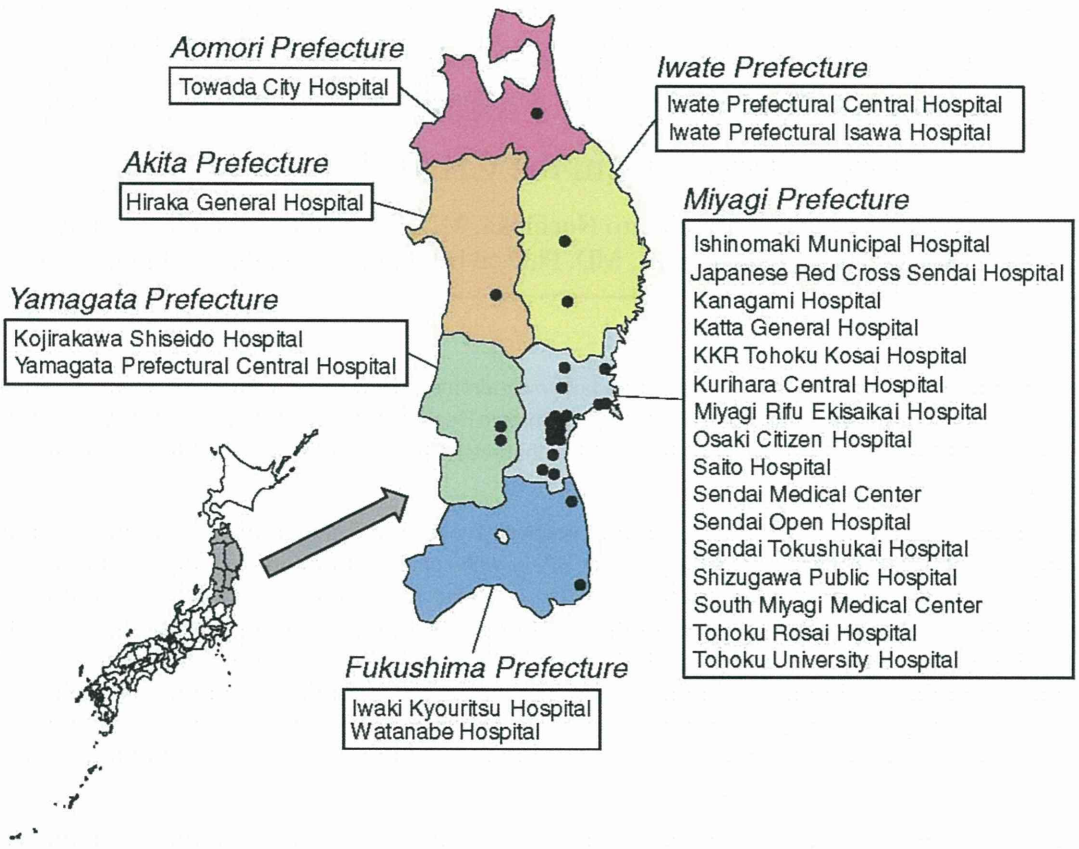

Figure 1. Map of the distribution of 24 collaborating hospitals in the Tohoku district.

patients with HF have preserved ejection fraction (pEF), as observed in the outpatient clinic, there is no evidence-based treatment guideline for such patients.[10,11] Patients with HFpEF are characterized as being more likely to be elderly, to be female and to have more comorbidities (eg, chronic kidney disease [CKD], chronic obstructive pulmonary disease, history of stroke and malignancy). Indeed, the pathophysiology of HFpEF is considered to be more closely related to those extracardiac factors compared with HF with reduced EF (HFrEF).[12,13] Another factor that is associated with the acceleration of the progression of CVD is the lower rate of achievement of clinical guideline-recommended treatment goals.[14,15] We need to regularly evaluate the penetration rate of evidence-based treatment and emphasize the appropriate adherence to the guidelines by physicians and patients.

Editorial p775

Thus, we started a large-scale multicenter prospective cohort study, named the Chronic Heart Failure Analysis and Registry in the Tohoku District 2 (CHART-2) Study, of consecutively enrolled patients at high risk for disease progression of CVD or HF due to the development of AHFS. In this first report of the CHART-2 Study, we examined the trend of etiology of HF patients and their characteristics as compared with the CHART-1 Study.[4,5]

Methods

Study Design and Specific Objectives

The CHART-2 Study is a prospective observational multicenter cohort study to identify the characteristics, mortality and prognostic risks of patients with overt HF and patients without HF but who are at high risk for disease progression of CVD. The purpose of the study was to evaluate the following: (1) characteristics of patients with overt HF and the associated prognostic risks; (2) characteristics of patients at risk for HF and the factors associated with CVD progression; (3) factors associated with the development of AHFS; (4) prevalence and prognostic impact of metabolic syndrome (MetS) in patients with overt HF; (5) the association between MetS and the development of AHFS; (6) the prevalence and prognostic impact of malignancy in patients with CVD; and (7) the prevalence of patients needing home nursing care and the characteristics of bedridden patients with CVD.

Information Disclosure

Rationale, design, and objectives of the CHART-2 Study were registered in clinicaltrials.gov (NCT00418041) and the University Hospital Medical Information Network (UMIN000000562) on the commencement of patient enrollment, and were updated instantly when modifications were made. Detailed information on the CHART-2 Study is available to the public on the Tohoku Heart Failure Association website (http://tohoku.cardiovascular-medicine.jp).

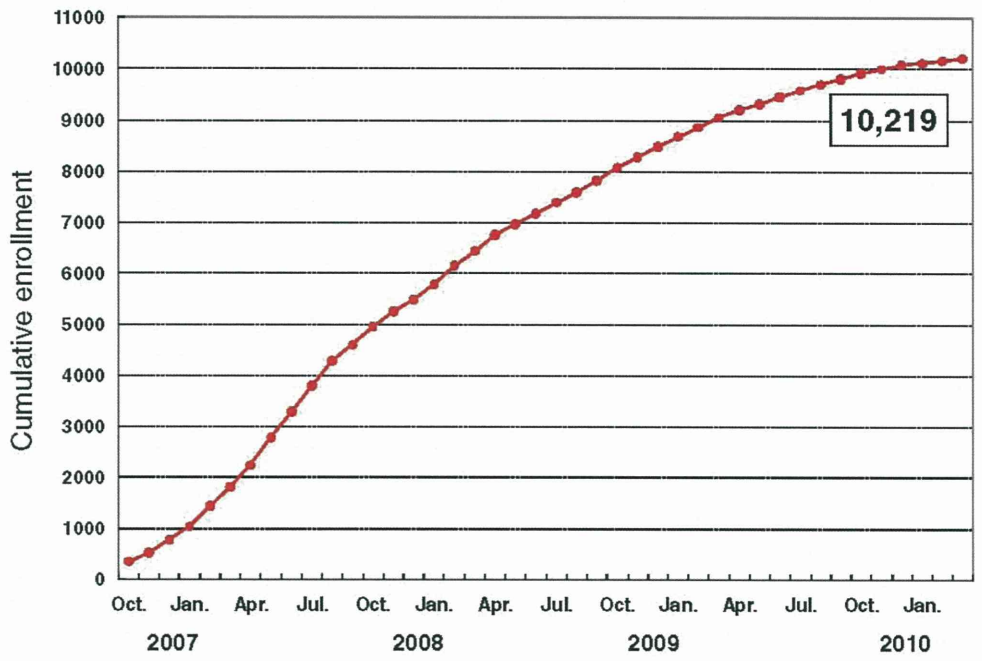

Figure 2. CHART-2 Study cumulative enrollment, showing a total of 10,219 registrations.

Site Selection

A total of 24 institutions, located in the Tohoku district, participated in the CHART-2 Study (**Figure 1**). A society was organized for the collaborating members and institutions, named the Tohoku Heart Failure Association, before the commencement of the study. The Tohoku district is located in the north-east of Japan and is composed of 6 prefectures, which include approximately 9.8 million individuals in total. The participating institutes and all collaborating members are listed in **Appendix 1**. Of 24 collaborating institutions, 15 hospitals also participated in the CHART-1 Study (**Appendix 1**). Patients enrolled in those 15 institutions accounted for 74.0% and 75.8% of the total subjects included in the CHART-1 and CHART-2 Studies, respectively.

Study Group

Stable patients were eligible for enrollment in the CHART-2 Study if they were aged ≥20 years with CAD or were in stage B, C or D defined according to the Guidelines for the Diagnosis and Management of Heart Failure in Adults authorized by the American College of Cardiology Foundation/American Heart Association.[2] In the present cohort study, patients who were asymptomatic but who had structural heart disease and/or impaired left ventricular (LV) function were categorized as being in stage B (**Appendix 2**). Stage C was defined as current or past symptoms of HF associated with underlying structural heart disease; and stage D was defined as refractory HF in which specialized and advanced treatment strategies were indicated.[2] HF was diagnosed according to the criteria of the Framingham Heart Study.[16] Patients who had been enrolled in the CHART-1 Study were not included in the CHART-2 Study. There were no other exclusion criteria in the present study. The CHART-2 Study was approved by the local ethics committee in each institution. Significant CAD was defined as either organic CAD requiring revascularization or vasospastic angina documented on electrocardiography or angiography. Eligible patients were consecutively recruited after written informed consent was obtained.

Data Collection and Processing

Eight clinical research coordinators (CRC) who belonged to the head office of the CHART-2 Study at Tohoku University visited collaborating hospitals regularly. They fully assisted attending physicians in registration, including candidate screening, explanation of the study design, obtainment of written informed consent, and data extraction from medical charts. Data were entered using a Web-based data collecting system (newly developed by Fujitsu Tohoku Systems) by CRC and trained keypunchers. An identification number was assigned to each enrolled patient and personal information was completely excluded. Data were recorded with regard to demographics, medical history, smoking history, alcohol use, family history of CVD, comorbidities for cardiovascular risks, laboratory findings, echocardiography reports, findings of coronary angiography, previous surgical treatments, and medications at entry. Anemia was defined as hemoglobin <12 g/dl in women and <13 g/dl in men, following the World Health Organization definition.[17] CKD was diagnosed when estimated glomerular filtration rate was $<60\,ml \cdot min^{-1} \cdot 1.73\,m^{-2}$, which was calculated using the formula for Japanese individuals.[18] MetS was defined according to the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome.[19]

Follow-up Survey and Study Outcomes

All follow-up data and events are surveyed once a year during the study period. Collected data were monitored at least twice yearly. Planned completion of the follow-up period is March 2013. Several predefined outcomes including development of AHFS, mortality and other events worsening HF status will be collected in the CHART-2 Study.

Table 1. Baseline Characteristics of the CHART-1 and CHART-2 Patients vs. HF Stage

	CHART-1 (Stage C/D, 2004)	P value*	CHART-2 (2010)				
			Total	Stage B or CAD without HF	Stage C	Stage D	P value**
No. patients	1,078		10,219	5,484 (53.7)	4,640 (45.4)	95 (0.9)	
Age (years), mean±SD	68.7±13.4	0.8	68.2±12.3	67.6±12.2	68.8±12.3	74.2±12.5	<0.001
<40 (%)	3.5	0.4	3.1	3.4	2.7	1.1	<0.001
40–64 (%)	29.2		29.0	29.6	28.5	21.1	
65–74 (%)	31.7		33.7	35.6	31.8	22.1	
≥75 (%)	35.6		34.2	31.4	37.0	55.8	
Male (%)	64.5	0.01	69.8	71.0	68.5	64.2	0.01
Outpatients (%)	NA	NA	79.5	80.3	79.0	60.6	<0.001
NYHA functional class (%)							
I	6.7	<0.001	47.4	68.3	23.4	9.5	<0.001
II	72.9		46.9	30.8	66.5	21.1	
III	19.5		5.3	0.8	9.8	43.2	
IV	0.9		0.4	0.0	0.3	26.3	
Blood pressure (mmHg), mean±SD							
Systolic	126.3±19.1	0.9	128.3±18.6	130.1±17.9	126.4±19.1	119.1±22.4	<0.001
Diastolic	71.5±11.0	0.08	73.5±11.8	74.5±11.5	72.3±11.9	69.2±13.2	<0.001
Heart rate (/min), mean±SD	74.7±14.3	<0.001	71.0±14.1	69.7±13.2	72.4±15.0	72.7±14.5	<0.001
BMI (kg/m²), mean±SD	23.0±3.7	<0.001	24.0±3.6	24.2±3.5	23.8±3.9	21.6±3.4	<0.001
<18.5 (%)	9.2	<0.001	6.6	4.8	8.3	20.0	<0.001
18.5–22.9 (%)	42.9		33.9	32.3	35.5	47.4	
23.0–24.9 (%)	20.6		23.5	25.0	21.9	21.1	
25.0–29.9 (%)	23.5		30.7	33.0	28.4	9.5	
≥30 (%)	3.7		5.3	4.9	5.9	2.1	
Waist circumference (cm), mean±SD	NA	NA	85.9±9.9	86.6±9.5	85.3±10.3	81.4±8.5	<0.001
Male	NA	NA	87.2±9.0	87.7±8.8	86.6±9.2	82.6±8.1	<0.001
Female	NA	NA	83.1±11.2	83.9±10.4	82.4±11.9	79.2±9.0	<0.001
Smoking (%)							
Never	NA	NA	52.7	51.7	53.7	63.2	0.052
Current	NA	NA	18.2	18.3	18.3	14.9	
Former	NA	NA	29.1	30.1	28.0	21.8	
Alcohol (%)							
Never	NA	NA	49.8	48.5	51.1	60.5	<0.001
Regular	NA	NA	27.7	30.0	25.1	19.8	
Chance	NA	NA	14.7	14.4	15.2	4.7	
Former	NA	NA	7.8	7.1	8.5	15.1	
Cardiothoracic ratio (%), mean±SD	NA	NA	52.1±6.5	50.7±5.8	53.6±6.9	57.0±8.1	<0.001
Laboratory findings, mean±SD							
Hemoglobin (g/dl)	13.0±2.2	0.007	13.4±2.0	13.6±1.8	13.2±2.2	12.0±2.5	<0.001
eGFR (ml·min^{-1}·1.73m^{-2})	60.9±30.7	0.9	64.5±22.6	67.5±21.2	61.1±23.5	53.2±29.6	<0.001
HDL-cholesterol (mg/dl)	NA	NA	52.2±15.4	52.9±15.3	51.5±15.6	50.8±14.9	<0.001
LDL-cholesterol (mg/dl)	NA	NA	105.7±30.0	106.3±29.4	105.3±30.9	93.7±26.2	0.001
Fast plasma glucose (mg/dl)	NA	NA	116.7±36.8	115.6±35.4	118.0±38.1	115.6±49.3	0.01
Hemoglobin A_{1c} (%)	NA	NA	5.8±1.0	5.8±0.9	5.9±1.0	5.8±1.1	<0.001
Uric acid (mg/dl)	NA	NA	5.9±1.6	5.7±1.5	6.2±1.8	6.6±2.2	<0.001
Other intervention							
CRT/ICD (%)	1.5	0.002	1.9	0.9	2.9	15.8	<0.001
Heart surgery (%)	NA	NA	14.4	10.9	18.6	18.9	<0.001
PCI (%)	NA	NA	36.8	40.6	32.6	26.3	<0.001
BNP (pg/ml), mean±SD	273.0±352.6	<0.001	145.4±249.3	97.6±188.1	191.4±283.5	454.3±555.6	<0.001
Urine albumin (mg/g·Cre), mean±SD	NA	NA	129.6±476.7	106.5±429.9	157.6±530.1	180.9±330.0	0.001

HF, heart failure; CAD, coronary artery disease; NYHA, New York Heart Association; BMI, body mass index; NA, not applicable; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRT, cardiac resynchronization therapy; ICD, implantable cardioverter defibrillator; PCI, percutaneous coronary intervention; BNP, B-type natriuretic peptide; Cre, creatinine.
*Comparison of stage C/D patients in the CHART-1 Study with those in the CHART-2 Study. **Comparison of stage B/CAD, stage C, and stage D in the CHART-2 Study.

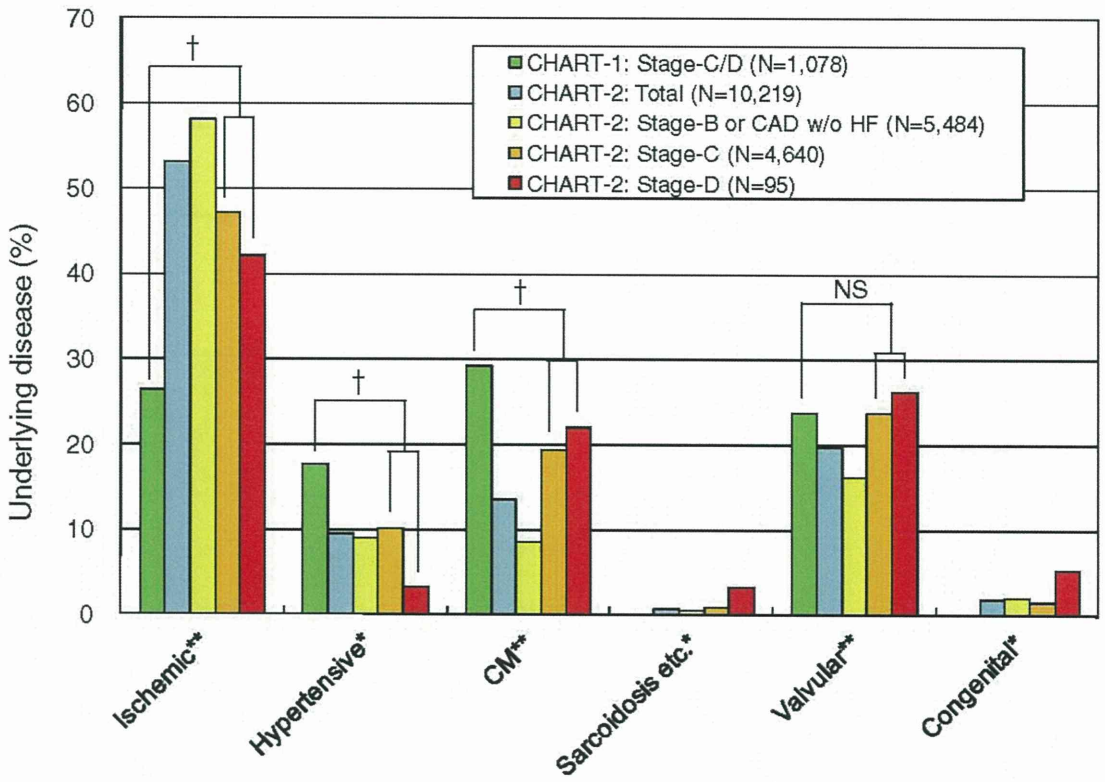

Figure 3. Underlying disease of the CHART-1 and the CHART-2 patients. CAD, coronary artery disease; CM, cardiomyopathy; HF, heart failure; w/o, without. *P<0.05 and **P<0.001 between patients in stage B/CAD, stage C, and stage D in the CHART-2 Study. †P<0.001 between stage C/D patients in the CHART-1 Study and those in the CHART-2 Study.

Statistical Analysis

We divided the study patients into 3 groups: patients with CAD but without HF or who were in stage B; those in stage C; and those in stage D. Comparisons of data between the 3 groups were performed using ANOVA test for continuous variables and chi-squared test for dichotomous variables. Continuous data are given as mean±SD. In order to elucidate the trend of HF in Japan, we selected overt HF patients from the CHART-1 Study (n=1,078, 84.4% of the total cohort), who were categorized as being in stages C or D. We then compared the characteristics of the stage C/D patients in the CHART-1 Study with those in the CHART-2 Study.[4,5] All statistical analyses were performed using IBM SPSS Statistics 19.0, and statistical significance was defined as 2-sided P<0.05.

Results

The enrollment of patients in the CHART-2 Study was started in October 2006. The registration period was prolonged once to achieve the target enrollment number. As of March 2010, a total of 10,219 patients have been enrolled at 24 institutions and the recruitment of patients has been closed, making the Study the largest multicenter prospective cohort of HF patients in Japan (**Figure 2**).

Clinical Profiles of the CHART-2 Patients at Registration

The mean age of the total study population was 68.2±12.3 years. Male patients accounted for 69.8%, and 79.5% of the total subjects were outpatients. In the present study, 5,484 patients (53.7%) did not have HF but had CAD or cardiac structural disorder. The stage C group included 4,640 patients and accounted for 45.4% of the entire cohort, while 95 patients (0.9%) were classified as being in stage D. Baseline characteristics of the CHART-1 stage C/D patients and the total CHART-2 subjects are given in **Table 1**. These data including age, sex, vital signs, HF symptoms, anthropometric data, history of smoking, alcohol use, and laboratory findings illustrate the difference in patient characteristics between the 2 studies performed at approximately 6-year intervals. Etiology, comorbidity, medication and echocardiographic findings at registry in the 2 studies are also given in **Figures 3–6**, respectively.

Baseline Characteristics and Different Clinical Profile vs. HF Stage

Clinical profiles of the CHART-2 patients were considerably different between the 3 HF stages. Mean age increased and HF symptoms became more severe as HF stage progressed (**Table 1**). Mean systolic/diastolic blood pressure at registration was 128.3/73.5 mmHg and decreased significantly with progression of HF stage. Mean body mass index was 24.0±3.6 kg/m^2 and mean waist circumference was 87.2±9.0 cm in men and 83.1±11.2 cm in women. The factors for obesity status significantly decreased with HF severity (**Table 1**). MetS as defined by the Japanese criteria was also significantly less frequent in patients in stage C or D compared with those in stage B or those who had CAD but without HF

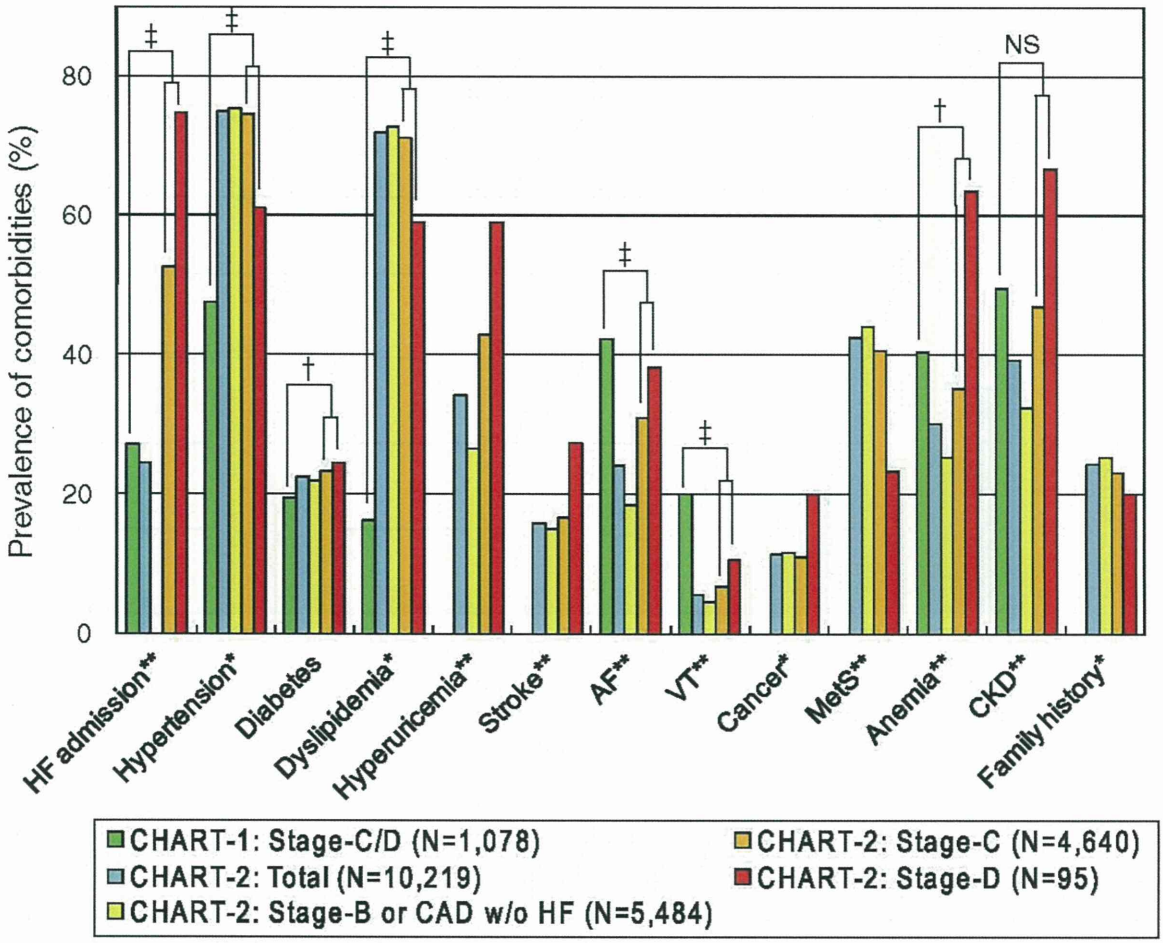

Figure 4. Comorbidities of the CHART-1 and the CHART-2 patients. AF, atrial fibrillation/flutter; CAD, coronary artery disease; CKD, chronic kidney disease; HF, heart failure; MetS, metabolic syndrome; VT, ventricular tachycardia; w/o, without. *P<0.05 and **P<0.001 between patients in stage B/CAD, stage C, and stage D in the CHART-2 Study. †P<0.05 and ‡P<0.001 between stage C/D patients in the CHART-1 Study and those in the CHART-2 Study.

(**Figure 4**). Approximately 18% of patients with CVD had a smoking habit and approximately 28% of the total patients were regular alcohol drinkers (**Table 1**).

Etiology of CVD in the CHART-2 patients is shown in **Figure 3**. CAD was the most prevalent etiology of CVD (53.1%), and approximately 20% of patients had valvular abnormalities as a cause of CVD. Cardiomyopathy accounted for 13.6% of the CHART-2 patients, and the prevalence increased as HF stage progressed. Myocardial diseases due to sarcoidosis or amyloidosis were observed in 0.7% of the total population.

Figure 4 illustrates comorbidities of the CHART-2 patients. The proportion of patients with a history of hospitalization for HF was 52.5% in stage C and 74.7% in stage D. Histories of hypertension or dyslipidemia were very common (74.9% and 71.8%), and diabetes was observed in 22.5% of the total population. Approximately 12% of patients had malignant neoplasm at enrollment. The prevalence of CKD increased significantly as HF stage progressed, accompanied by an increased percentage of patients with anemia and elevated urine albumin excretion (**Table 1**). Patients with overt HF, who were categorized in stages C or D, were also characterized by higher prevalence of atrial fibrillation/flutter, ventricular tachycardia and a history of stroke.

Heart surgery and percutaneous coronary intervention were performed in 14.4% and in 36.8% of the study population, respectively. The rates of use of implantable cardioverter defibrillator and cardiac resynchronization therapy were the highest in stage D (**Table 1**).

Figure 5 shows the usage rates of medication in the CHART-2 patients. A total of 64.6% of patients were treated with renin–angiotensin system (RAS) inhibitors, and β-blockers were used in 40.4% of patients. The penetration rates of such standard medication for HF were the highest in stage C but decreased in stage D patients. Aldosterone inhibitors, digitalis, warfarin, and amiodarone were used most frequently in stage D patients.

Echocardiographic findings and LVEF are shown in **Figure 6**. As HF stage progressed, LV end-diastolic dimension was increased, LVEF was decreased, and the percentage of patients with low EF was increased. Patients with HFpEF comprised 69.1% and 51.1% of stage C and D subjects, respectively. B-type natriuretic peptide (BNP) level was also increased as HF stage progressed (**Table 1**).

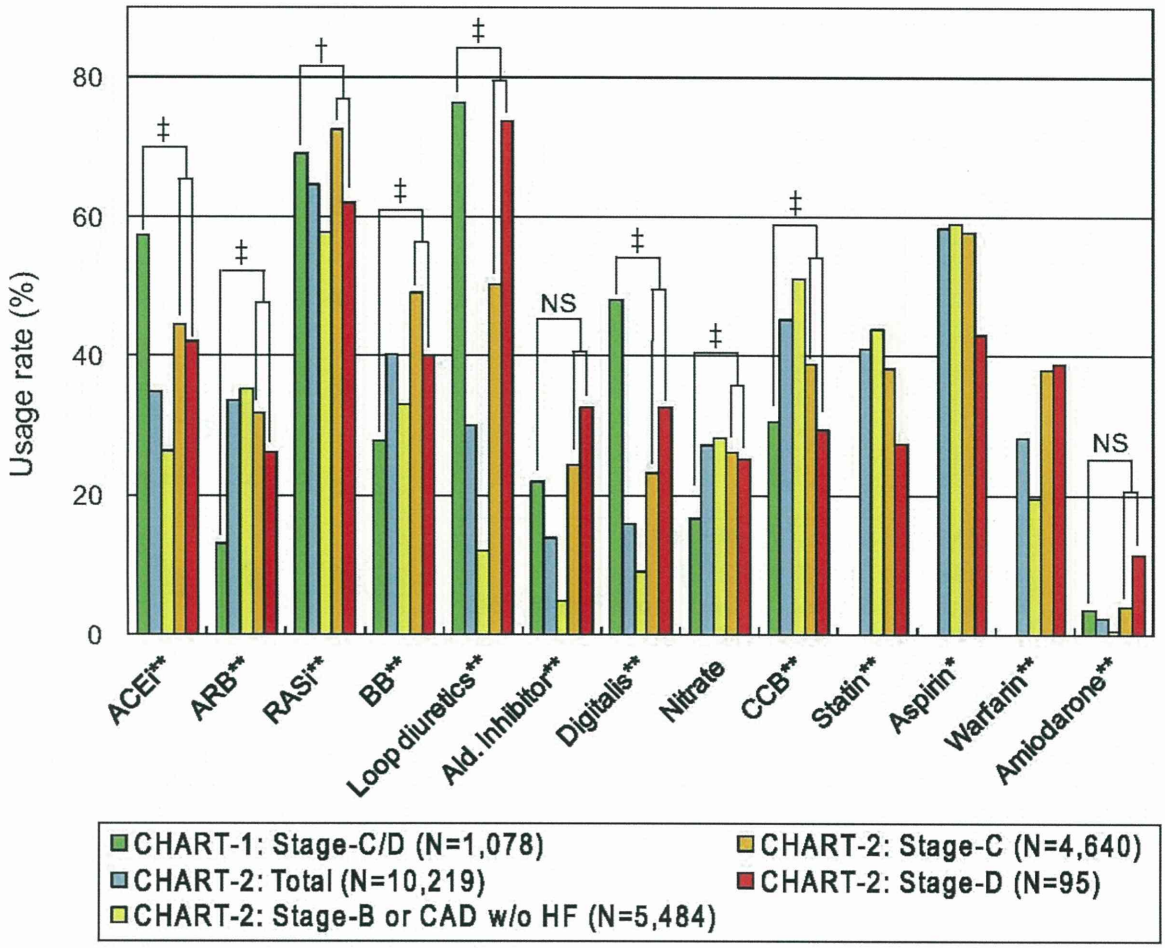

Figure 5. Medication of the CHART-1 and CHART-2 patients. ACEi, angiotensin-converting enzyme inhibitor; Ald., aldosterone; ARB, angiotensin II receptor blocker; BB, β-blocker; CAD, coronary artery disease; CCB, calcium channel blocker; RASi, renin–angiotensin system inhibitor; w/o, without. *P<0.05 and **P<0.001 between patients in stage B/CAD, stage C, and stage D in the CHART-2 Study. †P<0.05 and ‡P<0.001 between stage C/D patients in the CHART-1 Study and those in the CHART-2 Study.

Comparisons of Baseline Characteristics Between the CHART-1 Patients and the CHART-2 Patients or Those in Western Studies

The baseline characteristics of stage C/D patients enrolled in the previous CHART-1 Study[4,5] are given in **Table 1** and **Figures 3–6**. **Table 2** lists the comparisons of registration data in overt HF patients between CHART-1, CHART-2, and several observational Western cohort studies.

Mean age, blood pressure, and prevalence of CKD were similar between overt HF patients in the CHART-1 Study and those in the CHART-2 Study (**Tables 1,2**). As compared with the CHART-1 patients, however, those in the CHART-2 Study were characterized by a higher proportion having CAD as an etiology of HF (47.1%), the higher prevalence of histories of hypertension and diabetes (74.3% and 23.3%, respectively), more frequent HF admission history (53.0%), and a higher proportion having HFpEF (68.7%; **Table 2**; **Figures 3–5**). The usage rate of RAS inhibitors and β-blockers for overt HF patients in the CHART-1 and CHART-2 Studies increased from 69.1% to 72.3% and from 27.9% to 49.0%, respectively. In contrast, the usage rate of loop diuretics and digitalis decreased from 76.3% to 50.9% and from 48.1% to 23.5%, respectively (**Figure 5**).

Table 2 summarizes the baseline characteristics of overt HF patients in the CHART-1 Study, the CHART-2 Study, and Western observational cohort studies. Compared with Western patients, the CHART patients were characterized by less frequent ischemic etiology of HF, lower systolic blood pressure, less frequent diabetes, lower body mass index, and more frequent HFpEF. Usage rates of RAS inhibitors and β-blockers were similar between the CHART-2 patients and the Western HF patients except for the use of diuretics.

Characteristics of Patients in Stage B or Having CAD but Without HF

Patients in stage B or having CAD but without HF were characterized by younger age (67.6 years), a higher proportion of male patients (71.0%), less severe symptoms, and higher EF compared with patients in stages C or D (**Table 1**; **Figure 6**). The prevalence of cardiovascular risks such as hypertension, diabetes, and dyslipidemia, however, was similarly high (**Figure 4**), BNP was mildly elevated (**Table 1**), and the usage rate of standard HF treatment, such as RAS inhibitors and β-blockers, was too low in those patients (**Figure 5**).

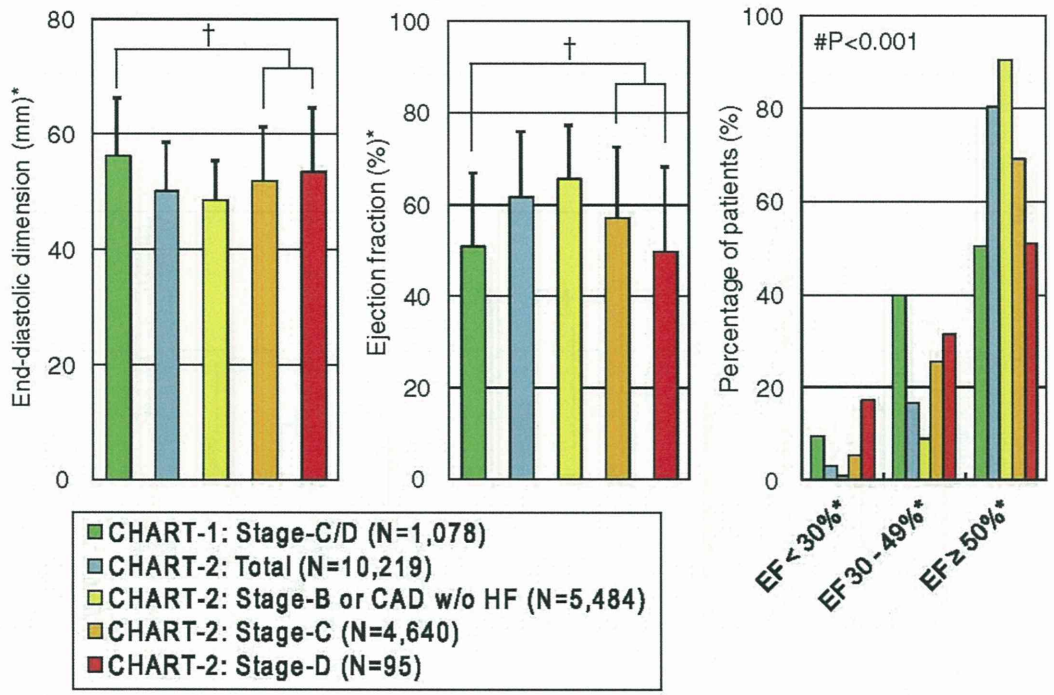

Figure 6. Left ventricular dimension and ejection fraction of the CHART-1 and CHART-2 patients. CAD, coronary artery disease; EF, ejection fraction. *P<0.001 between patients in stage B/CAD, stage C, and stage D in the CHART-2 Study. †P<0.001 between stage C/D patients in the CHART-1 Study and those in the CHART-2 Study. #P-value for the difference in distribution of ejection fraction between stage C/D patients in the CHART-1 Study and those in the CHART-2 Study.

Discussion

The clinical characteristics and prognosis of patients at high risk for disease progression due to development of AHFS have been poorly described, and thus epidemiological research involving such patients is extremely important in preventing the disease progression of HF and CVD. The CHART-2 Study is the first and the largest multicenter prospective cohort of consecutively enrolled patients at high risk for CVD progression due to AHFS in Japan. The Tohoku University head office and the CRC fulfilled their function to enroll patients in collaborating hospitals located in the Tohoku area, and the newly developed Web-based entry system also supported smooth entry of patient data.

Major Findings of the Present Analysis

Analysis of the registration data provides several new findings regarding patients with HF and those at risk of disease progression due to development of AHFS. First, when the CHART-2 patients were compared with the CHART-1 patients, a trend of increasing ischemic etiology and comorbidities of diabetes and hypertension was evident in Japanese patients with HF, whereas those risks had been more prominent in Western patients with HF (**Table 2**; **Figures 3,4**). Second, in the CHART-2 Study approximately 54% of patients were classified as being in stage B or having CAD without overt HF. In those patients, the plasma BNP concentration was mildly elevated and the cardiovascular risk profile was also similar to that of patients in stages C or D (**Table 1**; **Figures 3–5**). Third, the severity of prognostic risks including reduced EF, elevated BNP, comorbidity of CKD, and low hemoglobin level were exacerbated progressively as HF stage progressed in the CHART-2 patients (**Table 1**; **Figures 4,6**). Fourth, the prevalence of HFpEF patients was higher (68.7%) in the CHART-2 Study compared with the CHART-1 Study, demonstrating the trend of increasing prevalence of HFpEF (**Figure 6**).[12,13] Finally, the usage rates of standard medications in the CHART-2 patients were increased compared with the CHART-1 patients, but the usage was still too low, especially in the stage B patients (**Figure 5**).

Clear Trend of Increasing Prevalence of Ischemic HF in Japan

Several observational studies have previously demonstrated that the prevalence of CAD as an etiology in HF patients was 25–32% in Japan.[3,4,20,21] The prevalence of HF patients with ischemic etiology in the CHART-2 Study was dramatically increased compared with that in the CHART-1 Study, approaching the prevalence observed in Western subjects (**Table 2**, **Figure 3**). The prevalence of hypertension and diabetes, which are significant risks for developing CAD, similarly increased in the CHART-2 patients compared with the CHART-1 patients (**Table 2**, **Figure 4**). The report of the MIYAGI-AMI Registry Study showed the steady trend of increasing incidence of acute myocardial infarction in 30 years in Japan.[22] We speculate that the clear trend of increasing prevalence of CAD as an etiology of HF is due to the following reasons: (1) the number of CAD patients has been increasing due to accelerated westernization of lifestyle in Japanese people; and (2) the number of survivors after acute coronary event has dramatically increased due to the recent progress in treatment.

Table 2. Baseline Characteristics: CHART Patients vs. Previous Western HF Studies

	Framingham Study (1993)[16]	ADHERE (2005)[8]	EuroHeart Failure Survey II (2006)[9]	Owan et al (2006)[12]	Bhatia et al (2006)[13]	CHART-1 (Stage C/D, 2004)[4]	CHART-2 (Stage C/D, 2010)
No. patients	652	105,388	3,580	4,596	2,450	1,078	4,735
Age (years), mean±SD	70.0±10.8	72.4±14.0	69.9±12.5	73.0	73.1	68.7±13.4	68.9±12.3
Male (%)	51	48	61.3	55.5	52.4	64.5	68.4
Blood pressure (mmHg), mean±SD							
Systolic	150.9±27.6	144±32.6	NA	NA	150.0	126.3±19.1	126.3±19.2
Heart rate (/min), mean±SD	78.6±14.6	NA	NA	NA	NA	74.7±14.3	72.4±14.9
Comorbidity (%)							
Hypertension	74	73	NA	54.9	51.3	47.4	74.3
Diabetes	19	44	NA	33.7	36.3	19.5	23.3
Atrial fibrillation/flutter	NA	31	NA	34.5	26.6	42.3	31.0
Ventricular tachycardia	NA	8	NA	NA	NA	20.1	6.8
CKD	NA	30 (renal insufficiency)	NA	NA	20.1 (Cre <1.7 mg/dl)	49.5	47.3
History of HF admission	NA	NA	NA	NA	NA	27.2	53.0
Underlying disease (%)							
Ischemic	53.5	57	53.6	58.6	44.0	26.4	47.1
Hypertensive	23.6	NA	62.5	NA	NA	17.7	9.9
Valvular	16.0	NA	34.4	4.7	NA	23.8	23.8
BMI (kg/m²), mean±SD	27.2±5.3	NA	26.8	29.1	NA	23.0±3.7	23.8±3.9
LVEF (%), mean±SD	NA	34.4±16.1	38±15	44.1	39.0	50.9±16.0	56.9±15.5
≥50% (%)	NA	37†		47.2	35.9†	50.6	68.7
Medication (%)							
ACEI	NA	41	55.0	NA	NA	57.4	44.6
ARB	NA	12	9.3	NA	NA	13.1	31.8
β-blocker	NA	48	43.2	NA	NA	27.9	49.0
Loop diuretics	NA	70 (all diuretics)	71.2 (all diuretics)	NA	NA	76.3	50.9
Digitalis	NA	28	26.6	NA	NA	48.1	23.5
Nitrate	NA	26	NA	NA	NA	16.8	26.3
Amiodarone	NA	11 (all anti-arrhythmics)	12.9 (all anti-arrhythmics)	NA	NA	3.6	4.2

CKD, chronic kidney disease; LVEF, left ventricular ejection fraction; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker. Other abbreviations see in Table 1.
†Ejection fraction >40%.

Patients at High Risk for AHFS in the CHART-2 Study

Heart failure is classified according to the 4 stages of HF syndrome.[2] Stage A and stage B are pre-HF stages but appropriate identification and treatment are needed to prevent the progression to overt HF, which is equivalent to the development of de novo AHFS. In the present study, we enrolled patients without HF but with CAD, patients with structural heart disease but without HF (stage B), and patients with overt HF (stages C and D) in order to include patients at high risk for developing AHFS.

In Western HF patients, approximately 60–80% of patients hospitalized due to AHFS have a previous history of HF,[8,9,23] and the re-hospitalization rate following HF admission is 25% at 30 days after admission.[24] These findings suggest that patients in stages C or D are the most susceptible group to AHFS. Approximately one-third of AHFS cases are considered to be de novo AHF,[8,9,23] and the majority were related to CAD.[24,25] Other major comorbidities or cardiovascular risks in patients admitted with AHFS included hypertension, diabetes, arrhythmia and renal insufficiency.[8,9,23,25] In the present study, the stage B patients were characterized by a high number of cardiovascular risks along with some cardiac structural abnormalities, and 58.2% of those patients had CAD (**Figures 3, 4**). For these reasons, we also enrolled stage B patients and those with CAD but without HF, as patients at high risk for developing AHFS.

HF Stage Progression and Exacerbation of Cardiovascular Risk

Baseline characteristics of the CHART-2 patients showed the graded effects of HF stage on cardiovascular risk and comorbidity. As the HF stage progressed from stage B to stage D, mean age, number of female patients, heart rate, cardiothoracic ratio, LV dimension, and plasma BNP concentration increased significantly; whereas blood pressure, hemoglobin level, body mass index, waist circumference and EF decreased significantly (**Table 1**; **Figures 3–6**). In the present study the BNP level was mildly elevated in patients with CAD but without HF or in those in stage B, and was significantly increased with the decline of EF and exacerbation of HF stage (**Table 1**; **Figure 6**). It has also been reported that stage B patients had increased BNP level with heightened risk of mortality or cardiovascular events.[26,27] CKD is also an extensive public health problem and is more prevalent in patients with CVD or with CVD-related risk factors, such as hypertension, diabetes mellitus, dyslipidemia, and MetS.[28,29]

Furthermore, CKD is also a significant aggravating factor in those patients. As shown in **Figure 4**, the number of patients with CKD increased with the severity of HF stage. Anemia or low hemoglobin level is associated with poor prognosis in HF patients.[30] Hemoglobin level was decreased in the CHART-2 patients, reflecting the worsening in severity of HF and CKD in those patients (**Table 1**; **Figure 4**). MetS involves a cluster of important risk factors, including central obesity, elevated fasting plasma glucose, dyslipidemia, and high blood pressure and has become a leading health concern due to the strong link to CVD.[19] A recent meta-analysis of 87 studies reported that MetS is associated with a 2-fold increase in cardiovascular outcomes and a 1.5-fold increase in all-cause mortality.[31] Otherwise low body mass index has been consistently considered to be associated with the increased number of deaths in HF patients,[32] and the prognostic influence of MetS in those patients remains uncertain. The present study demonstrates that both body mass index and the prevalence of MetS in the CHART-2 patients were significantly decreased as HF stage progressed (**Table 1**; **Figure 4**).

Increasing Prevalence of HFpEF in the CHART-2 Study

Approximately half of the HF patients have normal or preserved EF, called HFpEF.[12,13,20] In the CHART-2 Study the prevalence of HFpEF was increased compared with the CHART-1 Study (68.7% vs. 50.6%; **Table 2**; **Figure 6**). Although the reason for the increasing prevalence of HFpEF remains unknown, we suggest the following: (1) the Japanese population is rapidly aging and the percentage of elderly HF patients has increased;[3] (2) the prevalence of hypertension has increased as a comorbidity of HF (**Table 2**); and (3) the recent progress in reperfusion therapy has contributed to preservation of EF after acute coronary events.[22]

Use of Standard Medication for CVD in the CHART-2 Patients

It has previously been reported that standard HF treatments were not used in patients who would have benefited from such medications.[33] The overall usage rates of RAS inhibitors or β-blockers in the CHART-2 patients were 64.6% and 40.4%, respectively (**Figure 5**). Although the penetration rate of such treatment was increased in overt HF patients in the CHART-2 Study compared with the CHART-1 Study (**Table 2**), it was still too low, especially in stage B patients (**Figure 5**). Further investigation is necessary to evaluate how such a low treatment rate of evidence-based medicine affects the prognosis of stage B patients.

Study Limitations

Several limitations in the design of the CHART-2 Study should be mentioned. First, the present study did not include data regarding physical inactivity, diet or nutrition, all of which are important modifiable risks for developing CVD. Second, all subjects in the CHART studies were Japanese people, which may limit extrapolation of the results to patients in Western countries. Third, the difference of the entry criteria in the CHART-1 and CHART-2 Studies might limit accurate comparison of enrolled patients in those 2 studies. Fourth, the primary design of the present study did not cover chronic lung disease, which has been recently recognized as one of the important cardiovascular risks.[34] In order to address this important issue, we started a retrospective survey on chronic obstructive pulmonary disease in the CHART-2 patients from April 2010.

Conclusions

The CHART-2 Study demonstrates the trend of increasing westernization of etiology, and the prevalence of hypertension and diabetes in HF patients in Japan. Although the number of HF patients is predicted to increase dramatically in the near future, the usage rate of standard medications in patients with CVD or HF is still too low, especially in stage B patients. Given the growing number of patients with CVD and HF in Japan, strategies preventing the development of CAD must be given top priority.

Acknowledgments

We thank all staff of the Department of Evidence-Based Cardiovascular Medicine, Tohoku University Graduate School of Medicine and all investigators at the collaborating institutions in the Tohoku Heart Failure Association. There are no conflicts of interest.

References

1. World Health Organization. Cardiovascular disease. http://www.who.int/cardiovascular_diseases/en/ (accessed 8 February, 2011).
2. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, et al. 2009 Focused update incorporated into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the International Society for Heart and Lung Transplantation. *J Am Coll Cardiol* 2009; **53:** e1–e90.
3. Shiba N, Shimokawa H. Chronic heart failure in Japan: Implications of the CHART studies. *Vasc Health Risk Manag* 2008; **4:** 103–113.
4. Shiba N, Watanabe J, Shinozaki T, Koseki Y, Sakuma M, Kagaya Y, et al. Analysis of chronic heart failure registry in the Tohoku district: Third year follow-up. *Circ J* 2004; **68:** 427–434.
5. Shiba N, Watanabe J, Shinozaki T, Koseki Y, Sakuma M, Kagaya Y, et al. Poor prognosis of Japanese patients with chronic heart failure following myocardial infarction: Comparison with nonischemic cardiomyopathy. *Circ J* 2005; **69:** 143–149.
6. Gheorghiade M, De Luca L, Fonarow GC, Filippatos G, Metra M, Francis GS. Pathophysiologic targets in the early phase of acute heart failure syndromes. *Am J Cardiol* 2005; **96:** 11G–17G.
7. Krumholz HM, Normand SL, Spertus JA, Shahian DM, Bradley EH. Measuring performance for treating heart attacks and heart failure: The case for outcomes measurement. *Health Aff (Millwood)* 2007; **26:** 75–85.
8. Adams KF, Fonarow GC, Emerman CL, LeJemtel TH, Costanzo MR, Abraham WT, et al. Characteristics and outcomes of patients hospitalized for heart failure in the United States: Rationale, design, and preliminary observations from the first 100,000 cases in the Acute Decompensated Heart Failure National Registry (ADHERE). *Am Heart J* 2005; **149:** 209–216.
9. Nieminen MS, Brutsaert D, Dickstein K, Drexler H, Follath F, Harjola VP, et al. EuroHeart Failure Survey II (EHFS II): A survey on hospitalized acute heart failure patients: Description of population. *Eur Heart J* 2006; **27:** 2725–2736.
10. Ouzounina M, Lee DS, LiuPP. Diastolic heart failure: Mechanisms and controversies. *Nat Clin Pract Cardiovasc Med* 2008; **5:** 375–386.
11. Paulus WJ, Tschöpe C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, et al. How to diagnose diastolic heart failure: A consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Association of the European Society of Cardiology. *Eur Heart J* 2007; **28:** 2539–2550.
12. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med* 2006; **355:** 251–259.
13. Bhatia RS, Tu JV, Lee DS, Austin PC, Fang J, Haouzi A, et al. Outcome of heart failure with preserved ejection fraction in a population-based study. *N Engl J Med* 2006; **355:** 260–269.
14. Kravitz RL, Hays RD, Sherbourne CD, DiMatteo MR, Rogers WH, Ordway L, et al. Recall of recommendations and adherence to advice among patients with chronic medical conditions. *Arch Intern Med* 1993; **153:** 1869–1878.
15. Mazzaglia G, Ambrosioni E, Alacqua M, Filippi A, Sessa E, Immordino V, et al. Adherence to antihypertensive medications and

cardiovascular morbidity among newly diagnosed hypertensive patients. *Circulation* 2009; **120:** 1598–1605.
16. Ho KK, Anderson KM, Kannel WB, Grossman W, Levy D. Survival after the onset of congestive heart failure in the Framingham Heart Study. *Circulation* 1993; **88:** 107–115.
17. Nutritional anaemias. Report of a WHO scientific group. *World Health Organ Tech Rep Ser* 1968; **405:** 5–37.
18. Japanese Society of Nephrology. Evidence-based practice guideline for the treatment of CKD. *Clin Exp Nephrol* 2009; **13:** 537–566.
19. Miura Y, Fukumoto Y, Shiba N, Miura T, Shimada K, Iwama Y, et al. Prevalence and clinical implication of metabolic syndrome in chronic heart failure. *Circ J* 2010; **74:** 2612–2621.
20. Tsuchihashi-Makaya M, Hamaguchi S, Kinugawa S, Yokota T, Goto D, Yokoshiki H, et al. Characteristics and outcomes of hospitalized patients with heart failure and reduced vs. preserved ejection fraction: A report from the Japanese Cardiac Registry of Heart Failure in Cardiology (JCARE-CARD). *Circ J* 2009; **73:** 1893–1900.
21. Tsutsui H, Tsuchihashi-Makaya M, Kinugwa S, Goto D, Takeshita A. Characteristics and outcomes of patients with heart failure in general practices and hospitals: Japanese Cardiac Registry of Heart Failure in General Practice (JCARE-GENERAL). *Circ J* 2007; **71:** 449–454.
22. Takii T, Yasuda S, Takahashi J, Ito K, Shiba N, Shirato K, et al. Trends in acute myocardial infarction incidence and mortality over 30 years in Japan: Report from the MIYAGI-AMI Registry Study. *Circ J* 2010; **74:** 93–100.
23. Gheorghiade M, Abraham WT, Albert NM, Greenberg BH, O'Connor CM, She L, et al. Systolic blood pressure at admission, clinical characteristics, and outcomes in patients hospitalized with acute heart failure. *JAMA* 2006; **296:** 2217–2226.
24. Ross JS, Chen J, Lin Z, Bueno H, Curtis JP, Keenan PS, et al. Recent national trends in readmission rates after heart failure hospitalization. *Circ Heart Fail* 2010; **3:** 97–103.
25. Zannad F, Mebazaa A, Juillie're Y, Cohen-Solal A, Guize L, Alla F, et al. Clinical profile, contemporary management and one-year mortality in patients with severe acute heart failure syndromes: The EFICA study. *Eur J Heart Fail* 2006; **8:** 697–705.
26. Ammar KA, Jacobsen SJ, Mahoney DW, Kors JA, Redfield MM, Burnett JC, et al. Prevalence and prognostic significance of heart failure stages: Application of the American College of Cardiology/American Heart Association Heart Failure staging criteria in the community. *Circulation* 2007; **115:** 1563–1570.
27. Daniels LB, Clopton P, Jiang K, Greenberg B, Maisel A. Prognosis of Stage A or B heart failure patients with elevated B-type natriuretic peptide levels. *J Card Fail* 2010; **16:** 93–98.
28. Shiba N, Shimokawa H. Chronic kidney disease and heart failure: Bidirectional close link and common therapeutic goal. *J Cardiol* 2011; **57:** 8–17.
29. Shiba N, Matsuki M, Takahashi J, Tada T, Watanabe J, Shimokawa H. Prognostic importance of chronic kidney disease in Japanese patients with chronic heart failure. *Circ J* 2008; **72:** 173–178.
30. Go AS, Yang J, Ackerson LM, Lepper K, Robbins S, Massie BM, et al. Hemoglobin level, chronic kidney disease, and the risks of death and hospitalization in adults with chronic heart failure: The Anemia in Chronic Heart Failure: Outcomes and Resource Utilization (ANCHOR) Study. *Circulation* 2006; **113:** 2713–2723.
31. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al. The metabolic syndrome and cardiovascular risk: A systematic review and meta-analysis. *J Am Coll Cardiol* 2010; **56:** 1113–1132.
32. Nochioka K, Shiba N, Kohno H, Miura M, Shimokawa H. Both high and low body mass indexes are prognostic risks in Japanese patients with chronic heart failure: Implications from the CHART study. *J Card Fail* 2010; **16:** 880–887.
33. Masoudi FA, Havranek EP, Wolfe P, Gross CP, Rathore SS, Steiner JF, et al. Most hospitalized older persons do not meet the enrollment criteria for clinical trials in heart failure. *Am Heart J* 2003; **146:** 250–257.
34. Kjøller E, Køber L, Iversen K, Torp-Pedersen C. Trace Study Group. Importance of chronic obstructive pulmonary disease for prognosis and diagnosis of congestive heart failure in patients with acute myocardial infarction. *Eur J Heart Fail* 2004; **6:** 71–77.

Appendix 1

Study Organization of The CHART-2 Study

Executive Committee

Hiroaki Shimokawa (Chair), Mitsumasa Fukuchi, Toshikazu Goto, Tetsuya Hiramoto, Kanichi Inoue, Atsushi Kato, Tatsuya Komaru, Masatoshi Ohe, Nobuyo Sekiguchi, Nobuyuki Shiba, Tsuyoshi Shinozaki, Masafumi Sugi, Kenji Tamaki.

Steering Committee

Tetsuya Hiramoto, Kanichi Inoue, Atsushi Kato, Masahiko Ogata, Syoichi Sato, Masafumi Sugi.

Collaborating Hospitals and Active Investigators by Prefecture

Aomori Prefecture

J. Kikuchi, S. Oyama, T. Tajima (Towada City Hospital).*

Iwate Prefecture

K. Tamaki, E. Nozaki, N. Hoshi (Iwate Prefectural Central Hospital).*
M. Nakagawa (Iwate Prefectural Isawa Hospital).*

Akita Prefecture

M. Hayashi, N. Sekiguchi, Y. Sugai, Y. Asaumi, K. Fukahori, S. Takeda, H. Endo, Y. Kokusho (Hiraka General Hospital).*

Yamagata Prefecture

M. Ohe, T. Kobayashi, M. Morita, K. Sakurai, T. Kagatani, A. Sato (Kojirakawa Shiseido Hospital).*
T. Goto, T. Yahagi, M. Matsui, Y. Tamada, A. Fukui, K. Takahashi, K. Takahashi, K. Oumi, K. Hao, S. Sasaki, A. Kikuchi, S. Suzuki, N. Yaoita (Yamagata Prefectural Central Hospital).

Miyagi Prefecture

K. Akai, Y. Konno, J. Demachi, T. Tajima, N. Takahashi (Ishinomaki Municipal Hospital).
Y. Konno (Japanese Red Cross Sendai Hospital).
N. Shiba, M. Wakayama (Kanagami Hospital).
H. Kanno, T. Ishizuka, J. Kaneko (Katta General Hospital).*
M. Fukuchi, N. Shiba, A. Sugimura, J. Otomo, H. Tada, Y. Ito (KKR Tohoku Kosai Hospital)*.
S. Komatsu, J. Suzuki (Kurihara Central Hospital).
D. Katayose (Miyagi Rifu Ekisaikai Hospital).*
S. Onodera, T. Hiramoto, M. Chida, K. Iwabuchi, M. Takeuchi, M. Takeda, K. Yahagi (Osaki Citizen Hospital).
Y. Kagaya, K. Otsuka, K. Komaki, Y. Koseki (Saito Hospital).
T. Shinozaki, T. Tanikawa, S. Baba, T. Tomioka, M. Tanaka, N. Onoue (Sendai Medical Center).*
M. Kanazawa, A. Kato, S. Namiuchi, B. H. Ong, C. Takahashi, T. Sugie, H. Tada (Sendai Open Hospital).*
S. Sato, M. Fukuchi, M. Ogata, K. Sakurai, A. Sugimura, T. Tomioka, O. Kitamukai (Sendai Tokushukai Hospital).*
Y. Fukumoto (Shizugawa Public Hospital).
K. Inoue, S. Horiguchi, J. Koyama, H. Shioiri, T. Tomioka (South Miyagi Medical Center).
T. Sakuma, T. Komaru, H. Kato, K. Saji (Tohoku Rosai Hospital)*.
Y. Kagaya, M. Miura, T. Komaru, N. Shiba, S. Yasuda, A. Karibe, J. Demachi, Y. Fukumoto, J. Nawata, K. Ito, M. Nakayama, K. Fukuda, M. Takeda, Y. Wakayama, R. Koshida, J. Takahashi, M. Wakayama, K. Kumagai, Y. Sugai, K. Sugimura, M. Hirose, K. Satoh, Y. Asaumi, H. Tada, K. Saji, T. Tada, M. Nakano, Y. Kokusho, S. Fukui, N. Yamaguchi, Y. Ito, R. Tsuburaya, K. Nochioka, Y. Miura, J. Ohashi, K. Aizawa, Y. Kikuchi, T. Takii, M. Kondo, K. Hao, K. Takahashi, Y. Takagi, S. Nakajima, S. Tatebe, T. Shimizu, S. Miyamichi, K. Noda (Tohoku University Hospital).*

Fukushima Prefecture

M. Sugi, Y. Yamamoto, Y. Minatoya, T. Tada, T. Shiroto (Iwaki Kyouritsu Hospital).*
K. Fukuda, M. Takeda (Watanabe Hospital).*
*Fifteen hospitals were collaborating institutions in the CHART-1 Study.

Head Office and Coordinating Center

N. Shiba, K. Nochioka, M. Miura, M. Matsuki, S. Osaki, D. Ito, K. Yukishita, Y. Kidoguchi, M. Hotta, H. Kohno, Y. Nakajima, E. Yanagisawa, A. Mori, M. Takahashi, R. Takeuchi, K. Takahashi, S. Toudera, M. Sugaya, M. Sato, C. Saga, J. Suenaga, M. Kikuchi, F. Mori.

Appendix 2

Subjects in stage B must meet at least one of the following criteria and must not have signs, symptoms, or history of hospitalization for heart failure.

(1) Enlarged left ventricular end-diastolic dimension (≥55 mm) measured on echocardiography.
(2) Impaired left ventricular ejection fraction (≤50%) measured on echocardiography.
(3) Thickened interventricular septum (>12 mm) and/or thickened left ventricular posterior wall (>12 mm) measured on echocardiography.
(4) Significant valvular stenosis/insufficiency.
(5) Significant myocardial abnormalities.
(6) Congenital abnormalities.
(7) Previous cardiac surgery.

European Heart Journal (2012) **33**, 436–443
doi:10.1093/eurheartj/ehr362

REVIEW

Clinical update

Fish oil and omega-3 fatty acids in cardiovascular disease: do they really work?

Daan Kromhout[1]*, Satoshi Yasuda[2], Johanna M. Geleijnse[1], and Hiroaki Shimokawa[2]*

[1]Division of Human Nutrition, Wageningen University, PO Box 8129, 6700 EV Wageningen, The Netherlands; and [2]Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan

Received 1 July 2011; revised 9 August 2011; accepted 19 August 2011; online publish-ahead-of-print 19 September 2011

Omega-3 fatty acids, which are found abundantly in fish oil, exert pleiotropic cardiometabolic effects with a diverse range of actions. The results of previous studies raised a lot of interest in the role of fish oil and omega-3 fatty acids in primary and secondary prevention of cardiovascular diseases. The present review will focus on the current clinical uses of omega-3 fatty acids and provide an update on their effects. Since recently published trials in patients with coronary artery diseases or post-myocardial infarction did not show an effect of omega-3 fatty acids on major cardiovascular endpoints, this review will examine the limitations of those data and suggest recommendations for the use of omega-3 fatty acids.

Keywords Fish oils • Cardiovascular disease

Introduction

In 1929, the essential fatty acids were discovered by the biochemists Evans and Burr.[1] They showed that mammals do not possess enzymes able to synthesize double bonds at the *n*-3 and *n*-6 positions of the carbon chain of a fatty acid. Therefore, humans must obtain the essential fatty acids linoleic acid (C18:2*n*-6) and alpha linolenic acid (ALA, C18:3*n*-3) from dietary sources. Alpha linolenic acid can be extended to eicosapentaenoic acid (EPA C20:5*n*-3) and docosahexaenoic acid (DHA C22:6*n*-3) through elongation and desaturation. Fish oil is a rich source of these omega-3 fatty acids.

In 1937, the British physiologist Hugh Sinclair visited Evans and became interested in the possibility that deficiencies in polyunsaturated fatty acids could cause coronary artery diseases (CAD). In 1944, he undertook his first visit to the Inuit and became convinced that their diet protects against atherosclerosis and Western diseases.[2] In a letter to the *Lancet*, he hypothesized in 1956 that omega-3 fatty acids may be responsible for the protective effect of their diet.[3] This view was contrary to the dogma of that time that all animal fats are harmful. In the 1970s, he joined the Danish investigators Bang and Dyerberg[4,5] during one of their expeditions to Greenland. They found that the Inuit consumed ~400 g of seafood per day and their average intake of omega-3 fatty acids was 14 g per day compared with 3 g per day among Danes. An epidemiological study showed that the incidence of myocardial infarction (MI) was 10 times lower among the Inuit compared with the Danes.[6]

The difference between the Inuit and the Danes in the intake of omega-3 fatty acids was reflected in their fatty acid composition of platelets. Differences were also observed in haemostatic factors, bleeding time, serum triglycerides, and high-density lipoprotein (HDL)—cholesterol levels. To show that these associations are causal, Sinclair put himself in 1977 on an Inuit diet for 100 days.[7] His bleeding time rose from 3–5 to 50 min and substantial decreases were observed in blood platelets, erythrocytes, packed cell volume, and haemoglobin. The triglyceride-rich very low-density lipoprotein (VLDL) fell and the HDL fraction increased considerably. A substantial increase in the EPA concentration and a marked decrease in the linoleic acid concentration of cholesteryl esters were noted. Sinclair concluded from this experiment that it is necessary to have the right balance of omega-3 and omega-6 fatty acids to prevent thrombotic disorders.

In 1985, Kromhout *et al.*[8] showed in the Zutphen Study, a prospective cohort study in the Netherlands, that eating fish once or twice per week was associated with a lower risk of fatal CAD

* Corresponding authors: Tel: +31 317 483054, Fax: +31 317 483342, Email: daan.kromhout@wur.nl (D.K.)/Tel: +81 22 717 7151, Fax: +81 22 717 7156, Email: shimo@cardio.med.tohoku.ac.jp (H.S.)

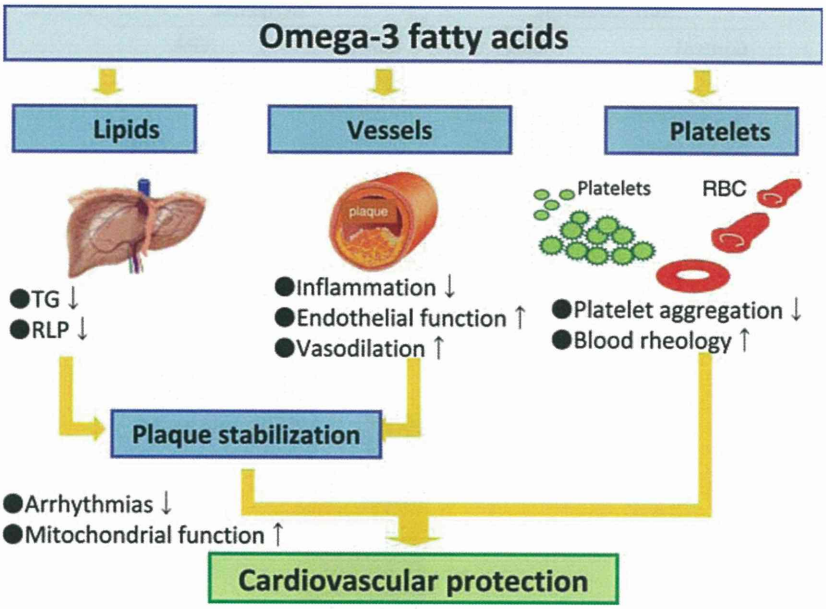

Figure 1 Beneficial effects of omega-3 fatty acids. TG, triglycerides; RLP, remnant lipoproteins; RBC, red blood cells.

compared with men who did not eat fish. Four years later in 1989, Burr *et al.*[9] showed in the Diet and Reinfarction Trial (DART) that cardiac patients who received an advice to add two fatty fish meals per week to their diet reduced CAD mortality significantly, compared with those who did not get a fish advice. The results of these studies raised a lot of interest in the role of fish oil and omega-3 fatty acids in primary and secondary prevention of cardiovascular diseases (CVD). In this article, we summarize the mechanisms of the action of omega-3 fatty acids and the results of cohort studies and clinical trials on omega-3 fatty acids and CVD. Finally, we draw conclusions on whether omega-3 fatty acids reduce the incidence of these diseases.

Mechanisms of action of omega-3 fatty acids

The cardiometabolic effects of omega-3 fatty acids continue to be extensively investigated and remain an active area of research. Omega-3 fatty acids can ultimately increase arrhythmic thresholds, reduce blood pressure, improve arterial and endothelial function, reduce platelet aggregation, and favourably affect autonomic tone (*Figure 1*). In this section, we briefly review recent studies that extend our knowledge on the cardioprotective effects of omega-3 fatty acids.[10]

Anti-inflammatory effects

Recently, the anti-inflammatory effects of omega-3 fatty acids have attracted much attention. Omega-3 fatty acids reduce the content of arachidonic acid (AA) in membrane phospholipids in platelets, endothelial cells, and inflammatory cells with a resultant reduced production of AA-derived pro-inflammatory mediators, including prostaglandin (PG)-E_2, thromboxane (TX)-B_2, leucotriene (LT)-B_4, hydroxyeicosatetraenoic acid (5-HETE), and LT-E_4. Importantly, EPA also acts as a substrate for cyclo-oxygenase and lipoxygenase enzymes, which could increase a different family of eicosanoids—the three-series PGs and TXs.[11] In addition to these anti-inflammatory effects, omega-3 fatty acids have a number of other effects that may occur either downstream of altered eicosanoid production or independent of this activity.[12] For example, the effects of omega-3 fatty acids on inflammatory cytokine expression could be at least in part through modulating intra-cellular signalling pathways that inactivates transcriptional factors.[12] Recent studies demonstrated that omega-3 fatty acids could down-regulate the activity of the nuclear factor (NF)-κB,[12] which plays a key role in the regulation of gene expression in inflammatory responses and has been implicated in the pathogenesis of CVD.[13] The inhibition of NF-κB activation can be mediated by the mechanism that is related to the activation of peroxisome proliferator-activated receptor (PPAR) or the inhibition of toll-like receptors.[13]

Rho-kinase is a downstream effector of the small GTPase Rho and mediates diverse cellular functions, such as smooth muscle cell contraction, cell migration, and proliferation.[14] Rho-kinase also up-regulates pro-inflammatory molecules and down-regulates endothelial nitric oxide (NO) synthase (eNOS).[15,16] It has been recently demonstrated that long-term treatment with EPA significantly inhibits Rho-kinase activation in the myocardium subjected to ischaemia–reperfusion *in vivo* (*Figure 2*).[17]

In addition, supplementation with EPA and DHA could exert a protective effect on the heart through improvement in mitochondrial function and the efficiency of ATP generation.[18] This effect may be due to changes in mitochondrial membrane phospholipids composition and improved efficiency of ATP generation.[18]

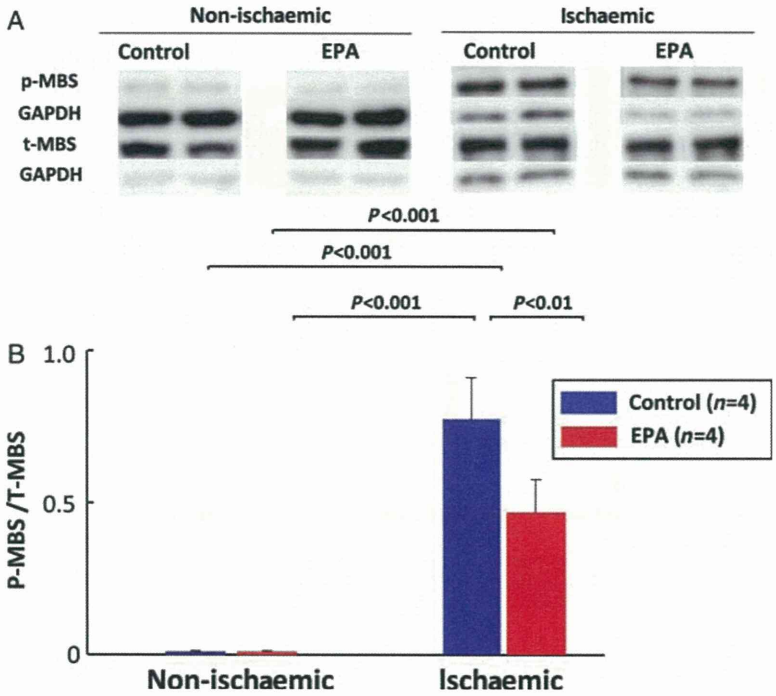

Figure 2 Long-term eicosapentaenoic acid treatment inhibits myocardial Rho-kinase activation induced by ischaemia/reperfusion. Male pigs were treated with either a control chow or eicosapentaenoic acid (600 mg/kg/day) for 3 weeks and were subjected to myocardial ischaemia by 90 min occlusion of the left circumflex coronary artery and subsequent 60 min reperfusion. The eicosapentaenoic acid group had increased eicosapentaenoic acid level in red blood cells (eicosapentaenoic acid 4.30 ± 0.63 mol%). The eicosapentaenoic acid treatment significantly ameliorated myocardial ischaemia/reperfusion injury and significantly inhibited myocardial Rho-kinase activity, assessed by the extent of myosin-binding subunit (MBS) phosphorylation. Top panel (*A*) shows representative western blots of myocardial expression of phosphorylated (p)- and total (t)-MBS. Bottom panel (*B*) shows quantitative results of the ratio of p-MBS to t-MBS, indicating Rho-kinase activity. Results are expressed as mean ± SD. (Reproduced from Gao *et al.*[17] with permission.)

Inhibition of platelet aggregation

Omega-3 fatty acids decrease the risk of thrombosis by inhibiting platelet aggregation. Importantly, omega-3 fatty acids inhibit platelet TXA_2 synthesis and acts as antagonists of the pro-aggregatory TXA_2/PG H_2 receptor in human platelets *in vitro*.[19] Supplementing a diet with omega-3 fatty acids down-regulate mRNA expression of platelet-derived growth factor-A and -B in mononuclear blood cells in humans.[20]

Triglyceride-lowering effects

Omega-3 fatty acids play an important role to regulate genes that are critical for controlling lipid homeostasis. Omega-3 fatty acids decrease VLDL assembly and secretion, resulting in diminished triacylglycerol production, through a decreased activity of sterol receptor element-binding protein-1c, which is the key switch in controlling lipogenesis.[21] In addition, omega-3 fatty acids could promote β-oxidation simultaneously in mitochondria and/or peroxisomes, possibly through the activation of peroxisome PPAR-α, leading to the reduction of fatty acids substrate for triglyceride synthesis.[21,22] The remnant lipoprotein (RLP), produced from the triacylglycerol-rich chylomicrons and VLDL, exerts potent pro-atherogenic effects and is thus regarded as an important risk factor of CVD.[22,23] The involvement of RLP has been suggested in the pathogenesis of sudden cardiac death[22] and restenosis after coronary angioplasty.[23] Although omega-3 fatty acids do not have a major effect on fasting total cholesterol and LDL cholesterol levels, EPA effectively reduces RLP in hyperlipidaemic patients.[24]

Improvement of endothelial function

Long-term treatment with fish oils augments endothelium-dependent relaxation of normal porcine coronary arteries,[25] for which EPA, a major omega-3 fatty acids of fish oils, is responsible for the augmentation.[26] This augmenting effect of EPA was also noted in porcine coronary microvessels.[27] Long-term treatment with fish oils improves endothelium-dependent relaxation of hypercholesterolaemic and atherosclerotic porcine coronary arteries[28] and femoral veins.[29] Eicosapentaenoic acid augments endothelium-dependent relaxation by NO as well as that by endothelium-derived hyperpolarizing factor.[30] Docosahexaenoic acid alters caveolae microenvironment not only by modifying membrane lipid composition, but also by changing distribution of major structural proteins, eventually increasing eNOS activity in human umbilical vein endothelial cells.[31] Nitric oxide also inhibits platelet aggregation and adhesion, leucocytes adhesion, and smooth muscle cell proliferation. In addition, in endothelial cells,

co-incubation with DHA following challenge with interleukin (IL)-1, IL-4, tumour necrosis-α, or lipopolysaccharide decreases expression of vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and E-selectin, and secretion of IL-6 and IL-8.[32]

Plaque stabilization

As mentioned above, through their anti-inflammatory effects, omega-3 fatty acids could not only prevent the plaque development but also contribute to the plaque stabilization.[33] The randomized clinical trial demonstrated that omega-3 fatty acids supplementation substantially increases tissue levels of EPA and DHA and decreases macrophage infiltration and thickened fibrous cap in human carotid arteries.[34] Exacerbated release of matrix metalloproteinase (MMP) by the activated endothelium and macrophages plays a pathological role in plaque progression and instabilization.[35] Eicosapentaenoic acid significantly suppresses the development of atherosclerotic lesions in ApoE−/− and LDL-receptor−/− mice with reduced production of MMPs by macrophages in a PPARα-dependent manner.[36]

Anti-arrhythmic effects

The omega-3 fatty acids are incorporated into cell membranes and affect the ion-channel function of myocytes. There are several mechanisms by which omega-3 fatty acids could exert anti-arrhythmic effects. Omega-3 fatty acids inhibit voltage-gated Na channels, prolonging relative refractory period and increased voltage that are required for membrane depolarization.[37] Omega-3 fatty acids also exhibit a modulatory action on L-type calcium Ca channels, resulting in lowered cytosolic free Ca and Ca influx rate and in preventing cytosolic Ca overload during ischaemic insult.[38] Long-term treatment with EPA reduces ischaemia-induced ventricular fibrillation in pigs *in vivo*, for which attenuation of shortening of monophasic action potential duration through suppression of cardiac K_{ATP} channels may be involved.[39] Anti-arrhythmic effect of omega-3 fatty acids may be mediated in part by their effects on autonomic control, especially by an increased vagal tone.[40] Through these mechanisms, omega-3 fatty acids may prevent ventricular tachyarrhythmias and hence decrease sudden cardiac death.[41]

Fish, omega-3 fatty acids, and coronary artery disease in cohort studies

Based on the ecological studies among the Inuit and those comparing farmers and fishermen in Japan, Kromhout *et al.*[8] hypothesized that a low level of fish consumption may reduce CAD mortality. They investigated this association in 852 middle-aged men free from CAD who were followed for 20 years. The average fish consumption in these men was 20 g per day, including those who did not eat fish (20%). About two-thirds of the fish was lean (e.g. cod and plaice) and one-third consisted of fatty fish (e.g. herring and mackerel). An inverse dose–response relationship was observed and CAD mortality was >50% lower among those who consumed at least 30 g of fish per day. Kromhout[42] deduced from the studies among the Inuit, Japanese fishermen and farmers, and the Zutphen men that two different mechanisms could be responsible for the association between fish consumption and CAD. He hypothesized an acute effect on fatal CAD in cultures with a low level of fish consumption and a chronic effect in cultures with a high level of fish consumption (*Figure 3*).

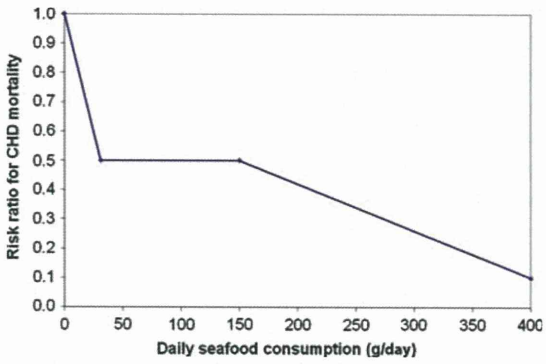

Figure 3 The association between seafood consumption and fatal coronary heart disease. (Reproduced from Kromhout[42] with permission.)

Since 1985, results of many prospective cohort studies on fish consumption and CAD have been published, with several studies showing a protective effect although others did not. The first quantitative review was published in 1999 by Marckmann and Gronbaek[43] and included 11 studies with 116 764 individuals. Four studies were judged to be of high quality, of which the two were performed in populations at high risk and the two in populations at low risk. In the high-risk populations, a protective association was found but not in the low-risk populations. The authors drew the conclusion that only in high-risk populations, a fish consumption of 40–60 g per day is associated with a markedly lower CAD mortality.

In 2004, two meta-analyses were published on fish consumption and fatal CAD.[44,45] The study of Whelton *et al.*[44] included both prospective cohort studies and case–control studies and the study by He *et al.*[45] only cohort studies. Case–control studies are more prone to selection and information bias and it is particularly difficult to obtain accurate data on fish consumption in patients before the occurrence of a CAD event. Therefore, only the results of the cohort studies are summarized here. The meta-analyses by Whelton *et al.*[44] and He *et al.*[45] were based on 14 and 13 cohort studies, respectively. Both had approximately 220 000 participants who were followed for ~12 years.

Whelton *et al.*[44] found a 17% lower incidence of fatal CAD (RR = 0.83, 95% CI 0.75–0.92) among those who consumed fish less than twice a week compared with those who ate little or no fish. A similar result was found by He *et al.*[45] for fish consumed once a week (RR = 0.85, 95% CI 0.76–0.96). He *et al.* observed a dose–response relationship between fish consumption and CAD death and individuals who consumed fish five or more times per week had a 38% lower risk of fatal CAD (RR = 0.62, 95% CI 0.46–0.82). These associations were confirmed in

cohort studies in which, besides fish consumption, information about the intake of the omega-3 fatty acids EPA and DHA was also obtained.[46–49]

There is less evidence for a relationship between fish consumption and non-fatal MI. Based on the results of their meta-analysis, He *et al.*[45] concluded that the evidence for an inverse association between fish consumption and non-fatal MI was weak, even though there was a significant association for those eating fish five times per week or more. This conclusion was confirmed by De Goede *et al.*,[49] who found that consuming fish less than once per month up to once per week was not associated with non-fatal MI in a population-based study in the Netherlands. However, a Japanese cohort study showed that a high level of fish consumption may be protective against non-fatal CAD. In the Japan Public Health Center-based Study, the relative risk (RR) of non-fatal MI was 0.43 (95% CI 0.23–0.81) in participants with a median fish consumption of 180 g per day compared with participants with a daily consumption of 23 g per day.[48] These results support the outcome of the meta-analysis of He *et al.*[45] that only a high level of fish consumption may reduce the risk of non-fatal MI.

Fish, omega-3 fatty acids, and sudden death in observational studies

The hypothesis that fish consumption may be protective against sudden cardiac death is derived from the DART trial. This secondary prevention trial showed a significant 33% reduction in CAD mortality in cardiac patients who consumed at least two portions of fatty fish per week and were followed for 2 years. The authors suggested that the protective effect of fatty fish may be due to preventing ventricular fibrillation during acute ischaemia. This hypothesis was tested in two population-based case–control studies.[50,51]

Siscovick *et al.*[50] identified 334 patients with primary cardiac arrest and 493 population-based controls. An average intake of 185 mg per day of EPA–DHA corresponding to eating fatty fish once a week was associated with a 50% lower risk of primary cardiac arrest (OR = 0.5, 95% CI 0.4–0.8). An even stronger association was observed for the corresponding quartile of red blood cell membrane omega-3 fatty acids (OR = 0.3, 95% CI 0.2–0.6). Similarly, a strong inverse relation was found between baseline blood levels of long-chain omega-3 fatty acids and sudden death in the Physicians' Health Study.[51] The RR value was 90% lower in those in the highest compared with the lowest quartile of omega-3 fatty acids (RR = 0.10, 95% CI 0.02–0.48).

The evidence from prospective cohort studies on fish, omega-3 fatty acids, and sudden cardiac death is less convincing than that from population-based case–control studies.[52–54] Albert *et al.*[53] showed, using again data from the Physicians' Health Study, that men who consumed one fish meal per week had a 52% lower risk of sudden cardiac death (RR = 0.48, 95% CI 0.24–0.96) compared with those who consumed fish less than once a month. A significant inverse dose–response relationship with sudden cardiac death was not observed for the intake of omega-3 fatty acids, although the data suggested that an intake of ~200 mg omega-3 fatty acids per day compared with ~10 mg per day was associated with a lower risk of sudden cardiac death.

In contrast to these findings, sudden cardiac death was not significantly inversely associated with fish consumption in the Western Electric Study.[52] In this study, information on causes of death was obtained only from death certificates. Sudden cardiac death was defined as death occurring no more than 12 h after the onset of the terminal acute illness. In the Physicians' Health Study, detailed information was available from next of kin, medical records, and autopsy reports; and sudden death was defined as death within 1 h of the onset of symptoms. This definition of sudden cardiac death is superior to the one used in the Western Electric Study.

The association between long-term fish consumption, omega-3 fatty acids, and sudden cardiac death was also investigated in the Zutphen Study.[54] Long-term fatty fish consumption was inversely associated with sudden coronary death, and men who consumed fatty fish had a 54% lower risk (RR = 0.46, 95% CI 0.27–0.78) than those who did not eat fatty fish. Lean fish consumption was not associated with sudden coronary death. The intake of omega-3 fatty acids was also inversely related to sudden coronary death but this association was not statistically significant.

In summary, the results of the population-based case–control and prospective cohort studies suggest a protective effect of fish consumption on cardiac arrest and sudden death. The two case–control studies showed the strongest effect for the omega-3 fatty acids measured in blood.

Fish oils and cardiovascular diseases in randomized trials

Several trials tested the hypothesis that omega-3 fatty acids reduce fatal CAD and sudden death. The first meta-analysis of these trials was published in 2002,[55] followed by others.[56–59] However, several meta-analyses included not only trials in which the effect of omega-3 fatty acids in fish oils was investigated but also trials in which a fish advice or margarines enriched with ALA were given.[55,56,58] One meta-analysis on fish oils included besides patients with MI, CAD, and heart failure also patients with peripheral vascular diseases, hypercholesterolaemia, and implanted cardioverter defibrillators (ICDs).[58] Only the meta-analysis by León *et al.*[57] evaluated the effect of EPA–DHA in a homogeneous group of patients with CAD or had had an MI. They used fatal CAD, sudden cardiac death, and severe arrhythmias as endpoints.

In three trials, patients with an ICD were included. In these trials, fish oil capsules containing an additional amount of 0.9–2.8 g omega-3 fatty acids per day reduced the risk of severe arrhythmias by 10% (OR = 0.90, 95% CI 0.55–1.46).[57] A similar result was found in a meta-analysis by Brouwer *et al.*[60] based on the same studies. Eight trials using fish oil capsules containing 0.9–2.8 g of EPA–DHA showed a significant 20% reduction of cardiac death (OR = 0.80, 95% CI 0.69–0.93).[57] In four trials, an additional amount of 0.9–2.4 g of EPA–DHA per day reduced the incidence of sudden cardiac death by 26% (OR = 0.74, 95% CI 0.59–0.92).[57] The results for fatal CAD and sudden death were dominated by

Table 1 Effects of fish oil on cardiovascular diseases in trials with patients with heart disease

	GISSI-P 1999 (41)	JELIS 2007 (64)	GISSI-HF 2008 (65)	Alpha Omega 2010 (61)	OMEGA 2010 (62)	SU.FOL.OM3 2010 (63)
Number	11 324	3664	7046	4837	3851	2501
Patients	Post-MI	CAD	HF	Post-MI	Post-MI	CAD
Post-event	<3 months			<10 years	3–14 days	<12 months
Design	Open label	Open label	Double-blind	Double-blind	Double-blind	Double-blind
Inclusion period	1993–95	1996–99	2002–05	2002–06	2003–07	2003–07
Follow-up (months)	42	55	47	41	12	56
Person-years	38 505			15 531		10 656
Dose EPA (mg)	289	1800	394	226	460	400
Dose DHA (mg)	577	0	472	150	380	200
Medication use (%)						
Antiplatelets	88		87	98	95	94
Antihypertensives				90		
Beta-blockers	41		65	69	94	68
ACE-I/ARBs	41		94	56	91	66
Statins	29	97	23	85	94	87
Number of events						
MCE	1115	355	4359	671	331	
Fatal CVD	639		1447	162		157[a]
Fatal CAD	479	39	236[a]	138		
Sudden death	286	26	632	57	57	
Relative risk						
MCE	0.80*	0.81*	0.92*	1.01	1.21	
Fatal CVD	0.70*		0.90*	0.98		1.08[a]
Fatal CAD	0.65*	0.87[a]	0.82[a]	0.95		
Sudden death	0.55*	1.02	0.93	0.90	0.95	

MI, myocardial infarction; CAD, coronary artery diseases; HF, heart failure; MCE, major cardiovascular event; CVD, cardiovascular diseases.
[a]Fatal and non-fatal events.
*$P < 0.05$.

those of the GISSI-Prevenzione trial[41] that contributed >85% to both endpoints.[57]

In 2010, the results of the Alpha Omega, OMEGA, and SU.FOL.OM3 trials were published.[61–63] The results of these trials and those of the large trials published before 2010—the GISSI-Prevenzione trial, the secondary prevention component of the JELIS trial, and the GISSI Heart Failure trial—will be discussed in detail[64,65] (*Table 1*). The GISSI-HF published in 2008[65] and the three trials published in 2010[61–63] were not included in the meta-analysis of León *et al.*[57]

The number of patients included in these trials ranged from 2501 to 11 324 with 15–26% females. Three trials included post-MI patients,[41,61,62] two trials CAD patients,[63,64] and one trial heart failure patients.[65] The average age of the patients varied between 59 and 69 years. Two trials recruited patients in the 1990s and used an open-label design.[41,64] The remaining trials were initiated between 2002 and 2007 and were double-blind.[61–63,65] The OMEGA trial had a 12-month follow-up and in the other trials the average follow-up period varied between 41 and 56 months. In four trials, the patients received fish oil capsules containing 600–900 mg of EPA–DHA per day and in the JELIS trial 1800 mg of EPA per day. In the Alpha Omega Trial, margarine spreads provided an average additional intake of EPA–DHA of ~400 mg per day.[61]

The most important commonly used endpoints in these trials were major cardiovascular events, fatal CVD, fatal CAD, and sudden death. The strongest effects were observed in the GISSI-P trial for patients surviving a recent MI. In this trial, an additional amount of EPA–DHA of 900 mg per day reduced significantly fatal CVD by 30%, fatal CAD by 35%, and sudden death by 45%.[41] In the GISSI-HF trial, in which heart failure patients were included, fatal CVD was significantly reduced by 10%, sudden death non-significantly by 7%, and first hospital admissions for ventricular arrhythmias significantly by 28%.[65] The JELIS trial showed that an additional intake of 1800 mg of EPA per day reduced only major coronary events (fatal and non-fatal CAD, unstable angina, percutaneous coronary intervention, and coronary artery bypass grafting).[64] The three trials published in 2010 included either post-MI or CAD patients.[61–63] Additional amounts of EPA–DHA varying from 400–800 mg/day did not reduce cardiovascular events.

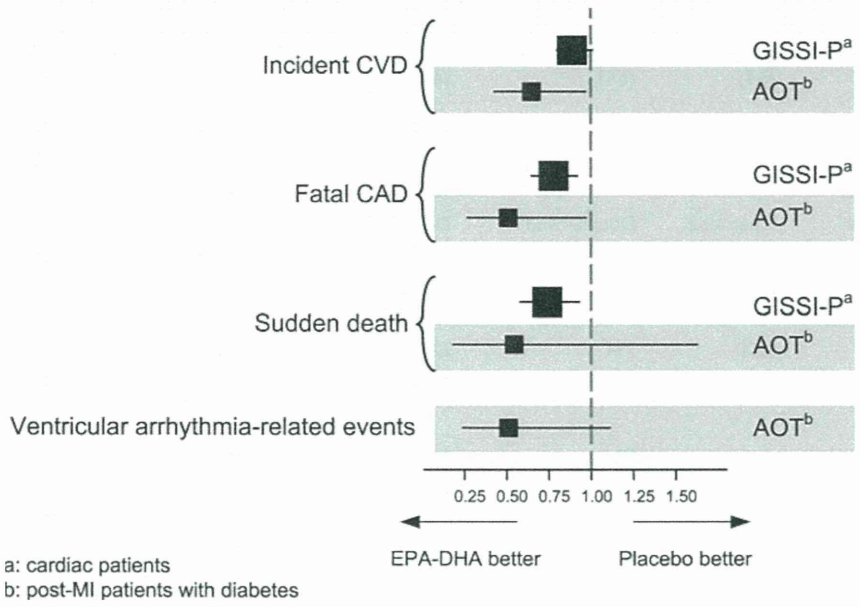

Figure 4 Effect of eicosapentaenoic acid–docosahexaenoic acid on cardiovascular diseases in the Alpha Omega Trial (AOT) and the GISSI-Prevenzione trial (GISSI-P). CVD, cardiovascular diseases; CAD, coronary artery diseases.

The strongest reductions in cardiovascular endpoints were obtained in the oldest trials. An explanation could be differences in study design. The GISSI-P and the JELIS trial used an open label design.[41,64] This may have confounded the results of these trials, because placebo capsules were lacking. Another explanation could be that the patients in the more recent trials were very well treated not only by antithrombotics but also by antihypertensives and statins. Compared with the recent trials, the treatment level with statins was low in the GISSI-P trial (29%).[41] This could be the reason for the high risk of fatal CAD and sudden death in the GISSI-P trial compared with the Alpha Omega Trial. The absolute risk for fatal CAD in the control group was 15.8/1000 person-years in the GISSI-P and 8.9/1000 person-years in the Alpha Omega Trial. For sudden death, the rates were 10.4/1000 person-years in the GISSI-P and 3.7/1000 person-years in the Alpha Omega Trial. A likely explanation is that these differences in absolute risk between the trials were responsible for the absence of an effect of EPA–DHA on fatal CAD and sudden death in the recent trials.

Those differences in absolute risk of fatal CAD and sudden death could play an important role in explaining the different results in the GISSI-P and the Alpha Omega Trial. This is supported by the results of the subgroup analysis of patients in the Alpha Omega Trial who also had diabetes.[61] The absolute risk of fatal CAD in the control group of diabetes patients in the Alpha Omega Trial was 17.1/1000 person-years. This is in the same order of magnitude as the absolute risk in the control group of the GISSI-P trial.[41] In the diabetes patients who received an additional amount of 400 mg of EPA–DHA per day, a significant reduction in fatal CAD was obtained comparable with the GISSI-P trial (*Figure 4*). Similar results were found in the Alpha Omega Trial for sudden death and ventricular arrhythmia-related events, although these effects were not statistically significant.

Emerging issues on the effects of fish oils/omega-3 fatty acids

Results of observational prospective cohort studies and randomized trials in subjects with or without CVD published before 2000 demonstrated that diets with higher amounts of omega-3 fatty acids or supplements with omega-3 fatty acids reduced cardiovascular mortality. These results formed the basis for recommendations, including the American Heart Association Guidelines, that patients with documented CAD should be advised to take 900–1000 mg of omega-3 fatty acids (EPA–DHA combined) per day.[66] However, this recommendation was challenged in a review and meta-analysis published by Hooper *et al.*[56] in 2006. They concluded that there was no clear benefit of additional amount of omega-3 fatty acids on cardiovascular events. In addition, the three recently published double-blind trials—the Alpha Omega, the OMEGA, and the SU.FOL.OM3—did not show an effect of an additional amount of EPA–DHA on major cardiovascular endpoints.[61–63] These negative results with omega-3 fatty acids supplementation were disappointing but were obtained in the current practice where other optimal conventional drug therapy was performed. It should be pointed out, however, that the OMEGA and the SU.FOL.OM3 trial were also underpowered.[62,63]

In addition, there is some evidence for possible pro-arrhythmic effects of omega-3 fatty acids in certain subgroups with CVD. In the three randomized controlled trials of patients with an

implantable cardioverter defibrillator (ICD) and a history of ventricular tachyarrhythmias, fish oil of omega-3 fatty acids did not show a significant benefit on the risk of appropriate ICD shocks.[57,60] In a trial of patients with stable angina pectoris without previous MI, a detrimental effect of omega-3 fatty acids on sudden death was observed.[67] Thus, further studies are needed to determine which patient population may or may not benefit from omega-3 fatty acids supplementation. Evidence is also insufficient regarding the optimal dose, source (oily fish or fish-oil supplements), and formulation of EPA and/or DHA in order to reduce cardiovascular events.[68]

Recently, a potential new indication of omega-3 fatty acids has been demonstrated, that is heart failure.[36] In the GISSI-HF trial,[65] a placebo-controlled trial of approximately 7000 patients with class II to IV heart failure, the patients were randomized to 1 g of omega-3 fatty acids (containing 850–882 mg of EPA plus DHA), rosuvastatin (10 mg), both of them, or dual placebo. This study was performed in addition to well-established current therapies, and the results showed a significant benefit of omega-3 fatty acids.[65] However, the optimal dose of omega-3 fatty acids remains to be determined depending on different stages and/or aetiology of heart failure and underlying mechanisms.[68] Growing evidence demonstrates anti-inflammatory effects of omega-3 fatty acids, including reduced circulating levels of inflammatory cytokines and AA-derived eicosanoids, and elevated plasma adiponectin.[18] In animal studies, fish oil favourably alters cardiac mitochondrial function.[18] All of these effects may work together to prevent the development and progression of heart failure.

Several issues remain to be elucidated. First, no evidence has been found for the optimal dosage, ratios of DHA to EPA, and ratios of omega-3 to omega-6. Second, whether dietary intake or therapeutic supplements are the best source of omega-3 fatty acids is yet to be determined. These issues remain to be clarified in future studies.

Conclusions

Omega-3 fatty acids exert pleiotropic, cardiometabolic effects with a diverse range of actions, most of which are beneficial for the cardiovascular system. Supplementation up to 1 g of omega-3 fatty acids per day is well tolerated except dysgeusia and does not increase the risk of bleeding. Recently published trials in patients with CAD or after MI did not show an effect of omega-3 fatty acids on major cardiovascular endpoints, probably due to state of the art drug treatment. However, as suggested by the current guidelines, the potential value of omega-3 fatty acids supplementation in patients with CAD or after MI and possibly in those with heart failure remains to be encouraged.

Funding

The work by the authors in this review article was supported in part by the Netherlands Heart Foundation, the Netherlands Prevention Foundation, the National Institutes of Health, USA, and an unrestricted grant of Unilever R&D (to D.K.), and the grants from the Japanese Ministry of Education, Sports, and Culture, Tokyo, Japan, and those from the Japanese Ministry of Health, Labour and Welfare, Tokyo, Japan (to H.S.).

Conflict of interest: none declared.

References

1. Burr ML. Lessons from the story of n-3 fatty acids. *Am J Clin Nutr* 2000;**71**(Suppl. 1):397S–398S.
2. Sinclair HM. The diet of Canadian Eskimos. *Proc Nutr Soc* 1953;**12**:69–82.
3. Kromann N, Green A. Epidemiological studies in the Upernavik district, Greenland. Incidence of some chronic diseases 1950–1974. *Acta Med Scand* 1980; **208**:401–406.
4. Bang HO, Dyerberg J. Plasma lipids and lipoproteins in Greenlandic west coast Eskimos. *Acta Med Scand* 1972;**192**:85–94.
5. Bang HO, Dyerberg J, Sinclair HM. The composition of the Eskimo food in north western Greenland. *Am J Clin Nutr* 1980;**33**:2657–2661.
6. Sinclair HM. Deficiency of essential fatty acids and atherosclerosis, etcetera. *Lancet* 1956;**270**:381–383.
7. Sinclair HM. Advantages and disadvantages of an Eskimo diet. In: Fumagalli R, Kritchevsky D, Peoletti R, eds. *Drugs Affecting Lipid Metabolism*. Amsterdam: Elsevier/North-Holland Biomedical Press; 1980. p363–370.
8. Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N Engl J Med* 1985;312:1205–1209.
9. Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC, Deadman NM. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: Diet and Reinfarction Trial (DART). *Lancet* 1989;**2**:757–761.
10. Shimokawa H. Beneficial effects of eicosapentaenoic acid on endothelial vasodilator functions in animals and humans. In: Hamazaki T, Okuyama H, eds. *Fatty Acids and Lipids - New Findings, World Review of Nutrition and Dietics*. Vol. 88. Basel, Switzerland: Karger; 2001. p100–108.
11. Calder PC. N-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. *Lipids* 2003;**38**:343–352.
12. Adkins Y, Kelley DS. Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. *J Nutr Biochem* 2010;**21**:781–792.
13. de Winther MP, Kanters E, Kraal G, Hofker MH. NF-κB signaling in atherogenesis. *Arterioscler Thromb Vasc Biol* 2005;**25**:904–914.
14. Shimokawa H, Takeshita A. Rho-kinase is an important therapeutic target in cardiovascular medicine. *Arterioscler Thromb Vasc Biol* 2005;**25**:1767–1775.
15. Takemoto M, Sun J, Hiroki J, Shimokawa H, Liao JK. Rho-kinase mediates hypoxia-induced downregulation of endothelial nitric oxide synthase. *Circulation* 2002;**106**:57–62.
16. Yada T, Shimokawa H, Hiramatsu O, Kajita T, Shigeto F, Tanaka E, Shinozaki Y, Mori H, Kiyooka T, Katsura M, Ohkuma S, Goto M, Ogasawara Y, Kajiya F. Beneficial effect of hydroxyfasudil, a specific Rho-kinase inhibitor, on ischemia/reperfusion injury in canine coronary microcirculation in vivo. *J Am Coll Cardiol* 2005;**45**:599–607.
17. Gao JY, Yasuda S, Tsuburaya R, Ito Y, Shiroto T, Hao K, Aizawa K, Kikuchi Y, Ito K, Shimokawa H. Long-term treatment with eicosapentaenoic acid ameliorates myocardial ischemia-reperfusion injury in pigs in vivo. Involvement of Rho-kinase pathway inhibition. *Circ J* 2011;**75**:1843–1851.
18. Duda MK, O'Shea KM, Stanley WC. Omega-3 polyunsaturated fatty acid supplementation for the treatment of heart failure: mechanisms and clinical potential. *Cardiovasc Res* 2009;**84**:33–41.
19. Swann PG, Venton DL, Le Breton GC. Eicosapentaenoic acid and docosahexaenoic acid are antagonists at the thromboxane A2/prostaglandin H2 receptor in human platelets. *FEBS Lett* 1989;**243**:244–246.
20. Kaminski WE, Jendraschak E, Kiefl R, von Schacky C. Dietary omega-3 fatty acids lower levels of platelet-derived growth factor mRNA in human mononuclear cells. *Blood* 1993;**81**:1871–1879.
21. Sampath H, Ntambi JM. Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Annu Rev Nutr* 2005;**25**:317–340.
22. Oi K, Shimokawa H, Hiroki J, Uwatoku T, Abe K, Matsumoto Y, Nakajima Y, Nakajima K, Takeichi S, Takeshita A. Remnant lipoproteins from patients with sudden cardiac death enhance coronary vasospastic activity through upregulation of Rho-kinase. *Arterioscler Thromb Vasc Biol* 2004;**24**:918–922.
23. Oi K, Shimokawa H, Hirakawa Y, Tashiro H, Nakaike R, Kozai T, Ohzono K, Yamamoto K, Koyanagi S, Okamatsu S, Tajimi T, Kikuchi Y, Takeshita A. Postprandial increase in plasma concentrations of remnant-like particles: an independent risk factor for restenosis after percutaneous coronary intervention. *J Cardiovasc Pharmacol* 2004;**44**:66–73.
24. Nakamura N, Hamazaki T, Ohta M, Okuda K, Urakaze M, Sawazaki S, Yamazaki K, Satoh A, Temaru R, Ishikura Y, Takata M, Kishida M, Kobayashi M. Joint effects of HMG-CoA reductase inhibitors and eicosapentaenoic acids on serum lipid profile and plasma fatty acid concentrations in patients with hyperlipidemia. *Int J Clin Lab Res* 1999;**29**:22–25.

25. Shimokawa H, Lam JYT, Chesebro JH, Bowie EJW, Vanhoutte PM. Effects of dietary supplementation with cod-liver oil on endothelium-dependent responses in porcine coronary arteries. *Circulation* 1987;**76**:898–905.
26. Shimokawa H, Vanhoutte PM. Dietary ω-3 fatty acids and endothelium-dependent relaxations in porcine coronary arteries. *Am J Physiol* 1989;**256**:H968–H973.
27. Shimokawa H, Aarhus LL, Vanhoutte PM. Dietary ω-3 polyunsaturated fatty acids augment endothelium-dependent relaxation to bradykinin in coronary microvessels of the pig. *Br J Pharmacol* 1988;**95**:1197–1203.
28. Shimokawa H, Vanhoutte PM. Dietary cod-liver oil improves endothelium-dependent responses in hypercholesterolemic and atherosclerotic porcine coronary arteries. *Circulation* 1988;**78**:1421–1430.
29. Komori K, Shimokawa H, Vanhoutte PM. Endothelium-dependent relaxation to aggregating platelets in porcine femoral veins and its modulation by diets. *Circulation* 1989;**80**:401–409.
30. Tagawa T, Hirooka Y, Shimokawa H, Hironaga K, Sakai K, Oyama J, Takeshita A. Long-term treatment with eicosapentaenoic acid improves exercise-induced vasodilation in patients with coronary artery disease. *Hypertens Res* 2002;**25**: 823–829.
31. Li Q, Zhang Q, Wang M, Liu F, Zhao S, Ma J, Luo N, Li N, Li Y, Xu G, Li J. Docosahexaenoic acid affects endothelial nitric oxide synthase in caveolae. *Arch Biochem Biophys* 2007;**466**:250–259.
32. De Caterina R, Cybulsky MI, Clinton SK, Gimbrone MA Jr, Libby P. The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. *Arterioscler Thromb* 1994;**14**:1829–1836.
33. Yasuda S, Shimokawa H. Potential usefulness of fish oil in the primary prevention of acute coronary syndrome. *Eur Heart J* 2010;**31**:15–16.
34. Thies F, Garry JM, Yaqoob P, Rerkasem K, Williams J, Shearman CP, Gallagher PJ, Calder PC, Grimble RF. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet* 2003;**361**: 477–485.
35. Morishige K, Shimokawa H, Matsumoto Y, Eto Y, Uwatoku T, Abe K, Sueishi K, Takeshita A. Overexpression of matrix metalloproteinase-9 promotes intravascular thrombus formation in porcine coronary arteries in vivo. *Cardiovasc Res* 2003; **57**:572–585.
36. Matsumoto M, Sata M, Fukuda D, Tanaka K, Soma M, Hirata Y, Nagai R. Orally administered eicosapentaenoic acid reduces and stabilizes atherosclerotic lesions in ApoE-deficient mice. *Atherosclerosis* 2008;**197**:524–533.
37. Billman GE, Kang JX, Leaf A. Prevention of sudden cardiac death by dietary pure omega-3 polyunsaturated fatty acids in dogs. *Circulation* 1999;**99**:2452–2457.
38. Hallaq H, Smith TW, Leaf A. Modulation of dihydropyridine-sensitive calcium channels in heart cells by fish oil fatty acids. *Proc Natl Acad Sci USA* 1992;**89**: 1760–1764.
39. Tsuburaya R, Yasuda S, Ito Y, Shiroto T, Gao JY, Ito K, Shimokawa H. Eicosapentaenoic acid reduces ischemic ventricular fibrillation via altering monophasic action potential in pigs. *J Mol Cell Cardiol* 2011;**51**:329–336.
40. Christensen JH, Schmidt EB. Autonomic nervous system, heart rate variability and n-3 fatty acids. *J Cardiovasc Med (Hagerstown)* 2007;**8**(Suppl. 1):S19–S22.
41. GISSI Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* 1999;**354**:447–455.
42. Kromhout D. N-3 fatty acids and coronary heart disease: epidemiology from Eskimos to Western populations. *J Intern Med Suppl* 1989;**731**:47–51.
43. Marckmann P, Gronbaek M. Fish consumption and coronary heart disease mortality. A systematic review of prospective cohort studies. *Eur J Clin Nutr* 1999; **53**:585–590.
44. Whelton SP, He J, Whelton PK, Muntner P. Meta-analysis of observational studies on fish intake and coronary heart disease. *Am J Cardiol* 2004;**93**:1119–1123.
45. He K, Song Y, Daviglus ML, Liu K, Van Horn L, Dyer AR, Greenland P. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. *Circulation* 2004;**109**:2705–2711.
46. Ascherio A, Rimm EB, Stampfer MJ, Giovannucci EL, Willett WC. Dietary intake of marine n-3 fatty acids, fish intake, and the risk of coronary disease among men. *N Engl J Med* 1995;**332**:977–982.
47. Hu FB, Bronner L, Willett WC, Stampfer MJ, Rexrode KM, Albert CM, Hunter D, Manson JE. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA* 2002;**287**:1815–1821.
48. Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kita Y, Kokubo Y, Tsugane S. 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.
49. de Goede J, Geleijnse JM, Boer JM, Kromhout D, Verschuren WM. Marine (n-3) fatty acids, fish consumption, and the 10-year risk of fatal and nonfatal coronary heart disease in a large population of Dutch adults with low fish intake. *J Nutr* 2010;**140**:1023–1028.
50. Siscovick DS, Raghunathan TE, King I, Weinmann S, Wicklund KG, Albright J, Bovbjerg V, Arbogast P, Smith H, Kushi LH, Cobb LA, Copass MK, Psaty BM, Lemaitre R, Retzlaff B, Childs M, Knopp RH. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA* 1995;**274**:1363–1367.
51. Albert CM, Campos H, Stampfer MJ, Ridker PM, Manson JE, Willett WC, Ma J. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med* 2002;**346**:1113–1118.
52. Daviglus ML, Stamler J, Orencia AJ, Dyer AR, Liu K, Greenland P, Walsh MK, Morris D, Shekelle RB. Fish consumption and the 30-year risk of fatal myocardial infarction. *N Engl J Med* 1997;**336**:1046–1053.
53. Albert CM, Hennekens CH, O'Donnell CJ, Ajani UA, Carey VJ, Willett WC, Ruskin JN, Manson JE. Fish consumption and risk of sudden cardiac death. *JAMA* 1998;**279**:23–28.
54. Streppel MT, Ocké MC, Boshuizen HC, Kok FJ, Kromhout D. Long-term fish consumption and n-3 fatty acid intake in relation to (sudden) coronary heart disease death: the Zutphen study. *Eur Heart J* 2008;**29**:2024–2030.
55. Bucher HC, Hengstler P, Schindler C, Meier G. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *Am J Med* 2002;**112**:298–304.
56. Hooper L, Thompson RL, Harrison RA, Summerbell CD, Ness AR, Moore HJ, Worthington HV, Durrington PN, Higgins JP, Capps NE, Riemersma RA, Ebrahim SB, Davey Smith G. Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. *BMJ* 2006;**332**:752–760.
57. León H, Shibata MC, Sivakumaran S, Dorgan M, Chatterley T, Tsuyuki RT. Effect of fish oil on arrhythmias and mortality: systematic review. *BMJ* 2008;**337**:a2931.
58. Marik PE, Varon J. Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. *Clin Cardiol* 2009;**32**:365–372.
59. Zhao YT, Chen Q, Sun YX, Li XB, Zhang P, Xu Y, Guo JH. Prevention of sudden cardiac death with omega-3 fatty acids in patients with coronary heart disease: a meta-analysis of randomized controlled trials. *Ann Med* 2009;**41**:301–310.
60. Brouwer IA, Raitt MH, Dullemeijer C, Kraemer DF, Zock PL, Morris C, Katan MB, Connor WE, Camm JA, Schouten EG, McAnulty J. Effect of fish oil on ventricular tachyarrhythmia in three studies in patients with implantable cardioverter defibrillators. *Eur Heart J* 2009;**30**:820–826.
61. Kromhout D, Giltay EJ, Geleijnse JM. n-3 Fatty acids and cardiovascular events after myocardial infarction. *N Engl J Med* 2010;**363**:2015–2026.
62. Rauch B, Schiele R, Schneider S, Diller F, Victor N, Gohlke H, Gottwik M, Steinbeck G, Del Castillo U, Sack R, Worth H, Katus H, Spitzer W, Sabin G, Senges J. 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.
63. Galan P, Kesse-Guyot E, Czernichow S, Briancon S, Blacher J, Hercberg S. Effects of B vitamins and omega 3 fatty acids on cardiovascular diseases: a randomised placebo controlled trial. *BMJ* 2010;**341**:c6273.
64. 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. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* 2007;**369**:1090–1098.
65. Tavazzi L, Maggioni AP, Marchioli R, Barlera S, Franzosi MG, Latini R, Lucci D, Nicolosi GL, Porcu M, Tognoni G. 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.
66. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW Jr, Kris-Etherton P, Goldberg IJ, Kotchen TA, Lichtenstein AH, Mitch WE, Mullis R, Robinson K, Wylie-Rosett J, St Jeor S, Suttie J, Tribble DL, Bazzarre TL. AHA Dietary Guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation* 2000;**102**:2284–2299.
67. Burr ML, Ashfield-Watt PA, Dunstan FD, Fehily AM, Breay P, Ashton T, Zotos PC, Haboubi NA, Elwood PC. Lack of benefit of dietary advice to men with angina: results of a controlled trial. *Eur J Clin Nutr* 2003;**57**:193–200.
68. Lavie CJ, Milani RV, Mehra MR, Ventura HO. Omega-3 polyunsaturated fatty acids and cardiovascular diseases. *J Am Coll Cardiol* 2009;**54**:585–594.