Heart and Vessels (2019) 34:368–374
https://doi.org/10.1007/s00380-018-1240-3

SHORT COMMUNICATION

Does administration of eicosapentaenoic acid increase soluble thrombomodulin level in statin-treated patients with stable coronary artery disease?

Shigemasa Tani[1,2,3] · **Rei Matsuo**[2,3] · **Atsushi Hirayama**[3]

Received: 20 February 2018 / Accepted: 17 August 2018 / Published online: 24 August 2018

Abstract
Interventions targeting the serum eicosapentaenoic acid (EPA)/arachidonic acid (AA) ratio could be useful for the prevention of coronary artery disease (CAD). Few data exist regarding the effects of administration of EPA on the serum levels of soluble thrombomodulin (sTM) as a marker of endothelial damage, or on the relationship between the sTM and EPA/AA ratio in patients with CAD receiving statin treatment. We assigned stable CAD patients already receiving statin therapy to an EPA group (1800 mg/day: $n=50$) or control group ($n=50$). A significant increase of the sTM level was observed in the EPA group as compared to that in the control group 0.40 (0.10/0.70) FU/mL vs. 0.20 (0/0.40) FU/mL, $p=0.004$ at the 6-month follow-up examination. Multivariate regression analysis after adjustments for coronary risk factors and changes of the serum lipid levels identified an increased EPA/AA ratio as an independent predictor of increased serum sTM level ($\beta=0.244$, $p=0.02$). The results suggest that an increased sTM level caused by additional administration of EPA to statin might be associated with an increased EPA/AA ratio. The increase of the serum sTM after administration of EPA might reflect an increase of the TM expression on the endothelial surface rather than endothelial damage in CAD patients under statin treatment.
Clinical Trial Registration Information UMIN (http://www.umin.ac.jp/), Study ID: UMIN000010452.

Keywords Eicosapentaenoic acid · Soluble thrombomodulin · Vascular endothelial cell

Introduction

Decreased mortality from coronary artery disease (CAD) has been ascribed to the beneficial effect of the two major n-3 polyunsaturated fatty acids (n-3PUFAs): eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both of which are contained in fish [1, 2].

The Japan EPA Lipid Intervention Study (JELIS) randomized as treatment with a statin alone or a statin plus highly purified EPA at a dose of 1800 mg/day demonstrated that the EPA group exhibited a 19% reduction in the incidence of major cardiovascular (CV) events [3]. This result suggests that interventions targeting the serum EPA/arachidonic acid (AA) ratio, particularly those involving an increase in EPA/AA ratio, could be useful for the prevention of CAD [4–9].

Thrombomodulin (TM) is an integral membrane glycoprotein and a high-affinity receptor for thrombin on the endothelial cell surface. It has been implicated in the endothelial regulation of fibrinolysis and coagulation [10]. After proteolytic cleavage of TM from the endothelial surface, soluble TM (sTM) can be detected in the circulation [11, 12]. Although sTM concentration is thought to reflect the degree of endothelial damage, the crucial physiological role of sTM is unknown [13].

That is, while there have been reports documenting that reinfarction and peripheral vascular disease were rather frequent among patients showing high sTM levels [14, 15], a follow-up survey in the Atherosclerosis Risk in Communities (ARIC) Study demonstrated a negative correlation

✉ Shigemasa Tani
tani.shigemasa@nihon-u.ac.jp

1 Department of Health Planning Center, Nihon University Hospital, 1-6 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8309, Japan

2 Department of Cardiology, Nihon University Hospital, Tokyo, Japan

3 Division of Cardiology, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan

between the serum sTM level and incidence rate of CAD in patients who had no history of CAD accounting for the myocardial infarction [16]. It would follow that the finding may reflect a higher rate of TM expression on the vascular endothelial cells, and hence a lower likelihood of vascular endothelial injury; in such patients, the absolute amount of circulating sTM is inevitably high, and CAD is less likely to develop [17, 18]. Few studies have attempted to evaluate the effects of EPA administration on serum sTM levels in stable CAD patients undergoing statin treatment.

The aim of this study was to investigate the effect of additional administration of EPA on the serum sTM levels with a particular focus on increased EPA/AA ratio, in stable CAD patients receiving statin treatment, in whom the serum lipid profiles are expected to be relatively well controlled.

Methods

Study design and populations

We conducted this 6-month, single-center, prospective, randomized open-label clinical trial to investigate the effect of the additional administration of EPA on sTM level, a marker of endothelial damage, in stable CAD patients receiving treatment with statins. The study was performed at Surugadai Nihon University Hospital between May 1, 2013, and June 30, 2014.

The primary efficacy parameter was the change in sTM level. The secondary endpoints were the relationship between the change in the EPA/AA ratio and the change in sTM level in statin-treated CAD patients receiving EPA therapy.

A total of 110 patients who provided consent to participate in this trial were randomized using a simple sealed-envelope method into two groups: an EPA group ($n=55$) and a non-EPA control group ($n=55$).

Subjects in the EPA group were treated with a 900-mg capsule containing EPA ethyl ester of > 98% purity twice a day (total daily dose, 1800 mg) (Mochida Pharmaceuticals, Tokyo, Japan). During the 6-month study, no restrictions were placed on concomitantly used drugs. Dietary counseling was provided for all patients as per the standards of the American College of Cardiology/American Heart Association guideline [19].

The subjects chosen for this study were all patients who had undergone coronary angiography for the evaluation of CAD. The decision to perform coronary angiography was made by the cardiologist in charge, based on the results of non-invasive clinical examinations. Patients who presented acutely with ST-elevation myocardial infarction, non-ST-elevation myocardial infarction, or unstable angina were excluded to ensure that the study consisted only of patients in stable condition. All patients had CAD, defined as a percent coronary stenosis of 50% or more in at least one coronary vessel.

The following exclusion criteria were applied: patients were not enrolled if they met any of the following exclusion criteria: a bleeding tendency, hepatic or renal dysfunction (creatinine ≥ 1.5 mg/dL, alanine aminotransferase and aspartate aminotransferase ≥ 2 times the upper limit of the normal values), known malignant disease, inflammatory disease, or current treatment with n-3 polyunsaturated fatty acids (n-3PUFAs).

A diagnosis of hypertension was made when systolic pressure was ≥ 140 mmHg and/or the diastolic pressure was ≥ 90 mmHg, or the subject reported a history of taking antihypertensive medication. Diabetes was defined as a fasting plasma glucose of ≥ 126 mg/dL and HbA1c of ≥ 6.5% or a current history of treatment with antidiabetic agents. Smoking was defined as current smoking or smoking cessation within 1 year prior to the start of the study. The estimated glomerular filtration rate (e-GFR) was calculated using the abbreviated Modification of Diet in Renal Disease (MDRD) Study equation modified using a Japanese coefficient [20].

The study protocol was approved by the Institutional Review Boards of our institutions (approval number: 20121202), and written informed consent was obtained from all the participants.

Measurement of laboratory parameters

Fasting blood samples were collected in the early morning after the subjects had fasted for 12 h. The serum sTM level was measured by an enzyme immunoassay (SRL Co., Ltd., Tokyo, Japan).The serum PUFAs composition was measured by capillary gas chromatography (SRL). The serum total cholesterol (TC), HDL-C, and TG levels were measured by the standard methods. The serum low-density lipoprotein cholesterol (LDL-C) level was estimated using the Friedewald formula [21]. The plasma PAI-1 level, including the tissue PA/PAI-1 complex and the active and latent forms of PAI-1, was determined using a latex photometric immunoassay method (kit, LSI Medience Co., Ltd., Tokyo, Japan). The concentration of fibrin was measured using the Clauss assay (SRL).

Statistical analysis

All statistical analyses were performed using the SPSS Window ver 12.0 software (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL). Data are expressed as the mean ± standard deviation for continuous variables and as percentages for discrete variables. Continuous variables between the groups were compared by Student's t test, and categoric variables were analyzed by χ^2 test. For cases in

which data did not show normal distribution, the data were expressed as medians (interquartile). Mann–Whitney's *U* test was used to evaluate the differences in data between groups, and Wilcoxon's signed-rank test was used to analyze the differences in data within the same group. A multivariate regression analysis was performed to identify the predictors associated with the absolute change in (Δ) serum sTM level at 6-month follow-up. Statistical significance was assumed at a p value of less than 0.05.

Results

Patients

There were no cardiovascular events during the 6-month follow-up period. Gastrointestinal disorder was noted as an adverse reaction in two of the patients, and 6-month follow-up data were not available for 3 patients in the EPA group. In the control group, pertinent baseline data were not available for 1 patient, and 6-month follow-up data were not available for 4 patients. After the exclusion of the above-mentioned patients, data for the remaining 100 patients were subjected to this analysis. The 2 groups exhibited similar baseline characteristics (Table 1). There were no changes in diet or medication treatment during the study period.

Changes in laboratory profile

A significant increase of the Δ serum sTM level was observed in the EPA group as compared to that in the control group (Fig. 1). The Δ serum TC and Δ non-HDL-C in the EPA group were significantly lower than those in the control group. No significant differences in the Δ LDL, Δ HDL-C, Δ TG, or Δ PAI-1 were noted between the 2 groups. The Δ EPA and EPA/AA ratio in the EPA group were significantly higher than those in the control group, whereas the Δ AA was significantly lower in the EPA group as compared to that in the control group (Table 2).

Multivariate regression analysis identified the serum Δ sTM

As shown in Table 3, a multivariate regression analysis with adjustments for atherosclerosis risk factors and Δ serum lipid levels identified the Δ EPA/AA ratio at the 6-month follow-up as an independent predictor of the Δ sTM level, indicating that patients with an increase in EPA/AA ratio at the 6-month follow-up also showed a greater degree of increase of the serum sTM level.

Association of sTM level and the EPA/AA ratio with severity of diseased coronary vessels

In the overall study population, the EPA/AA ratio was significantly lower in patients with multi-vessel disease (MVD, $n=58$) than in patients with single-vessel disease (SVD, $n=42$) (SVD vs. MVD: 0.400 [0.260/0.800] vs. 0.320 [0.200/0.480], $p=0.041$). Similar results were also obtained in both the randomized EPA group (SVD, $n=21$, MVD, $n=29$ and the randomized control group (SVD, $n=21$, MVD, $n=29$), with the EPA/AA ratio being lower in the patients with MVD than in those with SVD, although the differences between the patients with SVD and MVD failed to reach statistical significance (EPA group: 0.420 (0.253/0.808) vs. 0.290 (0.190/0.458), $p=0.111$; control group: 0.400 (0.255/0.690) vs. 0.320 (0.223/0.510), $p=0.216$). Furthermore, the serum sTM levels were also lower in the patients with MVD than in those with SVD in all of the overall study population (SVD vs. MVD, 2.73 ± 0.55 FU/mL vs. 2.93 ± 0.64 FU/mL, $p=0.108$), EPA group (2.67 ± 0.59 FU/mL vs. 2.92 ± 0.69 FU/mL, $p=0.177$), and Control group (2.79 ± 0.52 FU/mL vs. 2.93 ± 0.59 FU/mL, $p=0.392$); the difference in the level between the patients with SVD and MVD was not statistically significant in any of the groups.

Discussion

Administration of EPA as an additional agent resulted in elevation of the serum sTM concentrations in stable CAD patients receiving statin therapy. Increase of the EPA/AA ratio observed after 6 months of EPA administration in these patients proved to be an independent predictor of increased serum sTM levels.

The significant elevation of the serum sTM level even in the control group might be secondary to the release of sTM into the circulation due to increased expression of sTM on the vascular endothelial cells consequent on repair of vascular endothelial injury by statin treatment [22, 23]. Furthermore, not only the TM bound to the vascular endothelial cell membrane, but also sTM per se has anti-arteriosclerotic effects, including an anti-thrombotic effect and anti-inflammatory effect [24], and the usefulness of recombinant soluble TM in the treatment of disseminated intravascular coagulation (DIC) in the clinical practice setting has been demonstrated [25].

Serum sTM is generally recognized as a marker of vascular endothelial injury [26], and it has also been reported as a possible indicator of the degree of damage to inflammatory cells, or in other words, of the anti-inflammatory efficacy of TM [13]. That is, in cases receiving medical interventions such as statin therapy, the anti-inflammatory effect of TM

Table 1 Baseline patient characteristics

	Control group ($n=50$)	EPA group ($n=50$)	p value
Male/female, *n* (%)	42 (84)/8 (16)	46 (92)/4 (8)	0.218
Age (years)	67.3 ± 10.4	67.5 ± 10.1	0.925
BMI (kg/m^2)	24.8 ± 4.0	24.6 ± 3.2	0.702
Hypertension, *n* (%)	41 (82)	41 (82)	> 0.999
Diabetes Mellitus, *n* (%)	22 (44)	18 (36)	0.414
HbA1c (%)*	5.7 (5.4/6.0)	5.7 (5.4/6.1)	0.730
Cigarette smoking, *n* (%)	5 (10)	3 (6)	0.461
Hyperuricemia, *n* (%)	14 (28)	12 (24)	0.692
CKD, *n* (%)	19 (38)	17 (34)	0.732
eGFR (ml/min/1.73 m^2)	67.1 ± 16.3	67.1 ± 16.0	0.978
Number of diseased coronary vessels, *n* (%)			
Single-vessel/multi-vessels	21 (42)/29 (58)	21 (42)/29 (58)	> 0.999
Concomitant drugs *n* (%)			
Antiplatelets	46 (92)	49 (98)	0.169
ACEIs/ARBs	26 (52)	29 (58)	0.547
β blockers	19 (38)	22 (44)	0.542
Calcium channel blockers	31 (62)	31 (62)	> 0.999
Statins	50 (100)	50 (100)	> 0.999
Laboratory profile			
TC (mg/dL)	172 ± 21	167 ± 25	0.262
LDL-C (mg/dL)	94 ± 18	91 ± 18	0.435
HDL-C (mg/dL)	52 ± 15	53 ± 11	0.641
TG (mg/dL)*	114 (90/151)	100 (74/144)	0.100
non-HDL-C (mg/dL)	120 ± 23	113 ± 23	0.165
sTM (FU/mL)	2.87 ± 0.56	2.81 ± 0.65	0.628
PAI-1 (ng/mL)*	19 (15/31)	21 (15/29)	0.920
Fibrinogen (mg/dL)*	273 (246/321)	277 (250/321)	0.560
EPA (μg/mL)	70 ± 40	71 ± 45	0.909
AA (μg/mL)	175 ± 47	182 ± 54	0.549
EPA/AA ratio*	0.330(0.240/0.520)	0.360 (0.200/0.570)	0.914

Data are expressed as the mean ± SD, median (IQR)*, and percentage for variables

BMI body mass index, *Hb* hemoglobin, *CKD* chronic kidney disease, *e-GFR* estimated glomerular filtration rate, *ACEI* angiotensin converting enzyme inhibitor: *ARB* angiotensin receptor blocker, *TC* total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *TG* triglyceride, *Non-HDL-C* non-high-density lipoprotein, *sTM* soluble thrombomodulin, *PAI-1* plasminogen activator inhibitor 1, *EPA* eicosapentaenoic acid, *AA* arachidonic acid

might be predominant. However, it is not possible to differentiate between the two states by sTM assay alone in the clinical practice setting. Judgment should be based taking into consideration the pathophysiological state, drugs being administered, and degree of progression of arteriosclerosis. When considered with reference to the results of the JELIS Study and the study demonstrating the anti-arteriosclerotic effects of EPA [27], the increased sTM seen in response to EPA therapy may reflect the vascular endothelial function-improving effect of EPA.

In the present study, the baseline (prior to initiation of EPA therapy) EPA/AA ratio was lower in patients with multi-vessel disease than in patients with single-vessel disease. A previously reported virtual histologic study using intravascular ultrasound demonstrated a negative correlation between the EPA/AA ratio and the plaque volume [28], which represents evidence in support of the findings of the present study, even though the study method was different. In contrast, no significant correlation could be demonstrated between the serum sTM level and the number of (single vs. multiple) coronary arterial lesions, possibly on account of the limited sample sizes of the groups in this investigation. Whilst the serum sTM serves as an indicator of vascular endothelial damage, there is the possibility that a disparity occurred between the degree of vascular endothelial damage and the number of affected vessels in the present

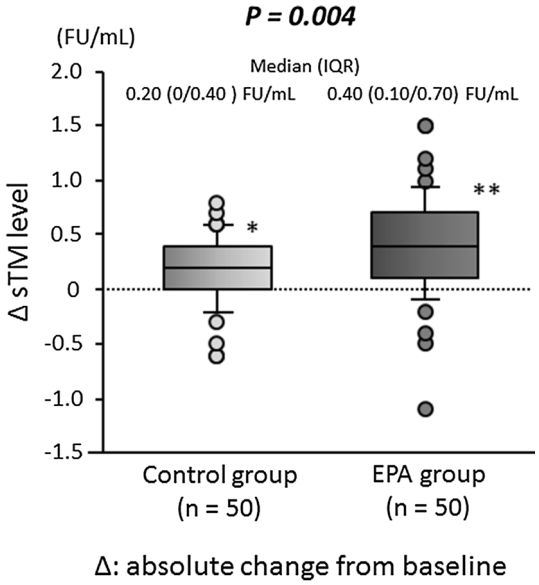

Fig. 1 Title: Comparison of sTM Level between the 2 Groups. IQR: interquartile range; Horizontal lines represent 75th, median, and 25th percentiles values; Whiskers represent 10th and 90th percentiles; sTM, soluble thrombomodulin. *$p < 0.05$ vs. from baseline. **$p < 0.0001$ vs. from baseline

study population, in which the interval from the onset of coronary artery disease varied among individual patients. This would be interpreted as implying that different results could be obtained if, for example, the study were confined to patients with onset of acute coronary syndrome just prior to the initiation of statin therapy.

No significant changes of the plasma level of PAI-1, which serves as a marker of vascular endothelial cell function, besides playing an important role within the coagulation–fibrinolytic system, were observed in patients receiving EPA therapy. Several studies reported elevation of the plasma PAI-1 level following administration of an n-3PUFA (combined EPA-DHA) preparation [29–31], and in another study, in which the changes in the plasma PAI-1 levels in subjects receiving EPA alone were investigated, increase of the levels in women and decrease of the levels in men of this plasma marker were observed, although the underlying mechanism remains unclear [32]. Thus, there is still no unanimity of opinion on the effects of EPA administration on the plasma PAI-1 level.

The JELIS Study showed a greater CV event-suppressing effect of statin therapy administered with an additional EPA agent, as compared to statin therapy alone. It would, thus, follow that attention focused solely on the significance of sTM as a predictive marker of re-infarction from the present study results may incur a contradiction with the results of the JELIS Study. Therefore, it would appear that its significance may differ from that of the increased sTM brought about by statin therapy with concomitant EPA administration being understood as a marker of vascular endothelial injury.

We previously investigated the relationship between the serum levels of n3PUFAs and the serum TM concentrations in a cross-sectional study, with the results indicating a negative correlation of the serum DHA level with the serum sTM level, but no correlation of the serum EPA level. This phenomenon observed in the investigation, which was a cross-sectional study, cannot be conclusive, but may suggest that EPA has no impact on the kinetics of sTM in the serum [33]. It is obvious, nevertheless, that the concentration of EPA in the serum resulting from administration of EPA is remarkably higher than that obtained by dietary fish intake. Superko

Table 2 Change in laboratory profile

	Control group $n = 50$	EPA group $n = 50$	§p value
ΔTC (mg/dL)	−7.1 ± 20.1*	−14.9 ± 17.6***	0.049
ΔLDL-C (mg/dL)	−3.4 ± 15.8*	−8.4 ± 14.5***	0.098
ΔHDL-C (mg/dL)*	1.5 (−6.0/2.0)	−3.5 (−6.0/1.0)**	0.488
ΔTG (mg/dL)*	−7.0 (−37.0/22.0)	−13.5 (−32.0/−2.0)	0.132
Δnon-HDL-C (mg/dL)	−5.0 ± 18.6	−12.4 ± 17.1***	0.042
ΔPAI-1 (ng/mL)	−0.13 ± 9.50	−0.40 ± 10.12	0.311
ΔEPA (μg/mL)	−6.3 ± 37.1	94.1 ± 54.1***	< 0.0001
ΔAA (μg/mL)	0.21 ± 24.0	−23.1 ± 29.8***	< 0.0001
ΔEPA/AA ratio	−0.036 ± 0.227	0.708 ± 0.428***	< 0.0001

Δ, absolute change from baseline. Abbreviations are the same as in Table 1

Data are expressed as the mean ± SD, or median (IQR)*

The figures shown in the columns for both the control group and the EPA group indicate the change between baseline and 6- month follow-up

*$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$ vs. from baseline

The figures shown in the §p value column indicate the p value comparison between the control group and the EPA group

Table 3 Mulivariate regression analysis identified predictor of Δ sTM

	β	p value
ΔLDL-C	0.043	0.668
ΔHDL-C	0.01	0.923
ΔTG	−0.019	0.869
ΔEPA/AA ratio	0.292	0.006

Δ, absolute change from baseline. Abbreviations are the same as in Table 1, β = standard partial regression coefficient, adjusted for age, sex, BMI, eGFR, hypertension (yes: 1,/no: 0), cigarette, smoking (yes: 1,/no: 0), hypertension (yes: 1,/no: 0), and diabetes mellitus (yes: 1,/no: 0). Multiple $R = 0.452$, $F = 2.002$, $p = 0.038$, $n = 100$

and colleagues have advocated in their review article that EPA exerts its bioactive effects in a blood level-dependent manner [34], so that such a characteristic might result in a discrepancy between the results of a cross-sectional study and those of an interventional study.

The results suggest the possibility that repeated serum sTM measurements over time in patients receiving EPA may enable monitoring not only for vascular endothelial cell damage, but also for any beneficial effects of EPA, in the clinical setting. However, further investigation together with evaluation of the vascular endothelial function is needed to clarify these issues.

Conclusion

While much remains yet to be clarified as to relationship of the serum sTM concentration with CAD events, the increased sTM brought about by EPA administration might be attributed to improvement of the integrity of the vascular endothelium when seen from the viewpoint of the proven add-on effect of EPA on statin therapy.

Acknowledgements This work was supported by KAKENHI Grant number 23590757 of the Japan Society for the Promotion of Science (JSPS). The authors would like to thank International Medical Information Center (www.imic.or.jp/services/translation.html) for the English language review. The authors would also like to thank Mr. Gary Cooper for his help in editing the English manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Mozaffarian D, Wu JH (2011) Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. J Am Coll Cardiol 58:2047–2067
2. Tani S, Matsuo R, Kawauchi K, Yagi T, Atsumi W, Hirayama A (2018) A cross-sectional and longitudinal study between association of n-3 polyunsaturated fatty acids derived from fish consumption and high-density lipoprotein heterogeneity. Heart Vessels 33:470–480
3. 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, Japan EPA lipid intervention study (JELIS) Investigators (2007) Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet 369:1090–1098
4. Itakura H, Yokoyama M, Matsuzaki M, Saito Y, Origasa H, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K, Matsuzawa Y, Investigators JELIS (2011) Relationships between plasma fatty acid composition and coronary artery disease. J Atheroscler Thromb 18:99–107
5. Nishizaki Y, Shimada K, Tani S, Ogawa T, Ando J, Takahashi M, Yamamoto M, Shinozaki T, Miyauchi K, Nagao K, Hirayama A, Yoshimura M, Komuro I, Nagai R, Daida H (2014) Significance of imbalance in the ratio of serum n-3 to n-6 polyunsaturated fatty acids in patients with acute coronary syndrome. Am J Cardiol 113:441–445
6. Tani S, Nagao K, Kawauchi K, Yagi T, Atsumi W, Matsuo R, Hirayama A (2017) The ratio of eicosapentaenoic acid (EPA) to arachidonic acid may be a residual risk marker in stable coronary artery disease patients receiving treatment with statin following EPA therapy. Am J Cardiovasc Drugs 17:409–420
7. Takahashi M, Ando J, Shimada K, Nishizaki Y, Tani S, Ogawa T, Yamamoto M, Nagao K, Hirayama A, Yoshimura M, Daida H, Nagai R, Komuro I (2017) The ratio of serum n-3 to n-6 polyunsaturated fatty acids is associated with diabetes mellitus in patients with prior myocardial infarction: a multicenter cross-sectional study. BMC Cardiovasc Disord 17:41
8. Tani S, Takahashi A, Nagao K, Hirayama A (2015) Association of fish consumption-derived ratio of serum n-3 to n-6 polyunsaturated fatty acids and cardiovascular risk with the prevalence of coronary artery disease. Int Heart J 56:260–268
9. Sakamoto A, Saotome M, Hosoya N, Kageyama S, Yoshizaki T, Takeuchi R, Murata K, Nawada R, Onodera T, Takizawa A, Satoh H, Hayashi H (2016) Aberrant serum polyunsaturated fatty acids profile is relevant with acute coronary syndrome. Heart Vessels 31:1209–1217
10. Sadler JE (1997) Thrombomodulin structure and function. Thromb Haemost 78:392–395
11. Ishii H, Majerus PW (1985) Thrombomodulin is present in human plasma and urine. J Clin Invest 76:2178–2181
12. Califano F, Giovanniello T, Pantone P, Campana E, Parlapiano C, Alegiani F, Vincentelli GM, Turchetti P (2000) Clinical importance of thrombomodulin serum levels. Eur Rev Med Pharmacol Sci 4:59–66
13. Martin FA, Murphy RP, Cummins PM (2013) Thrombomodulin and the vascular endothelium: insights into functional, regulatory, and therapeutic aspects. Am J Physiol Heart Circ Physiol 304:H1585–H1597
14. Blann AD, Amiral J, McCollum CN (1997) Prognostic value of increased soluble thrombomodulin and increased soluble E-selectin in ischaemic heart disease. Eur J Haematol 59:115–120
15. Blann AD, Seigneur M, Steiner M, Boisseau MR, McCollum CN (1997) Circulating endothelial cell markers in peripheral vascular disease: relationship to the location and extent of atherosclerotic disease. Eur J Clin Invest 27:916–921
16. Salomaa V, Matei C, Aleksic N, Sansores-Garcia L, Folsom AR, Juneja H, Chambless LE, Wu KK (1999) Soluble thrombomodulin as a predictor of incident coronary heart disease and

symptomless carotid artery atherosclerosis in the Atherosclerosis Risk in Communities (ARIC) Study: a case-cohort study. Lancet 353:1729–1734
17. Ireland H, Kunz G, Kyriakoulis K, Stubbs PJ, Lane DA (1997) Thrombomodulin gene mutations associated with myocardial infarction. Circulation 96:15–18
18. Ohlin AK, Norlund L, Marlar RA (1997) Thrombomodulin gene variations and thromboembolic disease. Thromb Haemost 78:396–400
19. Smith SC Jr, Blair SN, Bonow RO, Brass LM, Cerqueira MD, Dracup K, Fuster V, Gotto A, Grundy SM, Miller NH, Jacobs A, Jones D, Krauss RM, Mosca L, Ockene I, Pasternak RC, Pearson T, Pfeffer MA, Starke RD, Taubert KA (2001) AHA/ACC guidelines for preventing heart attack and death in patients with atherosclerotic cardiovascular disease: 2001 update. A statement for healthcare professionals from the American Heart Association and the American College of Cardiology. J Am Coll Cardiol 38:1581–1583
20. Imai E, Horio M, Nitta K, Yamagata K, Iseki K, Hara S, Ura N, Kiyohara Y, Hirakata H, Watanabe T, Moriyama T, Ando Y, Inaguma D, Narita I, Iso H, Wakai K, Yasuda Y, Tsukamoto Y, Ito S, Makino H, Hishida A, Matsuo S (2007) Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease. Clin Exp Nephrol 11:41–50
21. Sniderman AD, Blank D, Zakarian R, Bergeron J, Frohlich J (2003) Triglycerides and small dense LDL: the twin Achilles heels of the Friedewald formula. Clin Biochem 36:499–504
22. Lin SJ, Hsieh FY, Chen YH, Lin CC, Kuan II, Wang SH, Wu CC, Chien HF, Lin FY, Chen YL (2009) Atorvastatin induces thrombomodulin expression in the aorta of cholesterol-fed rabbits and in TNFalpha-treated human aortic endothelial cells. Histol Histopathol 24:1147–1159
23. Shi J, Wang J, Zheng H, Ling W, Joseph J, Li D, Mehta JL, Ponnappan U, Lin P, Fink LM, Hauer-Jensen M (2003) Statins increase thrombomodulin expression and function in human endothelial cells by a nitric oxide-dependent mechanism and counteract tumor necrosis factor alpha-induced thrombomodulin downregulation. Blood Coagul Fibrinolysis 14:575–585
24. Wu KK, Matijevic-Aleksic N (2000) Thrombomodulin a linker of coagulation and fibrinolysis and predictor of risk of arterial thrombosis. Ann Med 32(Suppl 1):73–77
25. Yamakawa K, Aihara M, Ogura H, Yuhara H, Hamasaki T, Shimazu T (2015) Recombinant human soluble thrombomodulin in severe sepsis: a systematic review and meta-analysis. J Thromb Haemost 13:508–519
26. Li YH, Shi GY, Wu HL (2009) Thrombomodulin in the treatment of atherothrombotic diseases. Front Biosci (Schol Ed) 1:33–38
27. Borow KM, Nelson JR, Mason RP (2015) Biologic plausibility, cellular effects, and molecular mechanisms of eicosapentaenoic acid (EPA) in atherosclerosis. Atherosclerosis 242:357–366
28. 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, Terashima M, Michishita I (2013) Effects of serum n-3 to n-6 polyunsaturated fatty acids ratios on coronary atherosclerosis in statin-treated patients with coronary artery disease. Am J Cardiol 111:6–11
29. Barcelli U, Glas-Greenwalt P, Pollak VE (1985) Enhancing effect of dietary supplementation with omega-3 fatty acids on plasma fibrinolysis in normal subjects. Thromb Res 39:307–312
30. Siasos G, Tousoulis D, Oikonomou E, Zaromitidou M, Verveniotis A, Plastiras A, Kioufis S, Maniatis K, Miliou A, Siasou Z, Stefanadis C, Papavassiliou AG (2013) Effects of Ω-3 fatty acids on endothelial function, arterial wall properties, inflammatory and fibrinolytic status in smokers: a cross over study. Int J Cardiol 166:340–346
31. Boberg M, Pollare T, Siegbahn A, Vessby B (1992) Supplementation with n-3 fatty acids reduces triglycerides but increases PAI-1 in non-insulin-dependent diabetes mellitus. Eur J Clin Invest 22:645–650
32. Phang M, Lincz LF, Garg ML (2013) Eicosapentaenoic and docosahexaenoic acid supplementations reduce platelet aggregation and hemostatic markers differentially in men and women. J Nutr 143:457–463
33. Kawauchi K, Tani S, Nagao K, Hirayama A (2014) Association of n-3 polyunsaturated fatty acids with soluble thrombomodulin as a marker of endothelial damage:cross-sectional pilot study. J Cardiol 64:312–317
34. Superko HR, Superko SM, Nasir K, Agatston A, Garrett BC (2013) Omega-3 fatty acid blood levels: clinical significance and controversy. Circulation 128:2154–2161