 (27%)     | 22 (27%) | 10 (26%) | 0.95    |
| Calcium channel blockers, n (%) | 12 (10%)     | 10 (12%) | 2 (5%)   | 0.24    |
| Ezetimibe, n (%)                | 6 (5%)       | 6 (7%)   | 0 (0%)   | 0.08    |
| Antidiabetics, n (%)            | 7 (6%)       | 5 (6%)   | 2 (5%)   | 0.86    |
| Anticoagulants, n (%)           | 22 (18%)     | 11 (13%) | 11 (28%) | 0.04    |

Abbreviations: ISR; in-stent restenosis; ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers

tected even in the absence of clinical symptoms or signs.

Blood samples were collected prior to the emergent PCI on admission and prior to the follow-up cath-

eter examination. The serum samples were stored at -30°C until assayed. Gas-liquid chromatography at a commercially available laboratory (SRL, Tokyo, Japan)

**Table 3.** Lipid and polyunsaturated fatty acids profiles at the onset and follow-up of acute coronary syndrome

| Variables                 | ACS onset   | Follow-up   | <i>P</i> -value |
|---------------------------|-------------|-------------|-----------------|
| TG (mg/dL)                | 122 ± 64    | 135 ± 60    | 0.05            |
| HDL-C (mg/dL)             | 51 ± 14     | 53 ± 12     | 0.06            |
| LDL-C (mg/dL)             | 121 ± 33    | 86 ± 23     | < 0.0001        |
| Fatty acid concentrations |             |             |                 |
| EPA (µg/mL)               | 57 ± 33     | 55 ± 31     | 0.39            |
| DHA (µg/mL)               | 134 ± 4     | 118 ± 42    | < 0.0001        |
| AA (µg/mL)                | 174 ± 52    | 180 ± 50    | 0.11            |
| EPA/AA                    | 0.37 ± 0.35 | 0.34 ± 0.31 | 0.15            |
| DHA/AA                    | 0.84 ± 0.44 | 0.71 ± 0.37 | < 0.0001        |
| (EPA + DHA)/AA            | 1.21 ± 0.75 | 1.05 ± 0.65 | < 0.0001        |

Abbreviations as in Table 1.

was used to measure the serum composition of PUFA, including the levels of EPA, DHA, and arachidonic acid (AA)<sup>8,9</sup>. The intra- and inter-assay coefficients of variation for the EPA, DHA, and AA measurements were 1.3% and 3.3%, 1.5% and 2.2%, and 1.1% and 2.2%, respectively<sup>17</sup>. In addition, other biochemical parameters, including low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured.

The Tokushima University Hospital Ethics Committee approved the study protocol (No. 2092), and the study was conducted in accordance with the Declaration of Helsinki.

### Statistical Analyses

The continuous variables were expressed as a mean ± standard deviation, while the categorical variables were expressed as percentages. The differences between CV risk factors with and without ISR were evaluated using Student's *t*-test, the Mann–Whitney *U* test, or the chi-square test according to their distributions. The paired *t*-test was used to evaluate the differences in the serum levels and the ratio of PUFA and the levels of LDL-C, HDL-C, and TG on the onset of ACS and on the follow-up coronary angiography. After stratification by dosage or type of statins, the differences in the levels or ratio of PUFA were determined by one-way analysis of variance. Pearson's correlation analysis was used to evaluate the correlation of changes in the serum levels of DHA with that of LDL-C. Single and multivariate logistic regression analysis was used to assess the degree of association between ISR and CV risk factors. JMP software version 10 (SAS, Cary, NC) was used to perform all the statistical analyses. Statistical significance was defined as a *P*-value of < 0.05.

## Results

### Clinical Characteristics of Subjects

Tables 1 and 2 show the characteristics of patients newly treated with statins stratified by ISR on admission. Follow-up coronary angiography was performed 7.3 ± 1.9 months following PCI on the onset of ACS. Follow-up coronary angiography revealed 38 ISR cases. Except for those in the levels of EPA and rate of anticoagulants use, no differences between the characteristics of the patients in the ISR and non-ISR groups were noted. In addition, no differences in the prevalence of drug-eluting stent (DES) implantation and the stent diameter or length were noted.

### Statin Therapy Decreases Levels of LDL-C in Patients with ACS

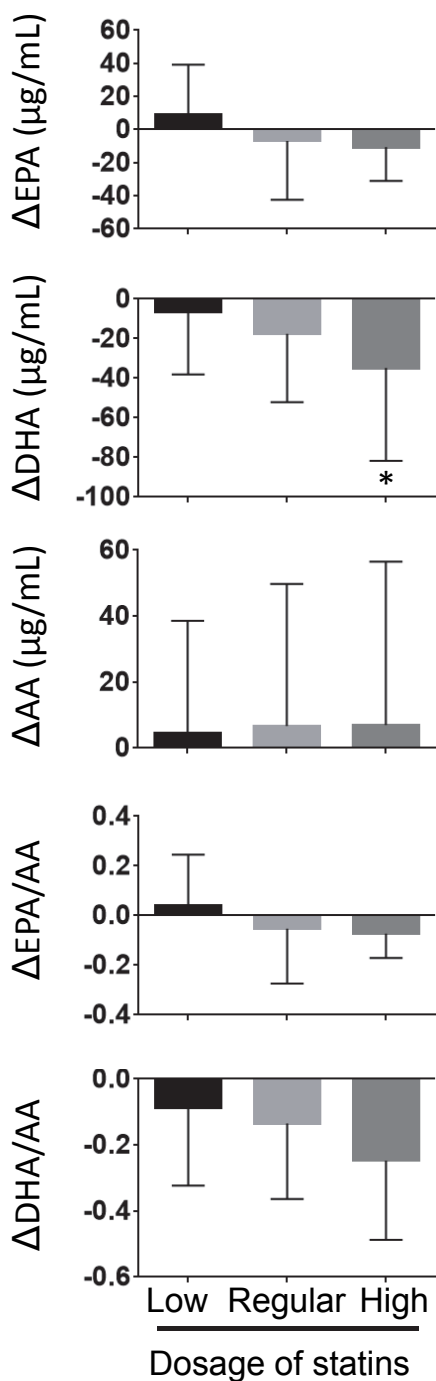
New statin therapy significantly decreased serum levels of LDL-C, while it did not change those of the TG and the HDL-C (Table 3).

### Statin Therapy Decreases Levels of DHA in Patients with ACS

New statin therapy significantly decreased serum levels of DHA, DHA/AA, and (EPA + DHA)/AA but did not change those of EPA, AA, and EPA/AA (Table 3). There were no differences in the change in EPA, AA, EPA/AA, and DHA/AA levels among the statin dosages (Fig. 2). However, statin therapy decreased serum level of DHA in a dose-dependent manner. In addition, there were no differences in the change in EPA, DHA, AA, EPA/AA, and DHA/AA levels among the types of statins including rosuvastatin, pitavastatin, atorvastatin, pravastatin, and fluvastatin/simvastatin (Fig. 3).

### Correlation between Changes in LDL-C and DHA

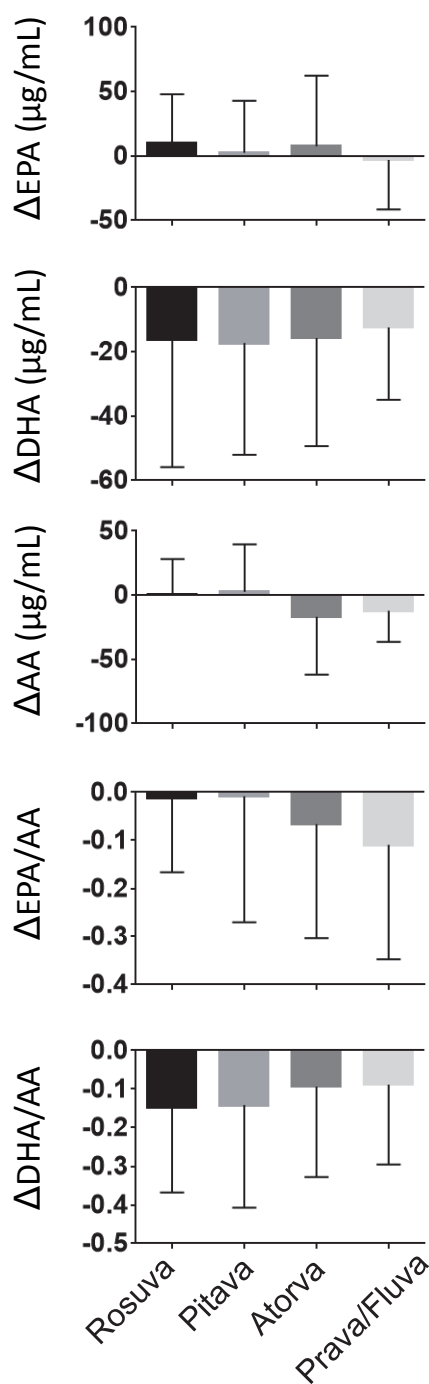
We investigated whether the changes in serum



**Fig. 2.** Change in the levels and ratio of PUFA after statin therapy stratified by statin dosage (\* $p < 0.05$  compared with low dosage)

Δ: levels and ratio of PUFA on follow-up–levels and ratio of PUFA on ACS onset

levels of DHA were induced by statins by recording the changes in the serum levels of LDL-C. In the patients newly treated with statins, Pearson’s correlation analysis



**Fig. 3.** Change in the levels and ratio of PUFA after statin therapy stratified by statin type

Δ: levels of and ratio of PUFA on follow-up–levels of and ratio of PUFA on ACS onset  
 Rosva, rosuvastatin; Pitava, pitavastatin; Atrova, atorvastatin; Prava, pravastatin; Fluva, fluvastatin.

revealed that changes in serum levels of LDL-C (LDL-C on follow-up–LDL-C on ACS onset) were positively correlated with changes in serum levels of DHA (DHA

on follow-up–DHA on ACS onset) ( $P < 0.01$ ), indicating that statins decrease DHA, which are reduced in parallel with the reduction of LDL-C levels (Fig. 4).

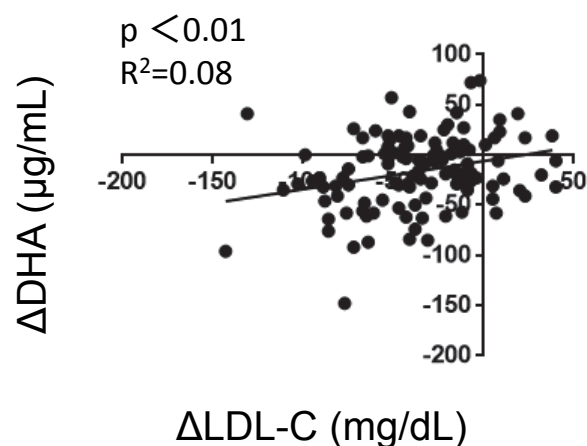
### Statin-Induced Decrease in DHA is a Risk Factor for ISR

We evaluated whether the serum levels of EPA and DHA on admission or on follow-up were risk factors for ISR. The single regression analysis revealed that the levels of EPA and EPA/AA ratios were significantly correlated with an increased prevalence of ISR on admission; however, this correlation was not significant on the follow-up (Table 4). In addition, there was no significant correlation between the prevalence of ISR and age, male gender, body mass index, hypertension, type 2 diabetes, estimate glomerular filtration rate, prevalence of DES implantation, stent diameter and length, and metabolic parameters of TG, HDL-C, LDL-C, and HbA1c both on admission and on follow-up catheter examination (Table 4). In addition, the ISR rate of bare metal stents and second- and third-generation DESs were 34%, 15%, and 32%, respectively; however, there were no significant differences in the prevalence of ISR among the stent types (data not shown). Stent diameter and length, type 2 diabetes, and renal dysfunction are traditional risk factors for ISR<sup>18–21</sup>. In addition, the serum levels of PUFA are influenced by age<sup>17</sup>. Multiple logistic regression analysis adjusted for those factors revealed that the decrease in DHA after statin therapy was an independent factor for increased prevalence of ISR. Furthermore, low serum level of EPA on admission was also an independent factor for increased prevalence of ISR (Table 5).

## Discussion

Our results revealed that ACS patients who were newly treated with statins had reduced serum levels of DHA, which were dependent on reduced LDL-C levels, indicating that statins alone can decrease serum levels of DHA but not EPA. In addition, a decreased DHA level after statin therapy and a low EPA level on admission of patients with ACS were risk factors for developing ISR. The results indicated that EPA level should be maintained before the onset of ACS for better outcome of ACS, and DHA should be maintained after statin therapy after onset of ACS.

ISR is the result of arterial damage with subsequent neointimal proliferation/migration of vascular smooth muscle cells from the bone marrow<sup>22</sup> and inflammatory changes in the form of macrophage accumulation, extensive neovascularization in response to stent-associated injuries<sup>16, 23, 24</sup>, and platelet aggregation<sup>25</sup>. Previous experimental studies have shown that



**Fig. 4.** Correlation between changes in low-density lipoprotein cholesterol and changes in docosahexaenoic acid

Changes in the serum levels of DHA (DHA on follow-up–DHA on ACS onset) are correlated with changes in the LDL-C (LDL-C on follow-up–LDL-C on ACS onset).

both EPA and DHA inhibit the proliferation/migration of vascular smooth muscle cells<sup>26–28</sup>, vascular inflammation<sup>29</sup>, neovascularization<sup>30, 31</sup>, and collagen-induced platelet aggregation<sup>25, 32</sup>. Thus, n-3 PUFA, including EPA and DHA, could reduce the incidence of ISR. However, because the effects of EPA and DHA on atherosclerosis are overlapping, the differential effects between DHA and EPA have been less well established. In an animal study, we showed that when combined with EPA, DHA treatment has additional anti-inflammatory and anti-atherosclerotic effects on Apoe<sup>-/-</sup> mice fed with a Western-type diet<sup>29</sup>. The result indicates that DHA can potentially prevent coronary atherosclerosis or ISR even when the EPA level is not low. Thus, EPA and/or DHA supplementation can potentially prevent ISR. However, no clinical studies have evaluated the effects of PUFA, including EPA and DHA on ISR. Prior to the stent-era, the accumulated evidence showed no beneficial effect of fish oils, including PUFA on coronary restenosis treated with balloon angioplasty in patients with stable angina; however, previous meta-analyses revealed a positive effect of fish oils on coronary restenosis<sup>33, 34</sup>. The CART study reported that initiating treatment with PUFA (2.7 g/d of EPA and 2.3 g/d of DHA) at least 2 weeks prior to an elective coronary balloon angioplasty for 6 months after did not prevent restenosis<sup>35</sup>. Similarly, the EMPAR Study and another study reported that fish oils (3.2 g/d of EPA and 2.2 g/d of DHA) and high doses of fish oils (4.1 g/d of EPA and 2.8 g/d of DHA), respectively, starting at least 1 or 2 weeks prior to an elective coronary balloon angioplasty did not prevent restenosis<sup>36, 37</sup>. This indicated that 1–2 weeks of PUFA therapy prior to coronary balloon an-

**Table 4.** Single regression analysis for in-stent restenosis in patients newly treated with statins

| Variables  | Univariate        |                 |
|--|-------------------|-----------------|
|  | OR (95% CI)       | <i>p</i> -value |
| Age  | 1.01 (0.97, 1.04) | 0.64            |
| Male   | 1.46 (0.60, 3.84) | 0.42            |
| Body mass index                                    | 1.02 (0.92, 1.14) | 0.70            |
| Hyperetension                                      | 1.00 (0.45, 2.28) | 0.99            |
| Type 2 diabetes eGFR (mL/min/1.73 m <sup>2</sup> ) | 0.94 (0.40, 2.12) | 0.88            |
| Stent  |                   |                 |
| Drug eluting stents                                | 0.68 (0.26, 1.66) | 0.42            |
| Diameter   | 0.63 (0.25, 1.56) | 0.33            |
| Length   | 0.93 (0.84, 1.02) | 0.13            |
| On admission                                       |                   |                 |
| TG (mg/dL)   | 1.00 (0.99, 1.00) | 0.46            |
| HDL-C (mg/dL)                                      | 1.00 (0.97, 1.03) | 0.84            |
| LDL-C (mg/dL)                                      | 1.00 (0.99, 1.01) | 0.75            |
| HbA1c (%)  | 1.05 (0.87, 1.53) | 0.32            |
| EPA (µg/mL)  | 0.98 (0.97, 0.99) | 0.02            |
| DHA (µg/mL)  | 0.98 (0.97, 1.00) | 0.06            |
| AA (µg/mL)   | 1.00 (0.99, 1.01) | 0.33            |
| EPA/AA   | 0.07 (0.01, 0.63) | 0.03            |
| DHA/AA   | 0.26 (0.05, 0.97) | 0.09            |
| Follow-up  |                   |                 |
| TG (mg/dL)   | 1.00 (0.99, 1.01) | 0.69            |
| HDL-C (mg/dL)                                      | 0.99 (0.95, 1.02) | 0.46            |
| LDL-C (mg/dL)                                      | 1.00 (0.97, 1.01) | 0.60            |
| HbA1c (%)  | 0.98 (0.59, 1.52) | 0.92            |
| EPA (µg/mL)  | 1.00 (0.98, 1.01) | 0.71            |
| DHA (µg/mL)  | 0.99 (0.98, 1.00) | 0.10            |
| AA (µg/mL)   | 1.00 (0.99, 1.00) | 0.39            |
| EPA/AA   | 0.83 (0.15, 2.93) | 0.79            |
| DHA/AA   | 0.63 (0.16, 1.85) | 0.45            |

Abbreviations as in Table 1.

gioplasties may not be long enough to prevent restenosis. Thus, further research is needed to evaluate the effects of PUFA on ISR especially in ACS patients at high risk of ISR.

In this study, follow-up coronary angiographies revealed that the serum levels of DHA were reduced in patients on statin therapy with a parallel reduction in LDL-C levels. In contrast, this was not observed with EPA. Statin therapy and diet reportedly modulate PUFA composition. Jula *et al.* reported that simvastatin significantly reduced the serum levels of DHA in patients with hyperlipidemia when compared to placebo; however it did not reduce the EPA levels<sup>15</sup>. Additionally, Nozue *et al.* reported that pitavastatin decreased serum DHA/AA ratio in patients with CV diseases, whereas it did not decrease EPA/AA ratio<sup>38</sup>. Although the mechanism by which statins reduce serum levels of

DHA has not been fully elucidated, it is speculated that statins can modulate the enzyme activity of PUFA synthesis, including fatty acid desaturases 1 and 2 and elongation of very long chain fatty acids protein 5<sup>39</sup>. Compared to EPA, DHA predominantly circulates in the blood. In addition, statins reduce LDL-C, a lipoprotein that includes EPA and DHA. Thus, higher concentrations of DHA in the serum may be more susceptible to a statin-induced LDL-C lowering.

Several studies reported that, in patients with myocardial infarctions, PUFA intake can decrease CV events. The DART study reported that, when compared to reducing fat intake and increasing cereal fiber intake in patients with myocardial infarction, eating fatty fish reduced the 2-year all-cause mortality by 29%<sup>40</sup>. The GISSI-Prevenzione study reported that PUFA intake resulted in a significant reduction in the total and CV-

**Table 5.** Multivariate regression analysis for in-stent restenosis in patients newly treated with statins

| Variables                          | Multivariate      |          |                   |          |                   |          |                   |          |
|------------------------------------|-------------------|----------|-------------------|----------|-------------------|----------|-------------------|----------|
|                                    | Model 1           |          | Model 2           |          | Model 3           |          | Model 4           |          |
|                                    | OR (95% CI)       | <i>p</i> | OR (95% CI)       | <i>p</i> | OR (95% CI)       | <i>p</i> | OR (95% CI)       | <i>p</i> |
| Age (y.o.)                         | 1.00 (0.98, 1.05) | 0.46     | 1.01 (0.97, 1.05) | 0.58     | 1.02 (0.98, 1.06) | 0.38     | 1.02 (0.98, 1.06) | 0.43     |
| Hyperetension                      | 1.14 (0.47, 2.83) | 0.76     | 1.13 (0.47, 2.79) | 0.78     | 0.99 (0.41, 2.43) | 0.97     | 0.94 (0.39, 2.31) | 0.90     |
| Type 2 diabetes                    | 0.83 (0.34, 1.98) | 0.68     | 0.81 (0.33, 1.92) | 0.64     | 0.88 (0.36, 2.10) | 0.78     | 0.90 (0.36, 2.13) | 0.90     |
| eGFR (mL/min/1.73 m <sup>2</sup> ) | 1.00 (0.98, 1.03) | 0.63     | 1.00 (0.98, 1.02) | 0.81     | 1.00 (0.98, 1.03) | 0.70     | 1.00 (0.98, 1.02) | 0.93     |
| Stents                             |                   |          |                   |          |                   |          |                   |          |
| Drug eluting stents                | 0.57 (0.20, 1.53) | 0.29     | 0.48 (0.17, 1.30) | 0.17     | 0.61 (0.21, 1.62) | 0.34     | 0.55 (0.19, 1.45) | 0.24     |
| Diameter (mm)                      | 0.68 (0.23, 1.91) | 0.41     | 0.62 (0.21, 1.74) | 0.38     | 0.71 (0.24, 1.98) | 0.51     | 0.67 (0.23, 1.84) | 0.44     |
| Length (mm)                        | 0.93 (0.83, 1.03) | 0.11     | 0.93 (0.84, 1.04) | 0.22     | 0.92 (0.82, 1.02) | 0.14     | 0.93 (0.83, 1.03) | 0.17     |
| On admission                       |                   |          |                   |          |                   |          |                   |          |
| EPA (µg/mL)                        | 0.98 (0.96, 0.99) | 0.02     |                   |          |                   |          |                   |          |
| DHA (µg/mL)                        |                   |          | 0.98 (0.97, 1.00) | 0.05     |                   |          |                   |          |
| EPA/AA                             |                   |          |                   |          | 0.06 (0.01, 0.56) | 0.03     |                   |          |
| DHA/AA                             |                   |          |                   |          |                   |          | 0.22 (0.04, 1.00) | 0.07     |

| Variables                          | Multivariate      |          |                   |          |                   |          |                   |          |
|------------------------------------|-------------------|----------|-------------------|----------|-------------------|----------|-------------------|----------|
|                                    | Model 5           |          | Model 6           |          | Model 7           |          | Model 8           |          |
|                                    | OR (95% CI)       | <i>p</i> | OR (95% CI)       | <i>p</i> | OR (95% CI)       | <i>p</i> | OR (95% CI)       | <i>p</i> |
| Age (y.o.)                         | 1.02 (0.97, 1.05) | 0.60     | 1.02 (0.98, 1.06) | 0.38     | 1.01 (0.97, 1.05) | 0.62     | 1.01 (0.98, 1.05) | 0.47     |
| Hyperetension                      | 0.99 (0.41, 2.39) | 0.97     | 1.10 (0.46, 2.70) | 0.83     | 0.96 (0.41, 2.33) | 0.93     | 0.99 (0.41, 2.39) | 0.97     |
| Type 2 diabetes                    | 0.83 (0.34, 1.94) | 0.67     | 0.78 (0.31, 1.84) | 0.57     | 0.78 (0.31, 1.84) | 0.72     | 0.86 (0.35, 2.02) | 0.73     |
| eGFR (mL/min/1.73 m <sup>2</sup> ) | 1.00 (0.98, 1.02) | 0.77     | 1.00 (0.98, 1.02) | 0.17     | 1.00 (0.98, 1.02) | 0.79     | 1.00 (0.98, 1.02) | 0.80     |
| Stents                             |                   |          |                   |          |                   |          |                   |          |
| Drug eluting stents                | 0.55 (0.19, 1.44) | 0.24     | 0.54 (0.18, 1.47) | 0.25     | 0.55 (0.19, 1.45) | 0.25     | 0.56 (0.19, 1.46) | 0.25     |
| Diameter (mm)                      | 0.58 (0.20, 1.62) | 0.31     | 0.50 (0.17, 1.44) | 0.41     | 0.58 (0.20, 1.62) | 0.32     | 0.59 (0.21, 1.63) | 0.32     |
| Length (mm)                        | 0.93 (0.98, 1.01) | 0.15     | 0.93 (0.83, 1.02) | 0.11     | 0.93 (0.84, 1.03) | 0.16     | 0.93 (0.83, 1.02) | 0.15     |
| On follow-up                       |                   |          |                   |          |                   |          |                   |          |
| EPA (µg/mL)                        | 1.00 (0.98, 1.01) | 0.51     |                   |          |                   |          |                   |          |
| DHA (µg/mL)                        |                   |          | 0.98 (0.98, 0.99) | 0.04     |                   |          |                   |          |
| EPA/AA                             |                   |          |                   |          | 0.71 (0.10, 2.93) | 0.69     |                   |          |
| DHA/AA                             |                   |          |                   |          |                   |          | 0.46 (0.08, 1.66) | 0.30     |

Abbreviations as in Table 1.

related mortality in addition to sudden cardiac death in patients following myocardial infarctions<sup>41</sup>). This suggests that administration of PUFA may prevent CV events, including ISR in patients who started statin therapy following the onset of ACS.

This study has several limitations. First, we used a retrospective design with a small sample size at a single center. Second, we did not evaluate the dietary intake of PUFA. Third, we excluded patients who did not receive follow-up catheter examinations, which may have led to a patient selection bias. Therefore, larger, prospective studies are needed to validate the effects of PUFA on ISR.

In conclusion, statin therapy decreased the serum

levels of DHA in parallel with a reduction in LDL-C levels in patients with ACS. Decreased DHA level after statin therapy and low eicosapentaenoic acid level on admission are risk factors for ISR, which indicated that decreased serum level of DHA may be a residual target for the prevention of ISR in patients with ACS.

### Conflicts of Interest

M. Sata received research funding from Tanabe-Mitsubishi, Takeda, Astellas, Bayer Healthcare, Daiichi-Sankyo, MSD, and Ono, and lecture fees from Astellas, Boehringer Ingelheim, Bayer Healthcare, Mochida, Takeda, Tanabe-Mitsubishi, Novartis, AstraZeneca, MSD,



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