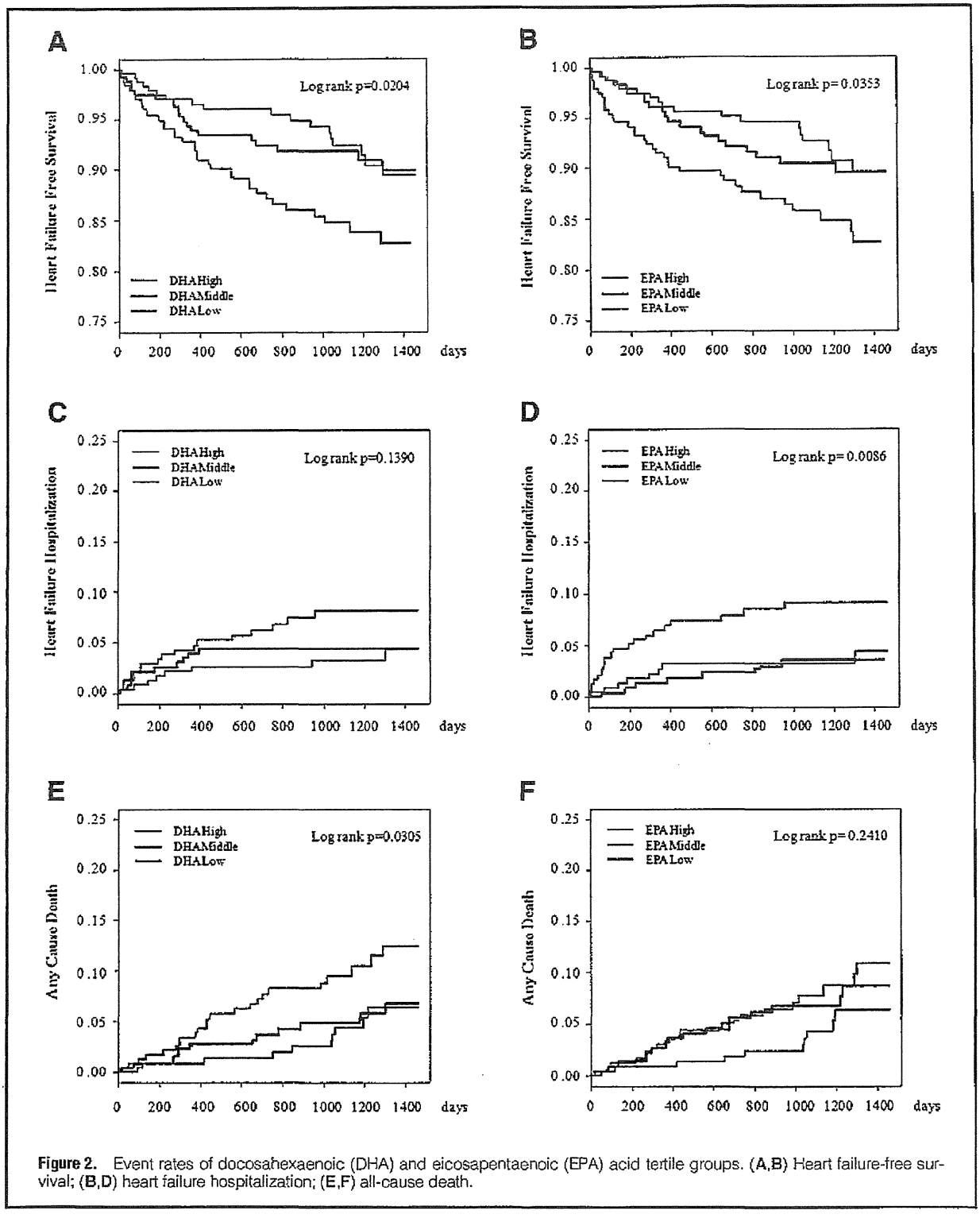

Figure 2. Event rates of docosahexaenoic (DHA) and eicosapentaenoic (EPA) acid tertile groups. (**A,B**) Heart failure-free survival; (**B,D**) heart failure hospitalization; (**E,F**) all-cause death.

cause death (P=0.0305; **Figures 2E,F**). Cause-specific cumulative death event rates among tertile groups of DHA or EPA were also evaluated (**Figure S2**). However, there were no statistical differences in event rates among the tertile groups of DHA or EPA possibly because of the low event rates.

Similar results to the Kaplan-Meier analyses were found by propensity-score-stratified Cox regression analysis (**Figures 3–5**), which revealed that low serum levels of DHA and EPA were both associated with a higher incidence of the composite of all-cause death and HF hospitalization at nearly the same

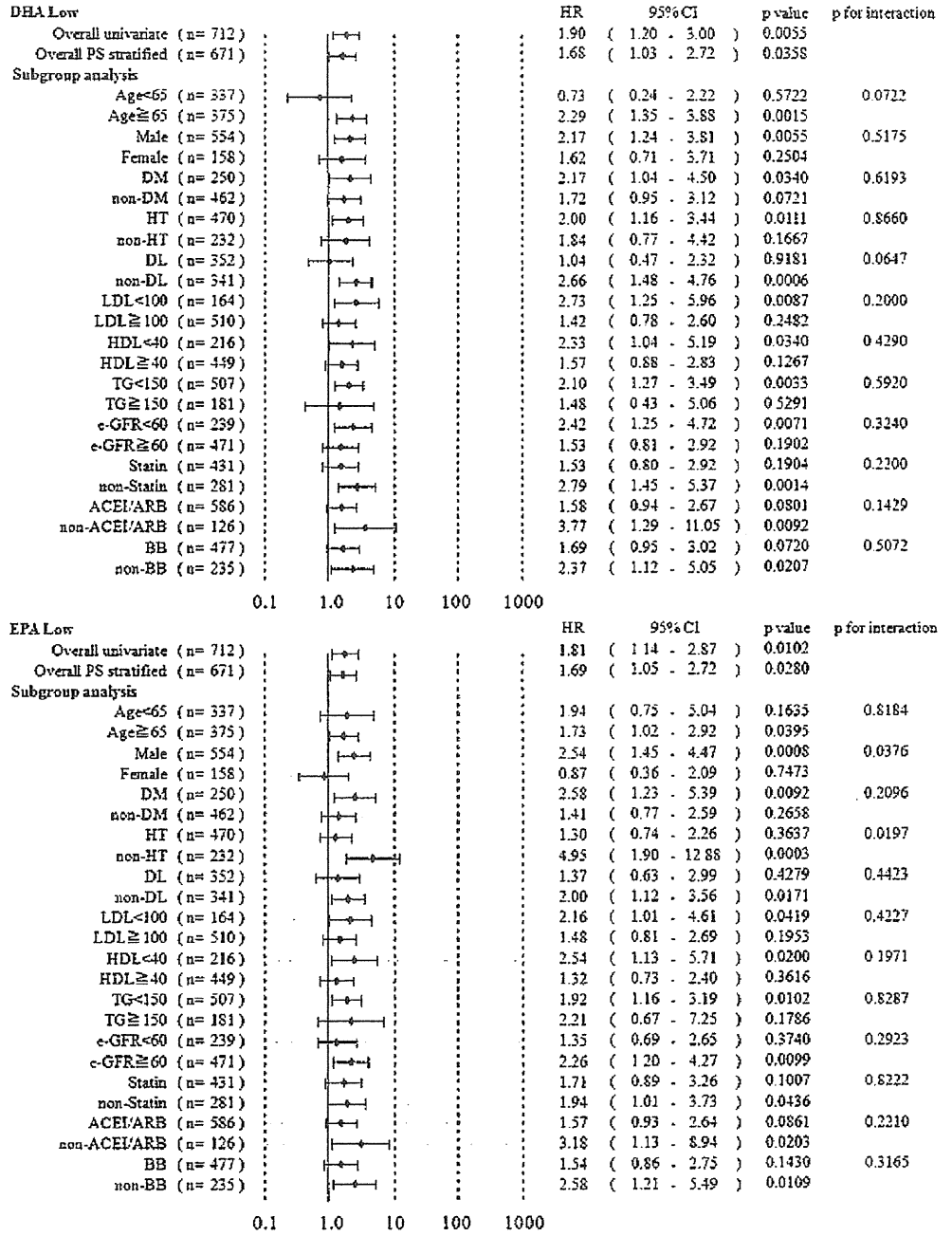

Figure 3. Results of Cox regression analysis for composite endpoint consisting of all-cause death and heart failure hospitalization. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin 2 receptor blocker; BB, β-blocker; CI, confidence interval; DHA, docosahexaenoic acid; DL, dyslipidemia; e-GFR, estimated glomerular filtration rate; EPA, eicosapentaenoic acid; HDL-chol, high-density lipoprotein cholesterol; HF, heart failure; HR, hazard ratio; LDL-chol, low-density lipoprotein cholesterol; PS, propensity score; T-chol, total cholesterol; TG, triglyceride.

significance level (HR 1.68, P=0.0358 for DHA; and HR 1.69, P=0.0280 for EPA) (Figure 3). However, low serum EPA was only associated with a higher risk of HF hospitalization (HR 2.40, P=0.0097) (Figure 4), whereas low DHA was only associated with a higher risk of all-cause death (HR 1.91, P=0.0386) (Figure 5). Thus, EPA appeared to be superior to DHA for estimating HF event risk after AMI whereas DHA appeared to be superior to EPA for estimating all-cause mortality after AMI.

Subgroup analyses demonstrated that the unfavorable effect

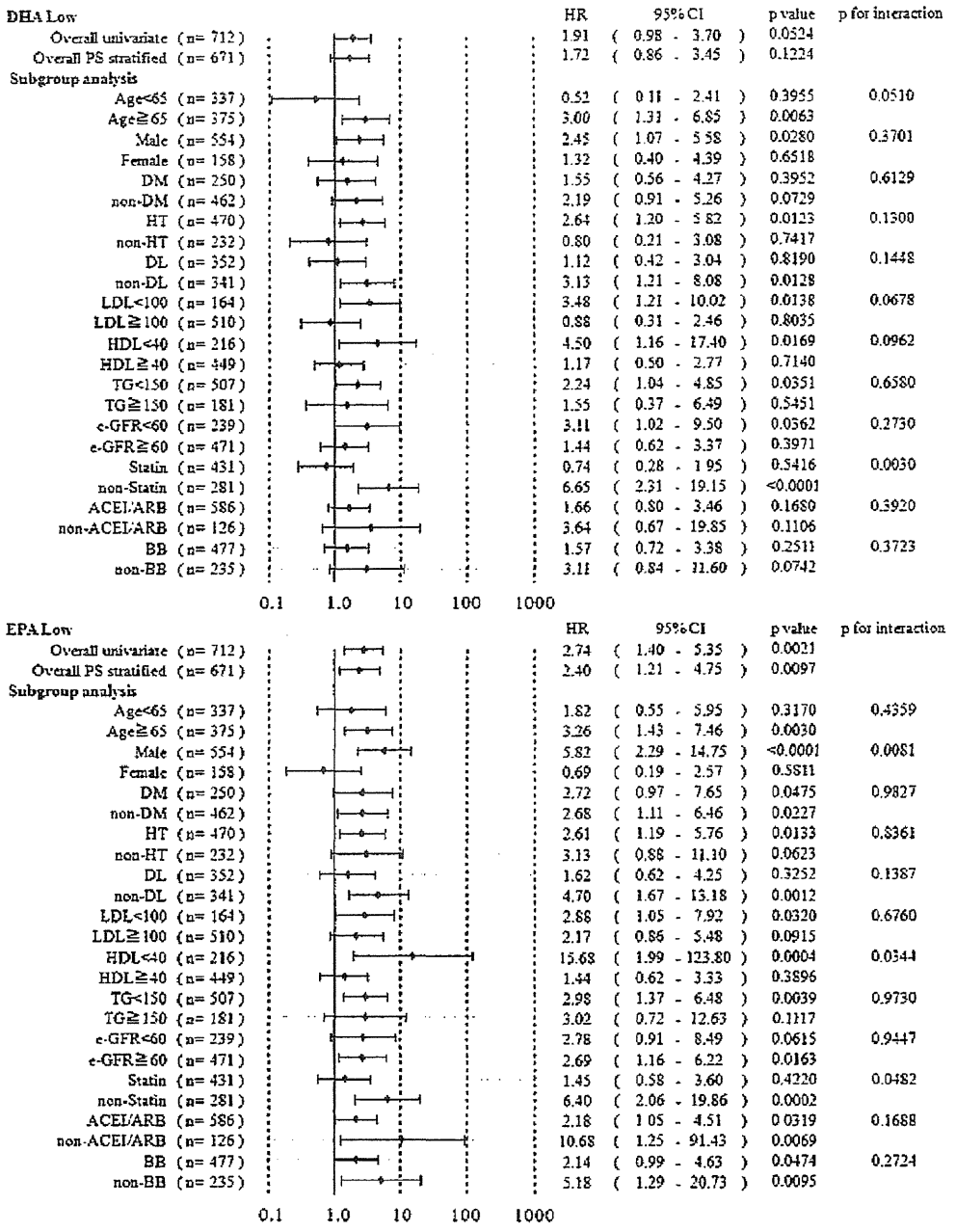

Figure 4. Results of Cox regression analysis for heart failure hospitalization. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin 2 receptor blocker; BB, β-blocker; CI, confidence interval; DHA, docosahexaenoic acid; DL, dyslipidemia; e-GFR, estimated glomerular filtration rate; EPA, eicosapentaenoic acid; HDL-chol, high-density lipoprotein cholesterol; HF, heart failure; HR, hazard ratio; LDL-chol, low-density lipoprotein cholesterol; PS, propensity score; T-chol, total cholesterol; TG, triglyceride.

of a low DHA or EPA serum level on the primary and secondary endpoints was generally common for all subgroups, with a few notable exceptions. For example, it was revealed that the effect of low EPA levels on HF hospitalizations was prominent in male patients (P for interaction=0.0081), those with low high-density lipoprotein (HDL) cholesterol levels (P for interaction=0.0344), and those without statin therapy (P for interaction=0.0482) (Figure 4). On the other hand, the effect of low DHA levels on all-cause death was common to all subgroups (Figure 5).

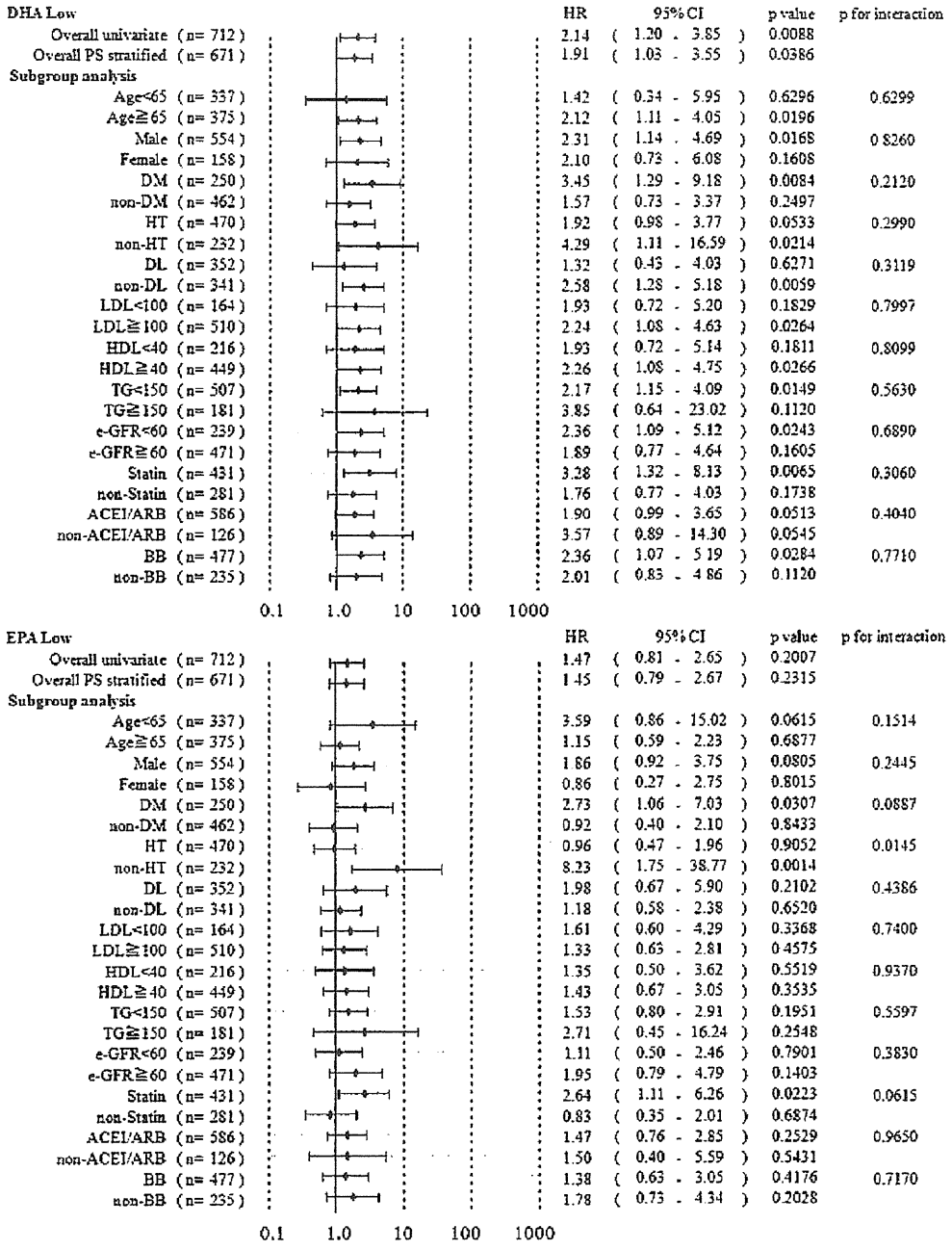

Figure 5. Results of Cox regression analysis for all-cause death. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin 2 receptor blocker; BB, β-blocker; CI, confidence interval; DHA, docosahexaenoic acid; DL, dyslipidemia; e-GFR, estimated glomerular filtration rate; EPA, eicosapentaenoic acid; HDL-chol, high-density lipoprotein cholesterol; HF, heart failure; HR, hazard ratio; LDL-chol, low-density lipoprotein cholesterol; PS, propensity score; T-chol, total cholesterol; TG, triglyceride.

Discussion

In the present study, we observed that low levels of both DHA and EPA were associated with a worse HF-free survival rate in the secondary prevention setting after AMI. In addition, an association with a higher risk of HF hospitalization was prominent for lower serum EPA levels, whereas there was a higher mortality risk with lower DHA. Although the effects of n-3 PUFA on atherosclerotic cardiovascular events have been intensively investigated, limited data are available regarding their salutary effect on the incidence of HF. To our knowledge, this is the first study to reveal the association between

decreased n-3 PUFA levels and worse HF-free survival in the secondary prevention setting after AMI. In addition, even though the relationship and underlying mechanisms remain unclear, our results also indicate that monitoring EPA serum levels may be useful for predicting HF events and monitoring DHA serum levels may be useful for predicting all-cause mortality following AMI. However, it should be noted that the EPA level also could be useful for predicting all-cause mortality if we change the cut-off level. As shown in **Figure 2F**, all-cause mortality estimate was clearly discerned between the lower 2 EPA tertile groups vs. the highest EPA tertile group, suggesting that the EPA level could be useful for estimating the all-cause mortality risk if we set the cut-off between the lower 2 tertiles and the highest tertile.

These observations are consistent with a recent report by Mozaffarian et al showing that total and individual n-3 PUFA concentrations are associated with incident congestive HF in the United States elderly population, with EPA having the highest correlation with HF events.[11] In comparison, 2 recently published large-scale randomized clinical trials of n-3 PUFA supplementation failed to demonstrate beneficial effects on major cardiovascular events in a secondary prevention setting after AMI, although they did not assess incidence of HF.[3,4] Those trials may suggest that contemporary evidence-based medications such as angiotensin-converting enzyme inhibitor or statins can surpass the beneficial effects of n-3 PUFA. Indeed, our result of subgroup analysis suggested that the beneficial effects of DHA and EPA on HF-free survival and HF hospitalization were mostly prominent in patients not treated with such medications (but not on all-cause death). Thus, it should be noted that effect of n-3 PUFA levels on cardiac events in patients administered recent evidence-based medications needs to be evaluated further, and we also emphasize that the benefit of n-3 PUFA should be determined in patients for whom these state-of-the-art medications are not available because of adverse effects or other reasons.[3,4]

The beneficial effect of both DHA and EPA in reducing HF hospitalizations and all-cause death after AMI is intuitively plausible considering the evidence for the cardiac benefits of n-3 PUFA intake.[1,2,10,15–21] For example, n-3 PUFA supplementation improves myocardial efficiency by reducing myocardial oxygen demand without a decrement in performance and clinically improves the left ventricular ejection fraction in patients with dilated cardiomyopathy or chronic HF.[10,15,16] In addition, n-3 PUFA intake is associated with lower blood pressure and heart rate.[17,18] Together these lines of evidence for the usefulness of n-3 PUFA supplementation in cardiovascular protection strongly support our observation that low levels of n-3 PUFA were associated with worse HF-free survival in patients with AMI.

In the subgroup analyses, the unfavorable effects of low serum DHA or EPA levels on HF-free survival appeared to be common to all of the subgroups, even for patients with well-controlled low-density lipoprotein (LDL) levels (<100 mg/dl). However, we also observed an inverse relationship between EPA level and the incidence of HF hospitalization prominently in male patients, patients with low HDL cholesterol levels, and those not receiving statin therapy. Thus, EPA supplementation following AMI may be beneficial for preventing HF hospitalization in these subpopulations, although the evidence is not yet available. Even though the salutary effects of n-3 PUFA supplementation on clinical outcomes have been demonstrated in patients with chronic HF,[9,10] an inverse correlation between serum EPA levels and HF risk after AMI has not been reported previously.

On the basis of currently available evidence, the American Heart Association recommends that patients with documented coronary heart disease consume approximately 1 g/day of DHA and EPA (combined) obtained from fish or fish-oil capsules.[22] Because our data suggest that EPA may be beneficial in preventing HF hospitalization and DHA in preventing all-cause death after AMI, the beneficial effects of higher serum levels of EPA and DHA on the secondary prevention of HF and all-cause death after AMI should be further studied in a randomized control trial.

Study Limitations

Our study has a few limitations that warrant mention. First, the measurement of serum n-3 PUFA levels was a 1-point assessment at discharge, and did not reflect temporal changes and everyday consumption of n-3 PUFA. This might lead to inaccurate estimation of the association between n-3 PUFA and HF events. In addition, because there were no available data regarding n-3 PUFA consumption, the association between serum n-3 PUFA levels and daily intake of n-3 PUFA could not be assessed. Second, there could be a selection bias in the present study as shown in **Table S1**. Third, it is possible that unmeasured confounding factors influenced the study outcomes because of the inherent nature of observational studies. In this regard, the data should be interpreted with caution.

Conclusions

Low levels of serum n-3 PUFA were associated with worse HF-free survival in patients with AMI. Notably, low levels of EPA and DHA were particularly associated with HF hospitalization and all-cause mortality, respectively.

Acknowledgements

We thank Mariko Kishida, Rie Nagai, Nanase Muraoka, Hiroko Takemori, Akiko Yamagishi, Kumiko Miyoshi, Chizuru Hamaguchi, Hiroko Machida, Mariko Yoneda, Nagisa Yoshioka, Mayuko Tomatsu, Kyoko Tatsumi, Tomoko Mizuoka, Shigemi Kohara, Junko Tsugawa, Junko Isotani, Sachiko Ashibe, and all other OACIS research coordinators and nurses for their excellent assistance with data collection.

Funding Sources and Disclosure

OACIS is supported by Grants-in-Aid for University and Society Collaboration (#19590816 and #19390215) from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan. None of the authors has a relationship with industry that requires disclosure or financial associations that might pose a conflict of interest in connection with the submitted article.

References

1. De Caterina R. n-3 fatty acids in cardiovascular disease. *N Engl J Med* 2011; **364:** 2439–2450.
2. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: A systematic review. *Atherosclerosis* 2006; **189:** 19–30.
3. Kromhout D, Giltay EJ, Geleijnse JM; Alpha Omega Trial Group. n-3 fatty acids and cardiovascular events after myocardial infarction. *N Engl J Med* 2010; **363:** 2015–2026.
4. Rauch B, Schiele R, Schneider S, Diller F, Victor N, Gohlke H, et al; OMEGA Study Group. OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction. *Circulation* 2010; **122:** 2152–2159.
5. Wang C, Harris WS, Chung M, Lichtenstein AH, Balk EM, Kupelnick B, et al. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: A systematic review. *Am J Clin Nutr* 2006; **84:** 5–17.
6. Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kita Y, et al; JPHC Study Group. Intake of fish and n3 fatty acids and risk of coronary

heart disease among Japanese: The Japan Public Health Center-Based (JPHC) Study Cohort I. *Circulation* 2006; **113:** 195–202.
7. Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, et al; Japan EPA Lipid Intervention Study (JELIS) Investigators. 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. Marchioli R, Barzi F, Bomba E, Chieffo C, Di Gregorio D, Di Mascio R, et al; GISSI-Prevenzione Investigators. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: Time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation* 2002; **105:** 1897–1903.
9. Gissi-HF Investigators, Tavazzi L, Maggioni AP, Marchioli R, Barlera S, Franzosi MG, Latini R, et al. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): A randomised, double-blind, placebo-controlled trial. *Lancet* 2008; **372:** 1223–1230.
10. Nodari S, Triggiani M, Campia U, Manerba A, Milesi G, Cesana BM, et al. Effects of n-3 polyunsaturated fatty acids on left ventricular function and functional capacity in patients with dilated cardiomyopathy. *J Am Coll Cardiol* 2011; **57:** 870–879.
11. Mozaffarian D, Lemaitre RN, King IB, Song X, Spiegelman D, Sacks FM, et al. Circulating long-chain ω-3 fatty acids and incidence of congestive heart failure in older adults: The cardiovascular health study: A cohort study. *Ann Intern Med* 2011; **155:** 160–170.
12. Nakatani D, Sakata Y, Mizuno H, Shimizu M, Suna S, Usami M, et al; Osaka Acute Coronary Insufficiency Study (OACIS) Group. Impact of diabetes mellitus on rehospitalization for heart failure among survivors of acute myocardial infarction in the percutaneous coronary intervention era. *Circ J* 2009; **73:** 662–666.
13. Shiozaki M, Iso H, Ohira T, Nakatani D, Shimizu M, Sakata Y, et al. Longitudinal risk of cardiovascular events in relation to depression symptoms after discharge among survivors of myocardial infarction: Osaka Acute Coronary Insufficiency Study. *Circ J* 2011; **75:** 2878–2884.
14. Rosenbaum PR, Rubin DB. Reducing bias in observational studies using subclassification on the propensity score. *J Am Stat Assoc* 1984; **79:** 516–524.
15. Peoples GE, McLennan PL, Howe PR, Groeller H. Fish oil reduces heart rate and oxygen consumption during exercise. *J Cardiovasc Pharmacol* 2008; **52:** 540–547.
16. Ghio S, Scelsi L, Latini R, Masson S, Eleuteri E, Palvarini M, et al; GISSI-HF investigators. Effects of n-3 polyunsaturated fatty acids and of rosuvastatin on left ventricular function in chronic heart failure: A substudy of GISSI-HF trial. *Eur J Heart Fail* 2010; **12:** 1345–1353.
17. Geleijnse JM, Giltay EJ, Grobbee DE, Donders AR, Kok FJ. Blood pressure response to fish oil supplementation: Metaregression analysis of randomized trials. *J Hypertens* 2002; **20:** 1493–1499.
18. Mozaffarian D, Geelen A, Brouwer IA, Geleijnse JM, Zock PL, Katan MB. Effect of fish oil on heart rate in humans: A meta-analysis of randomized controlled trials. *Circulation* 2005; **112:** 1945–1952.
19. Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: Evaluating the risks and the benefits. *JAMA* 2006; **296:** 1885–1899.
20. Saito Y, Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Ishikawa Y, et al; JELIS Investigators, Japan. Effects of EPA on coronary artery disease in hypercholesterolemic patients with multiple risk factors: Sub-analysis of primary prevention cases from the Japan EPA Lipid Intervention Study (JELIS). *Atherosclerosis* 2008; **200:** 135–140.
21. Domei T, Yokoi H, Kuramitsu S, Soga Y, Arita T, Ando K, et al. Ratio of serum n-3 to n-6 polyunsaturated fatty acids and the incidence of major adverse cardiac events in patients undergoing percutaneous coronary intervention. *Circ J* 2012; **76:** 423–429.
22. Kris-Etherton PM, Harris WS, Appel LJ; AHA Nutrition Committee. American Heart Association. Omega-3 fatty acids and cardiovascular disease: New recommendations from the American Heart Association. *Arterioscler Thromb Vasc Biol* 2003; **23:** 151–152.

Appendix

The OACIS Investigators

Chair: Issei Komuro: Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan.

Secretariats: Yasuhiko Sakata (Chief), Daisaku Nakatani, Shinichiro Suna, Masaya Usami, Sen Matsumoto, Masahiko Hara, Mariko Kishida, Rie Nagai: Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan.

Investigators (institutions listed in alphabetical order): Yoshiyuki Kijima, Yusuke Nakagawa, Minoru Ichikawa: Higashi-Osaka City General Hospital, Higashi-Osaka; Young-Jae Lim, Shigeo Kawano: Kawachi General Hospital, Higashi-Osaka; Hiroshi Sato: Kwansei Gakuin University, Nishinomiya; Takashi Shimazu, Hisakazu Fuji, Kazuhiro Aoki: Kobe Ekisaikai Hospital, Kobe; Seiki Nagata, Yoshio Ishida, Masaaki Uematsu, Tetsuya Watanabe, Masashi Fujita, Masaki Awata: Kansai Rosai Hospital, Amagasaki; Michio Sugii: Meiwa Hospital, Nishinomiya; Masatake Fukunami, Takahisa Yamada, Takashi Morita: Osaka General Medical Center, Osaka; Shinji Hasegawa, Nobuyuki Ogasawara: Osaka Kosei Nenkin Hospital, Osaka; Tatsuya Sasaki, Yoshinori Yasuoka: Osaka Minami Medical Center, National Hospital Organization, Kawachinagano; Hideo Kusuoka, Yukihiro Koretsune, Yoshio Yasumura, Keiji Hirooka: Osaka Medical Center, National Hospital Organization, Osaka; Masatsugu Hori (previous Chair): Osaka Prefectural Hospital Organization Osaka Medical Center for Cancer and Cardiovascular Diseases; Kazuhisa Kodama, Yasunori Ueda, Kazunori Kashiwase, Akio Hirata, Mayu Nishio, Mitsutoshi Asai: Osaka Police Hospital, Osaka; Yoshio Yamada, Jun Tanouchi, Masami Nishino, Hiroyasu Kato, Ryu Shutta: Osaka Rosai Hospital, Sakai; Shintaro Beppu, Akio Kohama, Hiroyoshi Yamamoto: Osaka Seamens Insurance Hospital, Osaka; Issei Komuro, Shinsuke Nanto, Kinya Otsu, Yasushi Matsumura, Kazuhiro Yamamoto, Tetsuo Minamino, Satoru Sumitsuji, Yasuhiko Sakata, Shungo Hikoso, Daisaku Nakatani: Osaka University Graduate School of Medicine, Suita; Toru Hayashi, Yasuji Doi, Ken-ichiro Okada: Noritoshi Ito: Saiseikai Senri Hospital, Suita; Kenshi Fujii, Katsuomi Iwakura, Atsushi Okamura, Motoo Date, Yoshiharu Higuchi: Sakurabashi Watanabe Hospital, Osaka; Noriyuki Akehi: Settsu Iseikai Hospital, Settsu; Eiji Hishida: Teramoto Memorial Hospital, Kawachinagano; and Shiro Hoshida, Kazuhiko Hashimura, Takayoshi Adachi: Yao Municipal Hospital, Yao, Japan.

Supplementary Files

Supplementary File 1

Figure S1. Flow chart of patient selection.

Figure S2. Cumulative cardiovasuclar (A,B) and non-cardiovascular (C,D) death event rates of the docosahexaenoic (DHA) and eicosapentaenoic (EPA) acid tertile groups.

Table S1. Comparison of the Patients' Backgrounds among the Present Study Population (A), Patients Without Eligible Serum Samples (B) and Patient Without Blood Sampling Agreement (C) who Registered in the Osaka Acute Coronary Insufficiency Study Between 2006 and 2009

Please find supplementary file(s);
http://dx.doi.org/10.1253/circj.CJ-12-0875