ORIGINAL PAPER

Nagoya J. Med. Sci. **85**. 592–601, 2023
doi:10.18999/nagjms.85.3.592

Role of serum n-6 polyunsaturated fatty acids in the development of acute coronary syndromes

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ABSTRACT

n-3 polyunsaturated fatty acids (PUFAs) have an inhibitory effect on the development of coronary artery disease (CAD). However, whether n-6 PUFAs, dihomo-gamma-linolenic acid (DGLA), and arachidonic acid (AA) play a role in the development of CAD remains unclear. This study investigated the association between PUFAs and the risk of developing acute coronary syndrome (ACS) using the lipid and PUFAs data of patients who received percutaneous coronary intervention (PCI) for either non-emergent conditions (staged group) or ACS (ACS group). We retrospectively evaluated 433 patients who underwent PCI between 2014 and 2021. The patients were divided into the ACS group (n = 18) and the staged group (n = 132). The lipid and PUFA values of each patient between the two groups were compared. Moreover, to investigate the correlation between n-6 PUFA levels and ACS, the effects of confounding factors such as the use of strong statins and low-density lipoprotein cholesterol (LDL-C) levels were adjusted. The ACS group had higher n-6 PUFAs levels than the staged group (DGLA: 36.8 μg/mL vs 29.6 μg/mL; AA: 203.3 μg/mL vs 145.8 μg/mL). Furthermore, the analysis of covariance adjusted for LDL-C levels showed a significant difference between the two groups in terms of DGLA and AA levels. The n-3 PUFA levels did not significantly differ between the staged and ACS groups. Moreover, the ACS group had higher DGLA and AA levels and lower n-3 PUFAs/AA ratios than the staged group. Therefore, excess n-6 PUFAs may be a risk factor for ACS.

Keywords: acute coronary syndrome, n-3 polyunsaturated fatty acids, n-6 polyunsaturated fatty acids, dihomo-gamma-linolenic acid, arachidonic acid

Abbreviations:
AA: arachidonic acid
ACS: acute coronary syndrome
DGLA: dihomo-gamma-linolenic acid
DHA: docosahexaenoic acid
EPA: eicosapentaenoic acid
PUFAs: polyunsaturated fatty acids

Received: May 2, 2022; accepted: November 1, 2022

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INTRODUCTION

With the advent of a super-aging society in Japan, the proportion of young people with acute coronary syndrome (ACS), has relatively decreased due to the increasing number of elderly people. On the contrary, the age-adjusted incidence of acute myocardial infarction (AMI) over the past 30 years has been increasing particularly among men.[1] In addition, the clinical characteristics and outcomes of ACS among individuals aged below 45 years differ from those of middle-aged and older patients due to differences in lifestyle habits including diet.[2] Therefore, the new risk factors for ACS correlated with lifestyle habits, particularly diet, should be identified.

Several investigators have shown that the relationship between coronary artery disease (CAD) and polyunsaturated fatty acids (PUFAs) converted from essential fatty acids such as linoleic acid (LA) and α-linolenic acid.[3-8] First, low serum n-3 PUFA levels are associated with a high incidence of cardiovascular events and mortality.[3,4] In particular, the eicosapentaenoic acid (EPA)/arachidonic acid (AA) ratio is a good indicator of cardiovascular events even in patients with diabetes mellitus who experienced post-myocardial infarction, as it is not affected by statins.[5] In addition, a low EPA/AA ratio is significantly correlated with the development of ACS.[6]

However, previous reports have focused on increasing the serum concentrations of n-3 PUFAs from seafood, plant sources, and supplements and, consequently, reducing the risk of developing CAD by preventing a decrease in the EPA/AA ratio.[3,4,6] Interestingly, one of these results showed that biomarkers of n-3 PUFAs are associated with a lower risk of incident fatal CAD but not nonfatal MI.[7] Furthermore, in a systematic review of the association between n-6 polyunsaturated fatty acids and CAD, the benefit of increasing n-6 remains unclear.[8]

This case-control study aimed to compare the PUFA levels between the ACS and staged groups. By comprehensively comparing serum n-6 PUFAs as well as n-3 PUFAs and multiple lipid levels and their respective ratios and the use of strong statins, we examined the use of serum n-6 PUFAs to prevent ACS.

PATIENTS AND METHODS

Patient population

This is a retrospective observational study conducted at a single institution (Chutoen General Medical Center, Kakegawa, Shizuoka, Japan). In total, 433 patients with ischemic heart disease who underwent optical coherence tomography (OCT)-guided percutaneous coronary intervention (PCI) at our institution from September 2014 to September 2021 were retrospectively evaluated. ACS included AMI and unstable angina. AMI was defined as a transient increase in the MB fraction of creatine kinase to a threshold that is three times higher than the 99th percentile of the upper reference limit after PCI with ischemic symptoms or typical electrocardiographic changes. Unstable angina was defined as angina at rest and accelerated exertional angina combined with typical electrographic changes. The exclusion criteria were patients with lipid and PUFA data examined on a day different from the day of PCI. Patients with ACS for more than 24 h after onset, stable circulatory status, and concomitant heart failure were excluded from the analysis based on the Japanese Circulation Society 2018 Guideline on the Diagnosis and Treatment of Acute Coronary Syndrome. Patients who developed ACS and underwent PCI during the study observation period were excluded from the staged group if they underwent staged PCI during the same period. The patients (n = 150) were divided into two groups for the final analysis: those who underwent PCI for non-emergent conditions (n = 132, staged group) and those who underwent PCI for ACS (n = 18, ACS group).

This study was performed by the standards of the Declaration of Helsinki and the current ethical guidelines, and it was approved by the institutional ethics board (Keni 166). Written informed consent was not obtained from the patients because this was not a clinical trial and data were retrospective in nature and were analyzed anonymously.

Assessment of lipid, PUFA, and MDA-LDL levels

The serum triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were assessed using standard laboratory procedures. To evaluate serum PUFA levels, a 10-mL blood sample was collected in a heparinized blood collection tube at the end of PCI. Serum samples were stored at −80 °C until measurement. The samples were then sent to a subcontractor (BML, Hamamatsu, Japan) on the same day for the analysis of fatty acid composition via gas chromatography. Blood samples were also collected and cryopreserved at the end of PCI to measure malondialdehyde-modified low-density lipoprotein (MDA-LDL) levels. Serum samples were collected after the blood had completely coagulated, frozen at −20 °C, and sent to a subcontractor for analysis (BML Corporation, Hamamatsu, Japan).

Treatment

OCT-guided PCI was performed. Patients commonly received aspirin (100 mg/day). Moreover, some were treated with ticlopidine (200 mg/day), clopidogrel (75 mg/day), or prasugrel (3.75 mg/day). Antiplatelet medication was not administered, except in a few patients in the ACS group. All patients were implanted with drug-eluting stents. Hence, two antiplatelet drugs (loading dose; aspirin 200 mg/day and prasugrel 20 mg/day or clopidogrel 300 mg/day) were administered at the end of PCI.

Statistical analyses

Continuous variables that follow a normal distribution were expressed as mean ± standard deviation and do not follow a normal distribution were expressed as median (interquartile ranges), as appropriate. Categorical variables were presented as numbers and percentages. Between-group comparisons were performed using the Mann–Whitney U test for continuous variables. Variables with a p-value of 0.05 in the univariate analysis were entered into the multivariate analysis. Multivariate analysis was performed via logistic regression analysis. The role of dihomo-gamma-linolenic acid (DGLA) and AA on the development of ACS was evaluated, with consideration of the difference in LDL-C, DGLA, AA, and LDL-C levels, which were natural log-transformed for the statistical analyses of covariance (ANCOVA). A p-value of < 0.05 was considered significant. All statistical analyses were performed with EZR[9] for R. More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.

In this study, because there was a difference in the number of patients in the ACS and staged groups, we calculated power using EZR as a post hoc validation. A p-value of 0.05 was considered statistically significant. The effect size was calculated, and the two-tailed test was performed. Missing values were analyzed only in samples with no missing values in the data required for individual statistical analysis.

RESULTS

Clinical characteristics of patients

Table 1 shows the baseline characteristics of the patients. The ACS group was younger than the staged group (57.8 ± 14.6 vs 68.4 ± 12.3 years). There was male predominance in both

groups. A previous history of PCI differed between the staged and ACS groups (5.9% vs 55.3%) but not diabetes mellitus, hypertension, and smokers. Table 1 shows differences in the use of antiplatelet and other oral medications. Moreover, CPK, Troponin I, LDH, and CRP levels remarkably differed.

Table 1 Baseline characteristics of participants

	ACS group (n = 18)	Staged group (n = 132)
Mean age ± SD, years	57.8 ± 14.6	68.4 ± 12.3
Male sex, n (%)	13 (72.2)	104 (78.8)
Diabetes mellitus, n (%)	8 (44.4)	64 (48.5)
Hypertension, n (%)	8 (44.4)	83 (62.9)
Smokers, n (%)	13 (72.2)	89 (67.4)
Previous coronary angioplasty, n (%)	1 (5.9)	73 (55.3)
Previous myocardial infarction, n (%)	1 (5.9)	48 (36.4)
Antiplatelet agent, n (%)	2 (11.1)	118 (89.4)
Strong statin, n (%)	5 (27.8)	91 (68.9)
Rosuvastatin 5 mg, n (%)	3 (60.0)	62 (68.1)
Atorvastatin 10 mg, n (%)	1 (20.0)	21 (23.1)
Pitavastatin 2 mg, n (%)	1 (20.0)	6 (6.6)
Pravastatin 10 mg, n (%)	0 (0.0)	2 (2.2)
Beta blocker, n (%)	1 (5.6)	53 (40.5)
ACE-I or ARB, n (%)	6 (33.3)	74 (56.1)
Insulin, n (%)	0 (0)	14 (10.6)
Creatinine level (mg/dL)	0.86 (0.67–0.95)	0.87 (0.73–1.04)
eGFR (mL/min/1.73 m^2)	70.9 (61.9–88.6)	65.3 (53.4–76.4)
Hemodialysis, n (%)	0 (0)	13 (9.8)
Hemoglobin level (g/dL)	14.5 ± 2.8	12.9 ± 1.8
HbA1c (%)	6.1 (5.8–7.8)	6.4 (5.8–7.1)
CRP level (mg/dL)	0.27 (0.05–0.76)	0.11 (0.04–0.34)
LDH level (IU/L)	259 (219–304)	183 (164–216)
CPK level (IU/L)	165 (93–264)	99 (71–143)
BNP level (pg/mL)	41.0 (15.3–84.1)	41.6 (14.1–165.8)
Troponin I level (ng/mL)	0.19 (0.04–0.56)	0.01 (0.01–0.04)

ACS: acute coronary syndrome
ACE-I: angiotensin-converting enzyme inhibitor
ARB: angiotensin II receptor blocker
eGFR: estimated glomerular filtration rate
CRP: C-reactive protein
LDH: lactate dehydrogenase
CPK: creatine phosphokinase
BNP: brain natriuretic peptide
Categorical variables were presented as number (%), and continuous variables as mean ± SD or median (interquartile ranges).

Comparison of each lipid and PUFAs levels in the staged and ACS groups

Table 2 shows the comparison of each lipid fraction between the ACS and staged groups. As shown in Table 2, there were no significant differences in triglyceride, HDL-C, EPA, and docosahexaenoic acid (DHA) levels. In addition, the ACS group had significantly higher total cholesterol, LDL-C, non-HDL-C, and MDA-LDL levels than the staged group, as shown in Table 2. Moreover, there was no statistical difference in terms of the levels of EPA and DHA, which are n-3 PUFAs (EPA: 38.9 vs 55.5, $p = 0.09$; DHA: 88.95 vs 98.25, $p = 0.86$, respectively). By contrast, the ACS group had significantly higher DGLA and AA levels than the staged group (DGLA: 36.75 vs 29.55, $p = 0.001$, AA: 203.30 vs 145.75, $p < 0.001$).

Table 2 Comparison of each lipid fraction between the ACS and staged groups

	ACS group (n = 18)	**Staged group (n = 132)**	**p-value**
Triglyceride level (mg/dL) [50–149]	112.5 (87.5–157.3)	90.5 (68.8–131.3)	0.13
Total cholesterol level (mg/dL) [150–219]	185 (160–216)	141 (120–167)	<0.001
HDL cholesterol level (mg/dL) [M:40–86, F:40–96]	40 (32–52)	39 (32–47)	0.65
LDL-cholesterol level (mg/dL) [70–139]	122.0 (91.0–140.8)	76.5 (64.0–100.0)	<0.001
non-HDL cholesterol level (mg/dL) [90–149]	138.5 (127.3–204.0)	101.0 (82.0–122.3)	<0.001
n-3 PUFAs			
EPA level (μg/mL) [9.0–128.5]	38.9 (28.3–59.4)	55.9 (33.1–82.5)	0.09
DHA level (μg/mL) [46.7–172.7]	89.0 (75.5–122.5)	98.3 (76.4–128.1)	0.86
n-6 PUFAs			
DGLA level (μg/mL) [16.7–58.2]	36.8 (30.7–45.5)	29.6 (22.0–36.5)	0.001
AA level (μg/mL) [112.7–237.9]	203.3(173.5–225.2)	145.8 (128.0–172.5)	<0.001
MDA-LDL level (mg/dL) [46–105]	109 (78–149)	76 (62–95)	0.003

ACS: acute coronary syndrome
HDL cholesterol: high-density lipoprotein cholesterol
LDL-cholesterol: low-density lipoprotein cholesterol
EPA: eicosapentaenoic acid
DHA: docosahexaenoic acid
DGLA: dihomo-gamma-linolenic acid
AA: arachidonic acid
MDA-LDL: malondialdehyde-modified low-density lipoprotein
PUFAs: polyunsaturated fatty acids
Data were presented as median (interquartile ranges) and [reference ranges].

EPA/AA, DHA/AA, EPA+DHA/AA, and AA/DGLA ratios

Table 3 shows the PUFA ratios. The ACS group had lower EPA, DHA, and EPA+DHA-to-AA ratios than the staged group, as shown in Table 3 (EPA/AA: $p = 0.003$, DHA/AA: $p = 0.008$, EPA+DHA/AA: $p = 0.005$). In addition, the AA/DGLA ratio, which was presented as the estimated delta-5 desaturase activity (D5D), did not significantly differ.

Table 3 Comparison of each PUFA ratio in the ACS and staged groups

	ACS group (n = 18)	Staged group (n = 132)	p-value
EPA/AA ratio [0.06–0.72]	0.23 (0.08–0.53)	0.38 (0.05–2.03)	0.003
DHA/AA ratio [0.27–1.07]	0.49 (0.25–1.24)	0.66 (0.11–2.35)	0.008
EPA+DHA/AA ratio [0.32–1.66]	0.75 (0.37–1.69)	1.08 (0.16–4.23)	0.005
AA/DGLA ratio	5.27 (4.31–6.20)	5.26 (4.30–6.67)	0.78

Data were presented as median (interquartile ranges). AA/DGLA ratio was expressed as estimated delta-5 desaturase activity. Study acronyms are explained in footnote to Table 2.

Effect of DGLA and AA on ACS with consideration of the difference in LDL-C levels and the use of strong statins

We performed ANCOVA to analyze the effect of DGLA and AA on the development of ACS, with consideration of the difference in LDL-C levels (Fig. 1A, B). Results showed statistically significant differences in DGLA and AA levels between the two groups based on ANCOVA adjusted for LDL-C levels (DGLA: $p = 0.02$, AA: $p = 0.003$).

In addition, univariate and multivariate analyses were performed to compare the DGLA and AA values and the n-3 PUFA/AA ratios between the ACS and staged groups with or without strong statin and LDL-C levels (Table 4). Hence, the adjusted ORs were 4.45 in DGLA levels (95% CI: 1.13–17.6, $p = 0.03$), 19.4 in AA levels (95% CI: 2.41–156, $p = 0.005$), and 0.26 in EPA/AA ratios (95% CI: 0.08–0.79, $p = 0.02$).

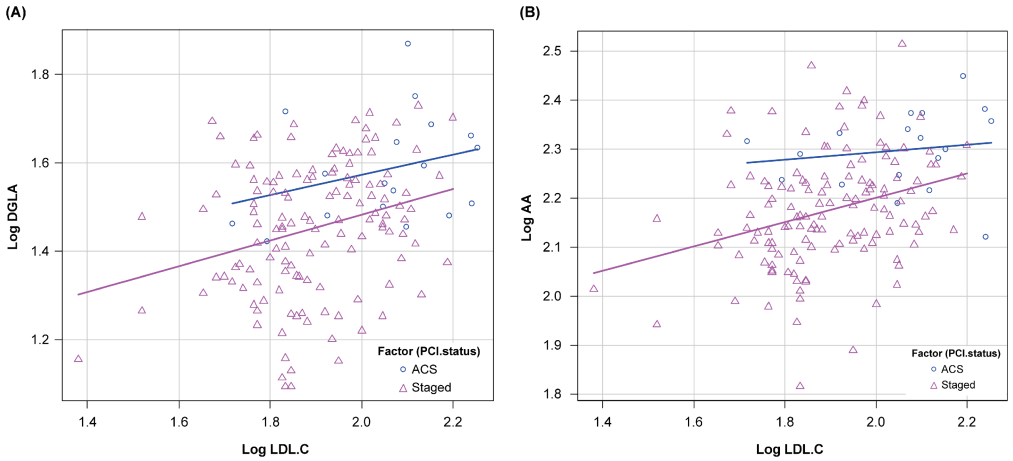

Fig. 1 Effect of DGLA and AA on ACS with consideration of the difference in LDL-C levels
An ANCOVA was performed to evaluate the effect of DGLA and AA levels on the development of ACS, with consideration of the difference in LDL-C levels. The DGLA (A) and AA levels (B) significant differences between the two groups based on ANCOVA adjusted for LDL-C levels (DGLA: $p = 0.02$, AA: $p = 0.003$).
ANCOVA: analyses of covariance
DGLA: dihomo-gamma-linolenic acid
AA: arachidonic acid
ACS: acute coronary syndrome
LDL-C: low-density lipoprotein cholesterol

Table 4 Logistic regression analysis of the onset of ACS

	Univariate analysis			Adjusted for LDL-C levels and the use of strong statin		
	OR	95% CI	p-value	OR	95% CI	p-value
DGLA	5.82	1.61–21.1	0.007	4.45	1.13–17.6	0.03
AA	21.7	2.80–168	0.003	19.4	2.41–156	0.005
EPA/AA	0.24	0.07–0.76	0.02	0.26	0.08–0.79	0.02
DHA/AA	0.43	0.15–1.21	0.11	0.28	0.09–0.89	0.03
EPA+DHA/AA	0.24	0.07–0.76	0.02	0.12	0.03–0.46	0.002

OR: odd ratio

CI: confidence interval

Other acronyms are explained in footnote to Table 2.

DISCUSSION

This study showed that the ACS group had higher DGLA and AA levels than the staged group. However, the EPA and DHA levels did not significantly differ between the two groups, and the ACS group had lower EPA/AA and DHA/AA ratios than the staged group. In particular, the median EPA/AA ratio of the ACS group was 0.23, and our results were consistent with a cutoff of 0.34, as shown in the study of Arashi H et al.[10] Furthermore, the significant differences in DGLA and AA levels in the ACS group were consistent with the factors influencing the development of ACS, such as the use of strong statins and LDL-C levels.

Previous reports have shown that reducing LDL-C levels suppresses CAD.[11] In relation to this reason, the AHA guidelines recommend the active use of strong statins for the secondary prevention of CAD,[12] and the control target set by ESC was < 70 mg/dL for LDL-C levels among high-risk patients.[13]

In addition, the serum oxidized LDL levels, including MDA-LDL, reflect the presence of vulnerable plaques. Therefore, MDA-LDL was found to play an important role in ACS.[14] In the current study, the ACS group had higher LDL-C and MDA-LDL levels than the staged group, which is consistent with previous reports.[11,14]

By contrast, about PUFAs, several recent RCTs have questioned the benefit of n-3 PUFAs supplementation.[15] The results of the current study support this finding, as there were no significant differences in EPA and DHA levels between the two groups. However, the EPA/AA and DHA/AA ratios remarkably differed, which is to the findings of a previous study.[16] Therefore, AA levels must be reduced to achieve higher EPA/AA ratios. Moreover, instead of EPA and DHA supplementation, DHA/AA may reduce coronary events. In addition, while there have been reports on the association between n-3 PUFAs alone and the n-3/n-6 PUFA ratios and ACS, there are few reports examining the association between n-6 PUFAs alone and the development of ACS. This is only speculation, but it is likely that the lack of reports on n-6 PUFAs, a known "proinflammatory" PUFA, is because n-3 PUFAs are easier to intervene with as drugs and are reasonably effective in maintaining high n-3/n-6 PUFA ratios, while n-6 PUFAs are only effective in terms of reducing food intake. The only intervention for n-6 PUFAs are to reduce the intake of foods.

The serum n-6 PUFA levels may lower LDL-C levels. However, it may have minimal or no effect on triglyceride and HDL-C levels.[17] Hence, there was no significant difference in

triglyceride or HDL levels between the ACS group with a high serum n-6 PUFA level and the staged group with a low serum n-6 PUFA levels. Previous studies have shown that LDL-C is strongly associated with the development of CAD.[11] As shown in Table 1, the use of strong statins was more common in the staged group than in the ACS group. Therefore, the staged group had lower LDL-C levels than the ACS group. In addition, ANCOVA was performed, with consideration of the possibility that the LDL-C-lowering effect via n-6 PUFAs may also affect the development of ACS. Therefore, high DGLA and AA levels may be a risk factor for the development of ACS, independent of other lipids and fatty acids, about serum n-6 PUFA levels.

This result is attributed to the fact that DGLA is the source of eicosanoids. However, when subjected to D5D, it is converted to AA, which ultimately leads to the production of bad eicosanoids that synthesize prostaglandin and leukotrienes and inflammation and thrombus formation.[18] However, D5D is affected by FADS1-2 gene locus variation.[19] In fact, there was no difference in D5D between the two groups in the current study. Therefore, the intake of linoleic acid (LA), which is located upstream of fatty acid metabolism, should be significantly reduced to prevent bad eicosanoids. Therefore, to inhibit the development of these events, LA intake, which is located upstream of fatty acid metabolism, should be remarkably reduced. This could lower DGLA and AA levels. Even though n-3 PUFAs are not actively consumed as supplements, the body will inevitably have a higher DHA/AA and EPA/AA ratio, which may relatively reduce the incidence of ACS, similar to the results of previous studies.

However, the benefits of n-6 PUFAs, including LA, are undeniable. Park S et al performed a Mendelian randomized analysis to examine the causal effect of n-3 and n-6 PUFAs on the risk of CAD.[20] Results showed that higher genetically predicted concentrations of EPA and DGLA were associated with a lower risk of CAD. In addition, a higher LA allele score was remarkably correlated with a lower risk of CAD. By contrast, consistent with the current study, AA showed a significant causal estimate of the higher risk of CAD. Therefore, D5D is important. Nevertheless, as mentioned above, it is determined by genetic predisposition. Therefore, in general, it is difficult to consider LA intake due to individual differences.

In summary, this study first showed that the incidence of ACS can be decreased by lowering n-6 PUFA levels, including AA, and increasing n-3 PUFAs/AA ratios (Fig. 2).

The current study had several limitations. First, this was a retrospective and single-center research. Thus, the risk of unintentional selection bias in the selection of patients could not be completed excluded, as noted above. Second, although the current study was conducted on patients with or without statin treatment, some types of statins might have affected the concentration of fatty acids.[11] However, rosuvastatin was commonly used in this research, and only a few patients were treated with atorvastatin, pitavastatin, and pravastatin. Hence, we could not evaluate the effect of the different types of statin on fatty acids.

The ACS group had significantly higher AA and DGLA levels than the staged group. This result was consistent even after adjusting for the effects of confounding factors such as the use of strong statins and LDL-C levels. Therefore, excess serum n-6 PUFA levels may be a risk factor for the development of ACS. In addition, supplementation with n-3 PUFAs may not be necessary for the prevention of ACS.

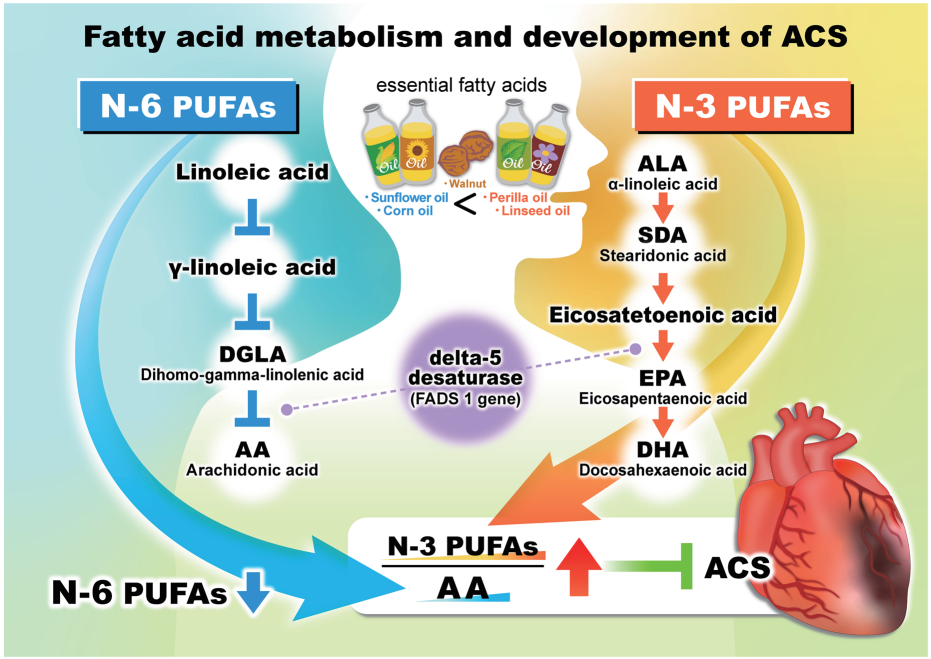

Fig. 2 Graphical abstract
The figure shows the metabolism of PUFAs. The incidence of ACS may be decreased by lowering n-6 PUFA levels, including AA, and increasing n-3 PUFAs/AA ratio.
AA: arachidonic acid
ACS: acute coronary syndrome
PUFAs: polyunsaturated fatty acids

AUTHOR CONTRIBUTIONS

Concept and design: Inoue, Murohara.
Data curation: Inoue.
Interpretation of data: Inoue, Murohara.
Drafting of the manuscript: Inoue, Murohara.
Critical revision of the manuscript: All authors.
Statistical analysis: Morikawa.
Supervision: Murohara.

ACKNOWLEDGMENTS

We would like to thank Dr Nobutake Kurebayashi for his advice regarding our lipid research.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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