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

Shusuke Yagi¹-², Daisuke Kondo³, Takayuki Ise¹, Daiju Fukuda⁴, Koji Yamaguchi¹, Tetsuso Wakatsuki¹, Yutaka Kawabata¹, Hiroyuki Ito¹, Yoshihiro Saijo¹, Hiromitsu Seno¹, Kumiko Sutou¹, Rie Ueno¹, Takaumi Todoroki¹, Kenya Kusunose¹, Tomomi Matsuura¹, Takeshi Tobiume¹, Hirotsugu Yamada¹, Takeshi Soeki¹, Michio Shimabukuro⁴-⁵, Ken-ichi Alhara⁶, Masashi Akaike⁷ and Masataka Sata¹

Shusuke Yagi and Daisuke Kondo contributed equally to this work.

¹ Department of Cardiovascular Medicine, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan
² Department of Community Medicine and Human Resource Development, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan
³ Student Laboratory, Faculty of Medicine, Tokushima University, Tokushima, Japan
⁴ Department of Cardio-Diabetes Medicine, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan
⁵ Department of Diabetes, Endocrinology and Metabolism, School of Medicine, Fukushima Medical University, Fukushima, Japan
⁶ Department of Community Medicine for Diabetes and Metabolic Disorders, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan
⁷ Department of Medical Education, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan

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)¹-⁴. 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 mortality⁵.⁶. In addition,
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. This may be attributed to the pleiotropic effects of PUFA including vascular anti-inflammatory effects and inhibitory effects on neointimal proliferation, 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.

Statin therapy is established for the primary and secondary prevention of CV events. In addition, they are speculated to modulate the activity of PUFA synthesis and to decrease the levels of EPA and DHA. 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 information 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). 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.

ISR was defined as binary angiographic restenosis when ≥ 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 ≥ 70 percent of luminal narrowing at follow-up angiography was detected.
ter examination. The serum samples were stored at −30°C until assayed. Gas-liquid chromatography at a commercially available laboratory (SRL, Tokyo, Japan) detected 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 catheter examination. The serum samples were stored at −30°C until assayed. Gas-liquid chromatography at a commercially available laboratory (SRL, Tokyo, Japan) detected even in the absence of clinical symptoms or signs.

---

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/m²)    | 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 m²)      | 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
was used to measure the serum composition of PUFA, including the levels of EPA, DHA, and arachidonic acid (AA)\textsuperscript{8,9}. 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\textsuperscript{17}. 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 $7.3 \pm 1.9$ 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

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

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

Abbreviations as in Table 1.
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 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 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 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
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. In addition, the serum levels of PUFA are influenced by age. 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 and inflammatory changes in the form of macrophage accumulation, extensive neovascularization in response to stent-associated injuries and platelet aggregation. Previous experimental studies have shown that both EPA and DHA inhibit the proliferation/migration of vascular smooth muscle cells, vascular inflammation, neovascularization, and collagen-induced platelet aggregation. 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 \(-/-\) mice fed with a Western-type diet. 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. 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. 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. This indicated that 1–2 weeks of PUFA therapy prior to coronary balloon an-
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 539. 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%40. The GISSI-Prevenzione study reported that PUFA intake resulted in a significant reduction in the total and CV-

| Table 4. Single regression analysis for in-stent restenosis in patients newly treated with statins |
|-----------------------------------------------|
| Variables | Univariate |
| 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 |
| Hyper tension | 1.00 (0.45, 2.28) | 0.99 |
| Type 2 diabetes eGFR (mL/min/1.73 m²) | 0.94 (0.40, 2.12) | 0.88 |
| Stent | 0.68 (0.26, 1.66) | 0.42 |
| Drug eluting stents | 0.63 (0.25, 1.56) | 0.33 |
| Diameter | 0.93 (0.84, 1.02) | 0.13 |
| Length | 0.60 (1.00, 0.00) | 0.46 |
| On admission | 1.00 (0.97, 1.03) | 0.84 |
| TG (mg/dL) | 1.00 (0.99, 1.01) | 0.75 |
| HDL-C (mg/dL) | 1.05 (0.87, 1.53) | 0.32 |
| LDL-C (mg/dL) | 0.98 (0.97, 0.99) | 0.02 |
| HbA1c (%) | 0.98 (0.97, 1.00) | 0.06 |
| EPA (µg/mL) | 1.00 (0.99, 1.01) | 0.33 |
| DHA (µg/mL) | 0.07 (0.01, 0.63) | 0.03 |
| AA (µg/mL) | 0.26 (0.05, 0.97) | 0.09 |
| EPA/AA | 1.00 (0.99, 1.00) | 0.69 |
| DHA/AA | 0.99 (0.95, 1.02) | 0.46 |
| Follow-up | 1.00 (0.97, 1.01) | 0.60 |
| TG (mg/dL) | 0.98 (0.95, 1.52) | 0.92 |
| HDL-C (mg/dL) | 1.00 (0.98, 1.01) | 0.71 |
| LDL-C (mg/dL) | 0.99 (0.98, 1.00) | 0.10 |
| HbA1c (%) | 0.99 (0.99, 1.00) | 0.39 |
| EPA (µg/mL) | 0.83 (0.15, 2.93) | 0.79 |
| DHA (µg/mL) | 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 levels15. Additionally, Nozue et al. reported that pitavastatin decreased serum DHA/AA ratio in patients with CV diseases, whereas it did not decrease EPA/AA ratio38. 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 539. 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%40. The GISSI-Prevenzione study reported that PUFA intake resulted in a significant reduction in the total and CV-
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, related mortality in addition to sudden cardiac death in patients following myocardial infarctions. 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

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

| Variables                          | Model 1 OR (95% CI) | Model 2 OR (95% CI) | Model 3 OR (95% CI) | Model 4 OR (95% CI) |
|------------------------------------|---------------------|---------------------|---------------------|---------------------|
| Age (y.o.)                          | 1.00 (0.98, 1.05)    | 1.01 (0.97, 1.05)    | 1.02 (0.98, 1.06)    | 1.02 (0.98, 1.06)    |
| Hyperension                        | 1.14 (0.47, 2.83)    | 1.13 (0.47, 2.79)    | 0.99 (0.41, 2.43)    | 0.94 (0.39, 2.31)    |
| Type 2 diabetes                    | 0.83 (0.34, 1.98)    | 0.81 (0.33, 1.92)    | 0.88 (0.36, 2.10)    | 0.90 (0.36, 2.13)    |
| eGFR (mL/min/1.73 m²)              | 1.00 (0.98, 1.03)    | 1.00 (0.98, 1.02)    | 1.00 (0.98, 1.03)    | 1.00 (0.98, 1.02)    |
| Drug eluting stents                | 0.57 (0.20, 1.53)    | 0.48 (0.17, 1.30)    | 0.61 (0.21, 1.62)    | 0.55 (0.19, 1.45)    |
| Diameter (mm)                      | 0.68 (0.23, 1.91)    | 0.62 (0.21, 1.74)    | 0.71 (0.24, 1.98)    | 0.67 (0.23, 1.84)    |
| Length (mm)                        | 0.93 (0.83, 1.03)    | 0.93 (0.84, 1.04)    | 0.92 (0.82, 1.02)    | 0.93 (0.83, 1.03)    |
| EPA (µg/mL)                        | 0.98 (0.96, 0.99)    | 0.98 (0.97, 1.00)    | 0.98 (0.97, 1.00)    | 0.98 (0.96, 0.99)    |
| DHA (µg/mL)                        | 1.00 (0.98, 1.02)    | 1.00 (0.98, 1.02)    | 1.00 (0.98, 1.02)    | 1.00 (0.98, 1.02)    |
| EPA/AA                             | 0.96 (0.41, 2.33)    | 0.96 (0.41, 2.33)    | 0.96 (0.41, 2.33)    | 0.96 (0.41, 2.33)    |
| DHA/AA                             | 0.93 (0.83, 1.03)    | 0.93 (0.84, 1.04)    | 0.92 (0.82, 1.02)    | 0.93 (0.83, 1.03)    |

**Abbreviations as in Table 1.**
and Shionogi. The Department of Cardio-Diabetes Medicine, Tokushima University Graduate School, is supported in part by unrestricted research grants from Actelion, Boehringer Ingelheim, Kowa, and Tanabe-Mitsubishi. The others declare no conflict of interest.

Acknowledgments

This work was partially supported by JSPS Kakenhi Grants (grant numbers 18K08040, 16H05299, and 26248050), the Fugaku Trust for Medical Research, and Takeda Science Foundation. We thank the staff of the Hospital Information Center at Tokushima University Hospital for extracting clinical data from medical records. We would like to thank Editage (www.editage.jp) for the English language editing.

References

1) Feinberg J, Nielsen EE, Greenhalgh J, Houssone J, Sethi NJ, Safi S, Gluud C and Jakobsen JC: Drug-eluting stents versus bare-metal stents for acute coronary syndrome. The Cochrane database of systematic reviews, 2017; 8: CD012481
2) Raber L, Kelbaek H, Ostojic M, Baumbach A, Heg D, Fuller D, von Birgelen C, Roffi M, Moschovitis A, Khattab AA, Wenaweser P, Bonvini R, Pedrazzini G, Kornowski R, Weber K, Trelle S, Luscher TF, Taniwaki M, Matter CM, Meier B, Juni P, Windecker S and Investigators CAT: Effect of biolimus-eluting stents with biodegradable polymer vs bare-metal stents on cardiovascular events among patients with acute myocardial infarction: the COMFORT-ABLE AMI randomized trial. JAMA, 2012; 308: 777-787
3) Sabate M, Cequier A, Iniguez A, Hernandez-Gomez-Pinedo R, Jula A, Marniemi J, Ronnemaa T, Virtanen A and Huupponen P: Effects of diet and simvastatin on fatty acid composition in hypercholesterolemic men: a randomized controlled trial. Nutrition and Metabolism, 2012; 9: 162545
4) Kocka V, Maly M, Tousek P, Budesinsky T, Lisa L, Prodanov P, Jarkovsky J and Widimsky P: Biodegradable vascular scaffolds in acute ST-segment elevation myocardial infarction: a prospective multicentre study ‘Prague 19’. Eur Heart J, 2014; 35: 787-794
5) Amano T, Matsubara T, Uetani T, Kato M, Kato B, Yoshida T, Harada K, Kumagai S, Kunimura A, Shinozaki Y, Kitagawa K, Ishii H and Murohara T: Impact of omega-3 polyunsaturated fatty acids on coronary plaque instability: an integrated backscatter intravascular ultrasound study. Atherosclerosis, 2011; 218: 110-116
6) Hara M, Sakata Y, Nakatani D, Suna S, Usami M, Matsumoto S, Hamasaki T, Doi Y, Nishino M, Sato H, Kitamura T, Nanto S, Hori M, Komuro I and Osaka Acute Coronary Insufficiency Study I: Low levels of serum n-3 polyunsaturated fatty acids are associated with worse heart failure-free survival in patients after acute myocardial infarction. Circulation journal: official journal of the Japanese Circulation Society, 2013; 77: 153-162
7) Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K and Japan EPAllis: Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet, 2007; 369: 1090-1098
8) Matsuzaki M, Yokoyama M, Saito Y, Origasa H, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K, Matsuzawa Y and Japan JFL: Incremental Effects of Eicosapentaenoic Acid on Cardiovascular Events in Statin-Treated Patients With Coronary Artery Disease - Secondary Prevention Analysis From JELIS. Circulation Journal, 2009; 73: 1283-1290
9) Ross R: Atherosclerosis—an inflammatory disease. The New England journal of medicine, 1999; 340: 115-126
10) Yagi S, Fukuda D, Aihara KI, Aikaie M, Shimabukuro M and Sata M: n-3 Polyunsaturated Fatty Acids: Promising Nutrients for Preventing Cardiovascular Disease. Journal of atherosclerosis and thrombosis, 2017; 24: 999-1010
11) Yagi S, Aihara K, Ikeda Y, Sumitomo Y, Yoshida S, Ise T, Iwase T, Ishikawa K, Azuma H, Aikaiie M and Matsumo T: Pitavastatin, an HMG-CoA reductase inhibitor, exerts eNOS-independent protective actions against angiotensin II induced cardiovascular remodeling and renal insufficiency. Circulation research, 2008; 102: 68-76
12) Yagi S, Aikaiie M, Aihara K, Ishikawa K, Iwase T, Ikeda Y, Soeki T, Yoshida S, Sumitomo-Ueda Y, Matsumoto T and Sata M: Endothelial nitric oxide synthase-independent protective action of statin against angiotensin II-induced atrial remodeling via reduced oxidant injury. Hypertension, 2010; 55: 918-923
13) Filion KB, El Khoury F, Bielinski M, Schiller I, Dendukuri N and Brophy JM: Omega-3 fatty acids in high-risk cardiovascular patients: a meta-analysis of randomized controlled trials. BMC cardiovascular disorders, 2010; 10: 24
14) Yagi S, Aihara K, Ikeda Y, Aikaie M, Sata M and Matsumoto T: Effects of statins on cardiorenal syndrome. International journal of vascular medicine, 2012; 2012: 162545
15) Jula A, Mariniaki J, Ronnemaa T, Virnætun A and Huupponen R: Effects of diet and simvastatin on fatty acid composition in hypercholesterolemic men: a randomized controlled trial. Arteriosclerosis, thrombosis, and vascular biology, 2005; 25: 1952-1959
16) Dangas GD, Claessen BE, Caixeta A, Sanidas EA, Mintz GS and Mehran R: In-stent restenosis in the drug-eluting stent era. J Am Coll Cardiol, 2010; 56: 1897-1907
17) Yagi S, Aihara K, Fukuda D, Takashima A, Bando M, Hara T, Nishimoto S, Ise T, Kusunose K, Yamaguchi K, Tobiume T, Iwase T, Yamada H, Soeki T, Wakatsuki T, Shimabukuro M, Aikaie M and Sata M: Reduced ratio of eicosapentaenoic acid and docosahexaenoic acid to arachidonic acid is associated with early onset of acute coronary syndrome. Nutr J, 2015; 14: 111
18) Kastrati A, Schomig A, Elezi S, Schuhlen H, Dirschinger J, Hadamitzky M, Wehinger A, Hausleiter J, Walter H and Neumann FJ: Predictive factors of restenosis after coronary stent placement. J Am Coll Cardiol, 1997; 30:
19) Kobayashi Y, De Gregorio J, Kobayashi N, Akiyama T, Reimers B, Finci L, Di Mario C and Colombo A: Stented segment length as an independent predictor of restenosis. J Am Coll Cardiol, 1999; 34: 651-659
20) de Feyter PJ, Kay P, Disco C and Serruys PW: Reference chart derived from post-stent-implantation intravascular ultrasound predictors of 6-month expected restenosis on quantitative coronary angiography. Circulation, 1999; 100: 1777-1783
21) Latif F, Kleinman NS, Cohen DJ, Pencina MJ, Yen CH, Cutlip DE, Moliterno DJ, Nassif D, Lopez JJ, Saucedo JF and Investigators E: In-hospital and 1-year outcomes among percutaneous coronary intervention patients with chronic kidney disease in the era of drug-eluting stents: a report from the EVENT (Evaluation of Drug Eluting Stents and Ischemic Events) registry. JACC Cardiovasc Interv, 2009; 2: 37-45
22) Sata M, Saiura A, Kunisato A, Tojo A, Okada S, Tokuhisa T, Hirai H, Makuuchi M, Hirata Y and Nagai R: Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. Nat Med, 2002; 8: 403-409
23) Goto K, Zhao Z, Matsumura M, Dohi T, Kobayashi N, Kirtane AJ, Rabbani LE, Collins MB, Parikh MA, Kodali SK, Leon MB, Moses JW, Mintz GS and Maehara A: Mechanisms and Patterns of Intravascular Ultrasound In-Stent Restenosis Among Bare Metal Stents and First- and Second-Generation Drug-Eluting Stents. The American journal of cardiology, 2015; 116: 1351-1357
24) Hoffmann R, Mintz GS, Dussaillant GR, Popma JJ, Pichard AD, Satler LF, Kent KM, Griffin J and Leon MB: Patterns and mechanisms of in-stent restenosis. A serial intravascular ultrasound study. Circulation, 1996; 94: 1247-1254
25) Takada K, Ishikawa S, Yokoyama N, Hosogoe N and Ishihiki T: Effects of eicosapentaenoic acid on platelet function in patients taking long-term aspirin following coronary stent implantation. International heart journal, 2014; 55: 228-233
26) Shiina T, Terano T, Saito J, Tamura Y and Yoshida S: Eicosapentaenoic acid and docosahexaenoic acid suppress the proliferation of vascular smooth muscle cells. Atherosclerosis, 1993; 104: 95-103
27) Terano T, Shiina T and Tamura Y: Eicosapentaenoic acid suppressed the proliferation of vascular smooth muscle cells through modulation of various steps of growth signals. Lipids, 1996; 31: S301-S304
28) Mizutani M, Asano M, Roy S, Nakajima T, Soma M, Yamashita K and Okuda Y: omega-3 polyunsaturated fatty acids inhibit migration of human vascular smooth muscle cells in vitro. Life Sci, 1997; 61: P269-P274
29) Takashima A, Fukuda D, Tanaka K, Higashikuni Y, Hirata Y, Nishimoto S, Yagi S, Yamada H, Soeki T, Wakatsuki T, Taketani Y, Shimabukuro M and Sata M: Combination of n-3 polyunsaturated fatty acids reduces atherogenesis in apolipoprotein E-deficient mice by inhibiting macrophage activation. Atherosclerosis, 2016; 254: 142-150
30) Kanayasu T, Morita I, Nakao-Hayashi J, Asuwa N, Fujisawa C, Ishii T, Ito H and Murota S: Eicosapentaenoic acid inhibits tube formation of vascular endothelial cells in vitro. Lipids, 1991; 26: 271-276
31) Zhang G, Panigrahy D, Mahakian LM, Yang J, Liu JY, Wettersten HI, Ulu A, Hu X, Tam S, Hwang SH, Ingham ES, Kieran MW, Weiss RH, Ferrara KW and Hammock BD: Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis. Proc Natl Acad Sci U S A, 2013; 110: 6530-6535
32) Nelson GJ, Schmidt PS, Bartolini GL, Kelley DS and Kyle D: The effect of dietary docosahexaenoic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. Lipids, 1997; 32: 1129-1136
33) O’Connor GT, Malenka DJ, Olmstead EM, Johnson PS and Hennekens CH: A meta-analysis of randomized trials of fish oil in prevention of restenosis following coronary angioplasty. American journal of preventive medicine, 1992; 8: 186-192
34) Gapinski JP, VanRuiswyk JV, Heudebert GR and Schectman GS: Preventing restenosis with fish oils following coronary angioplasty. A meta-analysis. Archives of internal medicine, 1993; 153: 1595-1601
35) Johansen O, Brekke M, Seljeffot I, Abdelnoor M and Arnesen H: n-3 fatty acids do not prevent restenosis after coronary angioplasty: Results from the CART study. J Am Coll Cardiol, 1999; 33: 1619-1626
36) Cairns JA, Gill J, Morton B, Roberts R, Gent M, Hirsh J, Holder D, Finnie K, Marquis JF, Naqvi S and Cohen E: Fish oils and low-molecular-weight heparin for the reduction of restenosis after percutaneous transluminal coronary angioplasty. The EMPAR Study. Circulation, 1996; 94: 1553-1560
37) Leaf A, Jorgensen MB, Jacobs AK, Cote G, Schoenfeld DA, Scheer J, Weiner BH, Slack JD, Kellett MA, Raizner AE and et al.: Do fish oils prevent restenosis after coronary angioplasty? Circulation, 1994; 90: 2248-2257
38) Nozue T, Yamamoto S, Tohyama S, Fukui K, Umezawa S, Onishi Y, Kunishima T, Sato A, Nozato T, Miyake S, Takeyama Y, Morino Y, Yamauchi T, Muramatsu T, Hibi K and Michishita I: Effects of statins on serum n-3 to n-6 polyunsaturated fatty acid ratios in patients with coronary artery disease. Journal of cardiovascular pharmacology and therapeutics, 2013; 18: 320-326
39) Ishihara N, Suzuki S, Tanaka S, Watanabe Y, Nagayama D, Saiki A, Tanaka T and Tatsuno I: Atorvastatin increases Fads1, Fads2 and Elov5 gene expression via the geranyl-geranyl pyrophosphate-dependent Rho kinase pathway in 3T3-L1 cells. Mol Med Rep, 2017; 16: 4756-4762
40) Burr ML, Feihly AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC and Deadman NM: Effects of changes in fat, fish, and fibre intakes on death and myocardial infarction in the multi-centre cohort study derived from post-stent-implantation intravascular ultrasound predictors of 6-month expected restenosis on quantitative coronary angiography. Circulation, 1999; 354: 447-455

Statins Reduce DHA in ACS