Original Article

Hypertension and lifetime risk of stroke

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Background: The lifetime risk (LTR) articulates the probability of disease in the residual lifetime for an index age. These estimates can be useful for general audience-targeted knowledge translation activities against hypertension. There are only a few reports on lifetime of impact of hypertension on stroke events in Asians in whom stroke incidence is higher than Westerners.

Methods: The Suita Study, a cohort study of cardiovascular diseases in Japan, was established in 1989. We included all participants who were stroke free at baseline. Age (in years) was used as the time scale. Age-specific incidence rates were calculated with person-year method within 10-year bands. We estimated the sex and index-age specific LTR of first-ever stroke with taking the competing risk of death into account.

Results: We followed 5783 men and women during 1989–2007 for 74 933 person-years. During the follow-up period, 276 (149 men and 127 women) participants had incident stroke. The majority of them were cerebral infarction; 166 (102 men and 64 women). The LTR of stroke, accounted for competing risk of death, at 45 years of age for men without hypertension was 17.21% and it was 32.79% for hypertensive men. Among the hypertensive patients, participants with stage 2 or greater hypertension had higher LTR of stroke than the participants with stage 1 hypertension. This increased LTR of stroke for hypertensive patients were also observed among women and across all index ages for stroke.

Conclusion: In this urban community-based population, we observed that hypertension has significant effect on the residual LTR of stroke among both men and women of middle age, specifically for ischemic stroke.

Keywords: cohort, hypertension, lifetime risk, stroke

Abbreviations: BP, blood pressure; LTR, lifetime risk

INTRODUCTION

In spite of the declining trend in the stroke mortality since the 1960s [1–3], stroke remains the third most common cause of death in Japan [4]. With the aging of the population and in the backdrop of major dietary changes and worsening some cardiovascular risk factors [1,2], stroke is likely to be still important health burden in Japan. Thus, prevention activities require urgent attention.

One of the major modifiable risk factor for stroke is high blood pressure (BP) or hypertension. The impact of hypertension on the burden of stroke needs to be presented in an easily understandable way to the lay audience, including at-risk population, patient population, health policy makers, and health educators. For example, clinical and scientific bodies are using this index in their knowledge translation materials [5]. Traditionally, measures of disease burden or risk estimates have primarily focused on the concepts of prevalence, incidence, or relative risk. Estimation of the lifetime risk (LTR) of stroke, which provides an absolute risk assessment, can be an important tool for knowledge translation because it would be more easily comprehensible by lay audience who are not that numerically savvy to apprehend the conventional measures of disease burden. This index would be helpful for public health education in motivating beneficial changes in lifestyle or health-related behaviors as well as social security policy such as health planning. In this study, we estimated the impact of hypertension on the short, intermediate-term risk and LTR of stroke in an urban population in central Japan.

POPULATION AND METHOD

Study sample

The Suita study, a cohort study for cardiovascular diseases among urban residents, was established in 1989. The details of this study have been described elsewhere [6–9]. Briefly, the cohort was formed from randomly sampled Suita city residents aged 30–79 years, stratified by sex and age class (10-year increments). From this sample, 6483 participated in a baseline survey at the National Cardiovascular Center between September 1989 and March 1994. After excluding

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participants with a previous history of stroke ($n = 98$) and those lost to follow-up ($n = 602$), data from the remaining 5783 participants (2722 men and 3061 women) were included in the analysis (Fig. 1). This cohort study was approved by the Institutional Review Board of the National Cardiovascular Center, Suita, Osaka, Japan.

Measurement of blood pressure and categories

Measurement of BP has been described elsewhere [10]. In brief, well trained physicians measured the BP of each individual three times in a seated position using a mercury column sphygmomanometer, an appropriately sized cuff, and a standard protocol. Before the initial BP reading was obtained, participants were seated at rest for at least 5 min. SBP and DBP were recorded as the average of the second and third measurements, which were taken more than 1 min apart. Hypertension was defined as SBP of at least 140 mmHg and/or DBP of at least 90 mmHg and/or on antihypertensive medication. Participants with SBP below 140 mmHg and DBP below 90 mmHg were defined as normotensive. We further categorized the hypertensive patients without regard to the use of antihypertensive medication according to the classification by the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [11] as follows: stage 1 hypertension, SBP 140–159 mmHg and/or DBP 90–99 mmHg; and stage 2 hypertension, SBP of at least 160 mmHg and/or DBP of at least 100 mmHg. We decided not to consider treatment of hypertension in this categorization in our analyses because we wanted to evaluate the effect of increased BP levels, which can also arise in hypertensive patients under treatment.

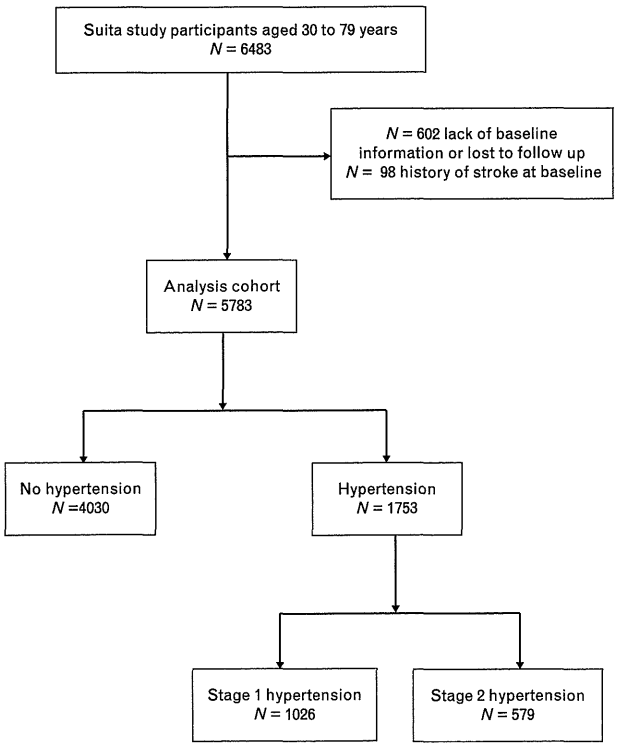

FIGURE 1 Cohort creation.

When SBP and DBP fell into different categories, the higher category was selected for the purposes of classification.

Endpoint ascertainment

The endpoints of the present study were the first stroke; death; or December 31, 2007. The first step in the survey involved checking the health status of all participants by repeated clinical visits every 2 years and yearly questionnaires by mail or telephone. In the second step, registered hospital physicians or research physicians reviewed in-hospital medical records of participants who were suspected of having a stroke. The reviewers were blinded to the baseline information. The criteria for stroke were defined according to the US National Survey of Stroke criteria [12]. For each stroke subtype (i.e. cerebral infarction, cerebral hemorrhage, and subarachnoid hemorrhagic), a definite diagnosis was established based on the computed tomography, MRI, or autopsy.

Statistical analysis

Age (in years) was used as the time scale [13,14]. Follow-up began at baseline or study entry age. Participants who were below 40 years of age at the beginning of the study period entered the sample on attainment of 40 years of age. The age categories began at the age of 40 years and the highest age category was set at age 90 years and over. The likelihood of failing from a particular cause at a given time is simply the product of the overall survival to that time. The follow-up ended either at stroke occurrence, on death, or on 31 December 2007, whichever came first.

We estimated cumulative stroke incidence conditional on survival to ages of 45, 55, 65, and 75 years. The estimation of cumulative incidence (the outcome of interest) is affected by the competing risk of death (death because of other causes). Participants who die of other causes of death during the observation period are treated as censored in traditional survival analytic technique, and their potential contribution to the outcome of interest is distributed among patients still at risk. Cause-specific survival is traditionally a net survival measure representing survival of a specified cause of event in the absence of other causes of death. However, the potential contribution of a participant who has died should not be zero, because to be at risk of event occurrence at a particular time, one must first survive from all causes until that time. Treating such participants as censored inflates the estimates of cumulative incidence. Therefore, to examine the actual risk during one's lifetime, we estimated cumulative stroke incidence conditional on survival to ages of 45, 55, 65, and 75 by executing double decrement taking into consideration both occurrence of outcome of interest and all-cause death [13–17].

Sex-specific 10, 20, 30, and 40-year risks and the LTR were estimated for stroke-free participants at different index ages for all stroke, cerebral infarction, and cerebral hemorrhage. The estimates were calculated using a modified technique of survival analysis from previously reported analyses methods [13,18]. All statistical analyses were done using SAS version 9.4 (SAS Institute, Cary, North Carolina, USA).

RESULTS

We had 74932.7 person-years of observation. During the follow-up period, 276 (149 men and 127 women) participants had incident stroke. The majority of them were cerebral infarction; 166 (102 men and 64 women). There were only 52 cerebral hemorrhage (27 men and 25 women) and 58 subarachnoid hemorrhage (25 men and 33 women). Owing to the very small number of cerebral hemorrhage and subarachnoid hemorrhage events, we could not estimate the short, intermediate, and LTR for these stroke subtypes.

Table 1 shows the basic characteristics of the participants with different hypertension statuses at the baseline. The proportion of hypertensive participants in the baseline survey was 33.36% for men and 27.61% for women. In men, 60.02% of the participants had stage 1 hypertension and 33.26% of the participants had stage 2 hypertension. In women, the respective proportions were 56.92 and 32.78%. Hypertensive patients were generally older and had higher mean plasma glucose and higher total blood cholesterol levels. This difference was observed in both men and women.

Figure 2 (and Supplementary Table 1, http://links.lww.com/HJH/A530) presents the 10, 20, 30, and 40-year risks and LTR of stroke by presence of hypertension in men and women of various index ages. The LTR of stroke, accounted for competing risk of death, for 45-year-old men without hypertension was 17.21% and was 32.79% for hypertensive men of the same index age. There was a graded increase in stroke risk with increasing time span. For stroke, 10-year risk at the age of 45 for normotensive patients was 0.34% and this increased across 20, 30, and 40-year risk categories as 1.74, 5.34, and 11.62%, respectively. This phenomenon was observed in both sexes and all index ages. Figure 3 (and Supplementary Table 2, http://links.lww.com/HJH/A530) presents the short, intermediate, and LTR of stroke by level of hypertension among hypertensive men and women. The LTR of stroke, accounted for competing risk of death, at 45 years of age for men with stage 1 hypertension was 20.21%, whereas the LTR of stroke for stage 2 hypertensive men of 45 years age was 48.33%. The graded increase in stroke risk with increasing time span was observed for both sexes and all index ages.

Table 2 presents the short, intermediate, and LTR of cerebral infarction by presence of hypertension in men and women of various index ages. The LTR of cerebral

TABLE 1. Baseline characteristics of Suita study participants with different hypertension status

		Blood pressure categories			
		No hypertension	Hypertension	Hypertension	
Sex	Variables			Stage 1	Stage 2
Men	Age (years) (s.d)	53.1 (13.2)	61.0 (11.6)	60.3 (11.8)	61.8 (11.3)
	BMI (kg/m^2) (s.d)	22.5 (2.8)	23.4 (3.1)	23.3 (3.0)	23.6 (3.3)
	Height (cm) (s.d)	165.9 (6.2)	164.0 (6.0)	164.2 (6.2)	163.8 (5.5)
	Weight (kg) (s.d)	62.1 (8.9)	63.1 (10.0)	63.1 (10.1)	63.5 (10.2)
	Plasma glucose (mg/dl) (s.d)	99.7 (18.8)	103.7 (21.2)	103.8 (22.3)	103.8 (17.0)
	Total cholesterol (mg/dl) (s.d)	199.6 (33.5)	204.9 (35.2)	205.5 (36.4)	204.5 (34.2)
	Serum creatinine (mg/dl) (s.d)	0.9 (0.2)	0.9 (0.2)	0.9 (0.3)	0.9 (0.2)
	Smoking, *n* (%)				
	Never smoker	338 (18.6)	169 (18.6)	105 (19.3)	58 (19.2)
	Current smoker	969 (53.4)	386 (42.5)	235 (43.1)	121 (40.1)
	Ex-smoker	485 (26.7)	334 (36.8)	194 (35.6)	116 (38.4)
	Unknown	22 (1.2)	19 (2.1)	11 (2.0)	7 (2.3)
	Drinking, *n* (%)				
	Never drinker	401 (22.1)	170 (18.7)	110 (20.2)	49 (16.2)
	Current drinker	1328 (73.2)	683 (75.2)	406 (74.5)	236 (78.2)
	Ex-drinker	64 (3.5)	40 (4.4)	22 (4.0)	10 (3.3)
	Unknown	21 (1.2)	15 (1.7)	7 (1.3)	7 (2.3)
Women	Age (years) (s.d)	51.2 (12.6)	62.6 (9.6)	61.8 (9.6)	63.3 (9.7)
	BMI (kg/m^2) (s.d)	21.8 (3.0)	23.5 (3.5)	23.2 (3.3)	23.8 (3.9)
	Height (cm) (s.d)	153.4 (5.7)	150.5 (5.7)	150.7 (5.7)	150.5 (5.5)
	Weight (kg) (s.d)	51.2 (7.6)	53.3 (9.0)	52.8 (8.5)	53.9 (9.8)
	Plasma glucose (mg/dl) (s.d)	94.4 (14.7)	101.6 (20.6)	100.8 (19.7)	102.7 (22.0)
	Total cholesterol (mg/dl) (s.d)	208.3 (37.6)	225.1 (37.0)	226.6 (36.4)	224.6 (38.8)
	Serum creatinine (mg/dl) (s.d)	0.7 (0.2)	0.7 (0.3)	0.7 (0.4)	0.7 (0.3)
	Smoking, *n* (%)				
	Never smoker	1794 (81.0)	705 (83.4)	408 (84.8)	231 (83.4)
	Current smoker	296 (13.4)	68 (8.1)	32 (6.7)	25 (9.0)
	Ex-smoker	78 (3.5)	35 (4.1)	23 (4.8)	7 (2.5)
	Unknown	48 (2.2)	37 (4.4)	18 (3.7)	14 (5.1)
	Drinking, *n* (%)				
	Never drinker	1392 (62.8)	571 (67.6)	316 (65.7)	194 (70.0)
	Current drinker	740 (33.4)	232 (27.5)	139 (28.9)	71 (25.6)
	Ex-drinker	38 (1.7)	14 (1.7)	10 (2.1)	4 (1.4)
	Unknown	46 (2.1)	28 (3.3)	16 (3.3)	8 (2.9)

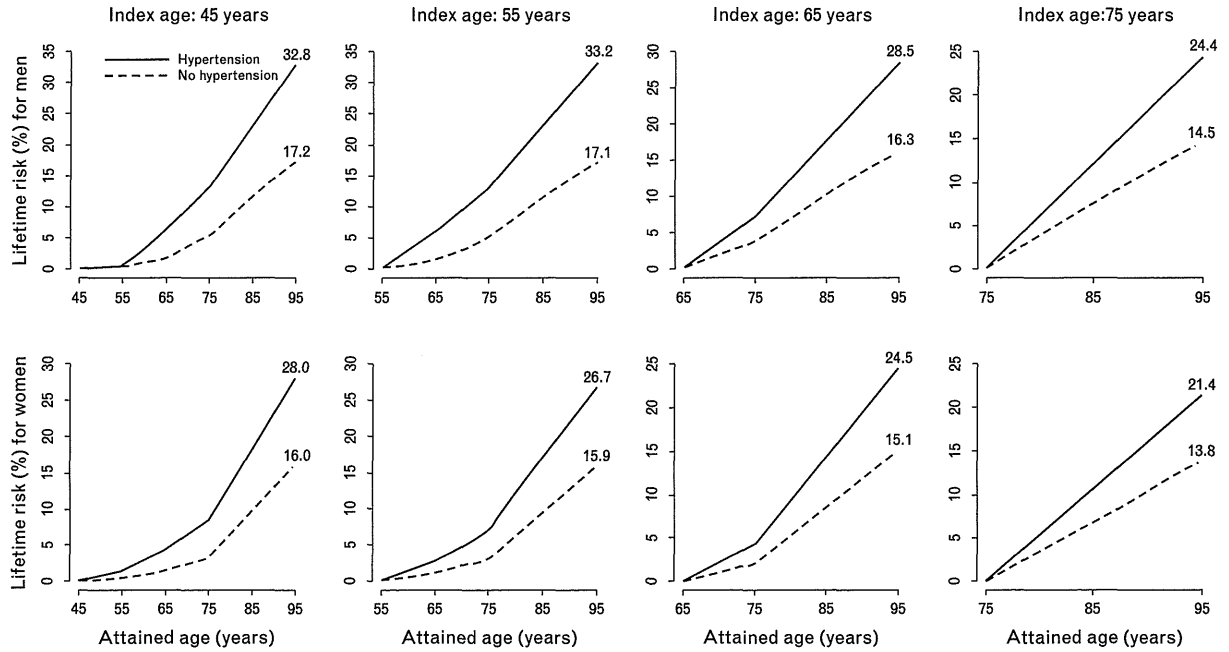

FIGURE 2 Lifetime risk estimates for stroke by presence of hypertension (adjusted for competing risk of death) across different index ages for men and women. The black line represents hypertension and the dotted line represents no hypertension.

infarction, accounted for competing risk of death, at 45 years of age for men without hypertension was 8.25%, whereas the LTR of cerebral infarction for hypertensive men aged 45 years was 19.01%. Table 3 presents the short, intermediate, and LTR of cerebral infarction by level of hypertension among hypertensive men and women. The LTR of cerebral infarction, accounting for competing risk of death, at 45 years of age for men with stage 1 hypertension was 14.75%, whereas the LTR of stroke for stage 2 hypertensive men of 45 years of age was 25.43%.

DISCUSSION

In this urban community-based population, we observed that hypertension has significant effect on the residual LTR of stroke among both men and women of middle age. Individuals with normal BP have significantly lower LTR for stroke in comparison with the individuals with hypertension. Among the hypertensive patients, though the differences were not that large, the individuals with stage 1 hypertension had a lower LTR of stroke in comparison with the individuals with

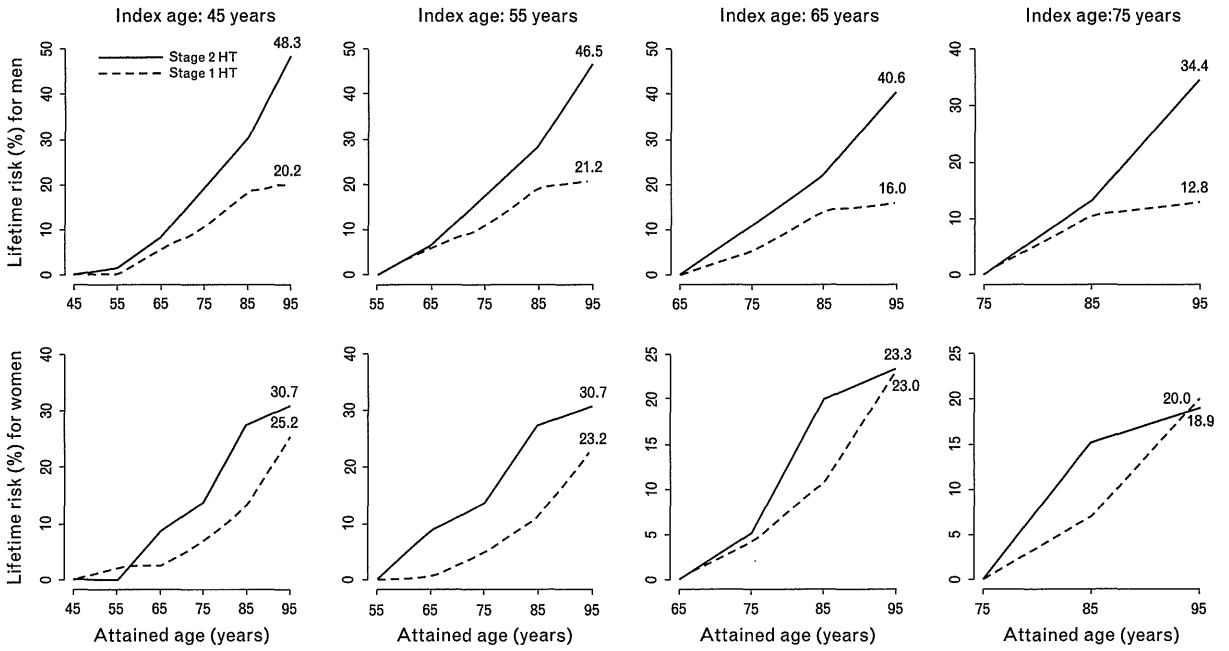

FIGURE 3 Lifetime risk estimates for stroke by level hypertension (adjusted for competing risk of death) across different index ages for men and women. The black line represents stage 2 hypertension and the dotted line represents stage 1 hypertension.

TABLE 2. Age and sex-specific 10, 20, 30, and 40-year and lifetime risk estimates for cerebral infarction by presence of hypertension (adjusted for competing risk of death)

Sex	Index age (years)	Short, intermediate term and lifetime risk (years)	No hypertension	Hypertension
Men	45	10	0.16 (0.00–0.48)	0.60 (0.00–1.77)
		20	1.1 (0.29–1.91)	5.29 (2.53–8.05)
		30	3.71 (2.22–5.2)	9.54 (6.28–12.81)
		40	7.24 (4.98–9.5)	17.95 (13.72–22.17)
		LTR	8.25 (5.28–11.22)	19.01 (14.35–23.68)
	55	10	0.95 (0.19–1.7)	4.84 (2.21–7.46)
		20	3.6 (2.12–5.07)	9.22 (6–12.44)
		30	7.17 (4.9–9.45)	17.89 (13.6–22.18)
		LTR	8.2 (5.2–11.2)	18.99 (14.25–23.74)
	65	10	2.75 (1.42–4.09)	4.57 (2.5–6.64)
		20	6.47 (4.22–8.72)	13.6 (9.86–17.34)
		LTR	7.54 (4.5–10.57)	14.75 (10.44–19.06)
	75	10	4.30 (2.16–6.43)	10.45 (6.7–14.2)
		LTR	5.52 (2.34–8.71)	11.78 (7.27–16.29)
Women	45	10	0.12 (0.00–0.34)	0.00 (0.00–0.00)
		20	0.48 (0.01–0.94)	0.83 (0.00–1.97)
		30	1.34 (0.51–2.18)	3.37 (1.51–5.22)
		40	4.33 (2.38–6.28)	9.94 (6.76–13.12)
		LTR	6.15 (3.01–9.29)	15.32 (7.59–23.06)
	55	10	0.37 (0.00–0.78)	0.83 (0.00–1.98)
		20	1.24 (0.43–2.06)	3.39 (1.52–5.25)
		30	4.28 (2.32–6.24)	10.00 (6.8–13.2)
		LTR	6.12 (2.95–9.3)	15.42 (7.64–23.19)
	65	10	0.90 (0.18–1.63)	2.62 (1.09–4.15)
		20	4.03 (2.05–6.01)	9.42 (6.32–12.52)
		LTR	5.93 (2.68–9.17)	14.98 (7.06–22.9)
	75	10	3.27 (1.33–5.21)	7.19 (4.27–10.11)
		LTR	5.26 (1.94–8.58)	13.08 (4.81–21.34)

LTR, lifetime risk.

stage 2 hypertension. These were observed across all the studied index ages as well as on both sexes.

Our estimates of LTR of stroke in the Suita study are also consistent with the reported LTR of stroke from the Netherlands [19], United States [20], United Kingdom [21], as well as another study from Japan [22]. The LTRs of stroke for middle-aged men and women were substantial. The observed probabilities from the Rotterdam [19], Framingham [20], Radiation Effects Research Foundation Adult Health [22], and Cardiovascular research using linked bespoke studies and electronic health records [21] studies illustrate that approximately one in five men and women of middle age will experience stroke in their remaining lifetime.

Although there are a number of reports regarding the LTR of cardiovascular diseases, including stroke [16,17, 23–26], only a few prior reports have presented the effect of hypertension on the LTR of stroke [20–22,27]. A recent publication from the UK, based on an analysis on electronic records of 1.25 million people during 1997 to 2010, reported that people aged 30 years or older with hypertension have a LTR for strokes compared with those with normal BP [21]. For ischemic stroke, the LTR was 7.6% among the hypertensive patients whereas the normotensive patients had a LTR of 6.5%. For intracerebral hemorrhage, the LTR was 1.3% among the hypertensive patients whereas the normotensive patients had a LTR of 0.9%. Another study from seven US cohorts pooled together estimated that increase or decrease in BP in middle age was associated with higher and lower remaining LTR of stroke [27]. Studying 61 585 men and women for 700 000 person-years, it was reported that LTR of stroke increased with increasing baseline BP categories for both men and women (normal BP, prehypertension, stage 1 hypertension, and stage 2 hypertension) [27]. Also, it was observed that individuals who maintained or decreased their BP to normal levels had the lowest remaining LTR for stroke, compared with individuals who had or developed hypertension by 55 years of age [27]. Authors from Japan, studying the Radiation Effects Research Foundation Adult Health Study cohort, showed that the LTR of stroke and its subtypes differed across the categories in BP among men and women of index age of 55 years [22]. The LTR of stroke for normotensive men and women was 13.8 and 16.0%, respectively, whereas the LTR of stroke for stage 2 hypertensive men and women were 25.8 and 30.5%, respectively [22]. Seshadri *et al.* [20], using Framingham study, reported that participants with a normal BP had a significantly lower LTR of stroke than participants with a high BP [20]. In men, these risks were 10 and 21%, respectively, whereas in women, these were 15 and 26% [20].

In our study, similar to other studies, the LTR for stroke was higher for the younger index ages (e.g. 45 years) in comparison to the older index ages (e.g. 65 years). This phenomenon was observed for both participants with and without hypertension. This finding is of very much importance because this points to the fact that the population-level stroke prevention activities need to be initiated early, especially among the patients with hypertension. This definitely provides supports for efforts to identify hypertension early and start treatment as early as possible.

TABLE 3. Age and sex-specific 10, 20, 30, and 40-year and lifetime risk estimates for cerebral infarction among the hypertensive patients by stages of hypertension (adjusted for competing risk of death)

Sex	Index age (years)	Short, intermediate term and lifetime risk (years)	Hypertension	
			Stage 1 hypertension	Stage 2 hypertension
Men	45	10	0.00 (0.00-0.00)	0.00 (0.00-0.00)
		20	4.65 (1.47–7.84)	7.42 (1.54–13.3)
		30	7.65 (3.95–11.36)	12.69 (5.97–19.4)
		40	14.75 (9.73–19.77)	21.88 (13.88–29.88)
		LTR	14.75 (9.73–19.77)	25.43 (15.26–35.61)
	55	10	4.88 (1.52–8.24)	5.60 (0.68–10.52)
		20	8.02 (4.11–11.93)	10.87 (4.79–16.95)
		30	15.47 (10.19–20.75)	20.06 (12.33–27.8)
		LTR	15.47 (10.19–20.75)	23.61 (13.57–33.66)
	65	10	3.31 (1.05–5.56)	5.38 (1.47–9.29)
		20	11.14 (6.62–15.67)	14.76 (8.24–21.29)
		LTR	11.14 (6.62–15.67)	18.39 (9.08–27.7)
	75	10	9.09 (4.45–13.74)	10.79 (4.43–17.16)
		LTR	9.09 (4.45–13.74)	14.96 (4.94–24.99)
Women	45	10	0.00 (0.00–0.00)	0.00 (0.00–0.00)
		20	0.00 (0.00–0.00)	2.71 (0.00–6.41)
		30	2.41 (0.5–4.31)	5.51 (1.00–10.01)
		40	4.92 (2.05–7.79)	16.94 (9.69–24.19)
		LTR	13.00 (0.00–26.32)	20.29 (11.98–28.6)
	55	10	0.00 (0.00–0.00)	2.71(0.00–6.41)
		20	2.43 (0.51–4.35)	5.51 (1.00–10.01)
		30	4.96 (2.07–7.86)	16.94 (9.69–24.19)
		LTR	13.12 (0.00–26.56)	20.29 (11.98–28.6)
	65	10	2.45 (0.51–4.39)	2.97 (0.10–5.84)
		20	5.01 (2.09–7.93)	15.11 (8.22–21.99)
		LTR	13.25 (0.00–26.82)	18.66 (10.49–26.83)
	75	10	2.72 (0.35–5.08)	12.73 (5.94–19.52)
		LTR	11.45 (0.00–25.73)	16.46 (8.21–24.72)

LTR, lifetime risk.

From a methodological standpoint, our study includes the use of a population-based cohort, the prospective ascertainment of endpoints using rigorous standardized and previously validated clinical diagnostic criteria, and the completeness of stroke event and mortality ascertainment. Thus, our estimates are based on simultaneously gathered data on both stroke incidence and other-cause mortality attributable to competing risk of death in the same cohort. Interpreting our observed estimates, few things need to be kept in mind. The Suita cohort is based on urban population, so the estimates might not represent Japanese population in general. But Japan has a high urbanization rate and in the urban environment changes in the lifestyle factors associated with the risk with stroke would be more prominent. Therefore, we believe that the LTR of stroke points toward risk in urban setting that itself also represents a large part of the population. On the contrary, time period and birth cohort effects could limit the external validity of our results. Temporal trends in life expectancy, risk factor prevalence and control among the study population, disease awareness, and the sensitivity of diagnostic tests could potentially alter the LTR of stroke. We also could not, because of the lack of enough number of outcomes, estimate the effect of hypertension on the LTRs of cerebral hemorrhage and subarachnoid hemorrhage.

Our findings have important clinical implications. Recently published clinical practice guidelines from the United States [28] and the United Kingdom [29] agree that the primary purpose of assessment of cardiovascular risk is to provide the basis of a risk discussion with the patient. These LTR estimates are specifically useful for public education because they are easier to comprehend than measures such as incidence, prevalence, or relative risk [30]. This will be a more commonsense approach to health education because problems with numeracy or low quantitative literacy are common [31]. A recent study using focus group discussions concluded that patients preferred health risks to be framed in absolute terms and lifetime estimate with a scale to 'x out of 100' [32]. In younger individuals with low short-term risks, the high LTR might be more useful to motivate lifestyle modifications with appropriate health education efforts aimed at prevention of stroke, thereby reducing the population burden of stroke.

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Conflicts of interest

There are no conflicts of interest.

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Reviewers' Summary Evaluations

Reviewer 1

Strengths: Stroke risk is usually calculated as relative risk. Estimation of the lifetime risk of stroke, as done in this study, provides an absolute risk assessment and seems to be more easily understandable by a lay audience. Therefore, it may improve adherence to preventive procedures. The current paper has therefore important clinical implications.

Weaknesses: The main limitation of the current study lies in its confirmatory role. Furthermore, as the study is based on a Japanese cohort, the number of ischemic stroke events is relatively small (276 patients with a stroke with only 166 with ischemic stroke), whereas the impact of ischemic stroke is largely the most relevant in western populations. This limits the potential clinical implications of the study.

Reviewer 2

The main strengths of this paper are its longitudinal follow-up for close to 20 years and its novel mode of quantifying the increase in the risk of stroke in people with hypertension. The main limitations are firstly, that the notion that hypertension increases the risk of stroke is not really new, and secondly that the number of subjects with stroke was small (276).

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Original Article

Association Between Serum Long-Chain n-3 and n-6 Polyunsaturated Fatty Acid Profiles and Glomerular Filtration Rate Assessed by Serum Creatinine and Cystatin C Levels in Japanese Community-Dwellers

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ABSTRACT

Background: Plasma concentration of n-3 polyunsaturated fatty acids (PUFAs) has been reported to be associated with renal function in Western populations. However, few studies have investigated the association between serum long-chain n-3 and n-6 PUFA profiles and renal function in a Japanese population with high marine-derived long-chain n-3 PUFA intake.

Methods: A cross-sectional study was performed in 549 Japanese rural community-dwellers aged 40 to 64 years. In adjusted analysis of covariance, we assessed the relationship between estimated glomerular filtration rate (eGFR) and tertiles of serum long-chain n-3 and n-6 PUFA profiles ([eicosapentaenoic acid {EPA} + docosahexaenoic acid {DHA}]:arachidonic acid [AA]). GFR was estimated by Japanese specific equations using serum creatinine and cystatin C ($eGFR_{cre}$ and $eGFR_{cys}$). Using multivariate-adjusted linear regression models, we also assessed the relationships between eGFRs and several n-3 and n-6 PUFAs, which have been suggested to be associated with renal function.

Results: In all participants, higher dietary fish intake as assessed by a semi-quantitative questionnaire was associated with higher serum value of (EPA+DHA):AA. Participants in the higher (EPA+DHA):AA tertiles had non-significantly higher $eGFR_{cre}$ and significantly higher $eGFR_{cys}$ ($P = 0.016$). In addition, $eGFR_{cys}$ in T_2+T_3 of (EPA+DHA):AA was significantly higher than that in T_1 (adjusted mean $eGFR_{cys}$, T_1: 87 ml/min/1.73 m^2, T_2+T_3: 91 ml/min/1.73 m^2; $P < 0.01$). Among the PUFAs, only (EPA+DHA) was significantly associated with $eGFR_{cys}$.

Conclusions: Serum (EPA+DHA):AA, which reflects an individual's fish intake, might be associated with $eGFR_{cys}$ in Japanese community-dwellers.

Key words: epidemiology; (EPA+DHA):AA; population-based study

INTRODUCTION

N-3 polyunsaturated fatty acids (PUFAs) have been suggested to be protective against the development of renal dysfunction. According to a previous community-based study in Italy, plasma concentration of n-3 PUFAs was inversely associated with age-associated decline in estimated glomerular filtration rate (eGFR).[1] The Japanese population is unique because it has particularly high fish intake; consequently, Japanese people tend to have high serum long-chain n-3 PUFA levels,[2] which may be associated with low risk of coronary artery disease.[3,4] However, the relationship between serum long-chain n-3 PUFA levels and renal function has not been investigated in Japanese community-dwellers.

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Arachidonic acid (AA), which is classified as an n-6 PUFA, has been considered to have inflammatory and thrombotic effects because many (though not all) eicosanoids derived from AA are considered to be inflammatory, whereas EPA- and DHA-derived eicosanoids are considered to be protective against inflammation induced by AA.[5] Accordingly, previous studies in Japanese patients have investigated the relationship between cardiac events and serum n-3 PUFA:AA ratios, which are markers for balance of n-3 PUFAs and AA.[5] These studies have shown that higher EPA:AA and (EPA+DHA):AA ratios were associated with lower risk of cardiac events.[5–7] However, few community-based epidemiological studies have investigated the relationship between kidney function and long-chain n-3 PUFA:AA ratios.

The Japanese Society of Nephrology has developed 2 equations to estimate GFR, using serum creatinine (Cre) and cystatin C (Cys C) levels.[8] Serum Cys C is currently being considered as a potential replacement for Cre as a filtration marker because it is not affected by dietary intake and muscle mass.[9,10]

To investigate the relationships between eGFR and serum long-chain n-3 and n-6 PUFA profiles in community-dwellers, we performed a cross-sectional study in 549 Japanese men and women aged 40–64 years. GFR was estimated by 2 equations for the Japanese population, using serum Cre and Cys C.

METHODS

Study participants

The data from the baseline survey of the Sasayama study were analyzed. The Sasayama study is a population-based cohort study in which the endpoints are increased medical expenditures, worsening of quality of life, or cerebral and cardiovascular disease (CVD) risk factors, such as hypertension, diabetes mellitus, and dyslipidemia.

The study participants consisted of Japanese national health insurance (NHI) beneficiaries living in Sasayama City in Western Japan's Hyogo Prefecture who had undergone a medical examination between May 2012 and February 2013. The NHI system is one of the insurance systems in Japan, which is for non-employees, such as self-employed individuals, farmers, fishermen, and their dependents. During this time period, a total of 1131 NHI beneficiaries aged 40–64 years underwent a medical examination, and 675 individuals agreed to participate in the study. Written informed consent was obtained from each participant. Of these 675 participants, 126 were excluded due to 1 or more of the following reasons: non-fasting visit ($n = 82$), missing data ($n = 37$), or triglyceride level ≥400 mg/dL ($n = 7$). The remaining 549 individuals (237 men and 312 women, mean [standard deviation {SD}] age: 57 [7] years) were included in the present study. The present study was approved by the Hyogo College of Medicine Ethics Committee.

Data collection and standardization

Height and weight while wearing socks and light clothing were measured, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Blood pressure was measured using an automatic sphygmomanometer after a 5-minute rest. Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg and/or use of medication for hypertension.

The participants were asked to respond to questionnaires about lifestyle-related factors, such as medication, smoking (current smoker or not), alcohol consumption (current drinker or not), and fish intake. The questionnaires included question about the frequency of fish intake per week, and the portion size of fish consumed in his or her typical meal using full-scale photos of 80 g of cooked fish. Then, each participant's total fish intake per week was calculated by summing the values that were calculated by multiplying the frequency and portion size.

Blood samples after an overnight fast were obtained from all participants. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides, and glucose levels were measured by enzymatic methods. Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's formula. Diabetes was defined as fasting blood glucose ≥126 mg/dL and/or HbA1c ≥ 6.5% (measured according to National Glycohemoglobin Standardization Program [NGSP] standards) and/or current use of insulin or oral medication for diabetes.

Fatty acid concentrations were measured using gas chromatography (GC-17A; Shimazu Corp, Kyoto, Japan) in the same commissioned clinical laboratory center (SRL Inc., Tokyo, Japan).[11] Serum total PUFA concentration was calculated as the sum of n-6 PUFA concentration (linoleic acid [LA, 18:2n-6], γ-linolenic acid [18:3n6], dihomo-γ-linolenic acid [20:3n6], and arachidonic acid [AA, 20:4n6]) and n-3 PUFA concentration ([α-linolenic acid [18:3n3], eicosapentaenoic acid [EPA, 20:5n3], docosapentaenoic acid [22:5n3], and docosahexaenoic acid [DHA, 22:6n3]).[2] Long-chain n-3 PUFAs were calculated as the sum of EPA, docosapentaenoic acid, and DHA.

Serum Cre was measured using the enzymatic method, and serum Cys C was measured using the colloidal gold technique.[12] GFR (mL/min/1.73 m^2) was estimated using the following 2 equations, which were developed by the Japanese Society of Nephrology: equation 1: $eGFR_{cre} = 194 \times Cre^{-1.094} \times age^{-0.287}$ (× 0.739 if female),[8] and equation 2: $eGFR_{cys} = 104 \times Cys\ C^{-1.019} \times 0.996^{age}$ (× 0.929 if female) − 8.[8,13]

Statistical analysis

Sex-specific and sex-combined analyses were performed. To show the characteristics of the study participants classified according to tertiles of the (EPA+DHA):AA ratio, mean (SD) or median were calculated for continuous variables, and the

percentage was calculated for dichotomous variables. The crude and age- and sex-adjusted geometric means of fish intake per week were compared among the tertiles of the (EPA+DHA):AA ratio with Bonferroni's correction for multiple post-hoc comparisons.

To investigate which variables among the long-chain n-3 and n-6 PUFA profiles show large standardized coefficients in relation to $eGFR_{cre}$ and $eGFR_{cys}$, linear regression models were used after adjusting for age, sex, BMI, hypertension, diabetes, HDL-C, LDL-C, medication for dyslipidemia, and current smoking and drinking. In these models, the long-chain n-3 and n-6 PUFA profiles included the serum concentrations of EPA, DHA, EPA+DHA, and long-chain n-3 PUFA, as well as EPA:AA, DHA:AA, (EPA+DHA):AA, and long-chain n-3 PUFA:AA ratios.

Among the tertiles of the (EPA+DHA):AA ratio, $eGFR_{cre}$ and $eGFR_{cys}$ were compared by analysis of covariance (ANCOVA) with Bonferroni's correction for multiple post-hoc comparisons after adjusting for the following confounders: Model 1 included age, sex, BMI, hypertension, diabetes, HDL-C, LDL-C, medication for dyslipidemia, and current smoking and drinking; Model 2 included variables in Model 1 plus log-transformed C-reactive protein (CRP) measured using a high-sensitivity CRP assay. Because the fish intake of Japanese population was generally higher than that providing the maximal preventive effect for CVD in the previous studies,[14] $eGFR_{cre}$ and $eGFR_{cys}$ were also compared among the participants in the lowest tertile (T_1) and those in the other tertiles (T_2+T_3) of the (EPA+DHA):AA ratio after adjusting for the same confounders mentioned above.

Because several n-3 and n-6 PUFAs have been suggested to be associated with renal function in previous studies,[1,15] multiple linear regression models were used to confirm the contribution of serum PUFA concentration to $eGFR_{cre}$ and $eGFR_{cys}$ after adjusting for age, sex, BMI, hypertension, diabetes, HDL-C, LDL-C, medication for dyslipidemia, and current smoking and drinking. Serum concentrations of EPA+DHA, AA, linoleic acid, and α-linolenic acid were included in Model 1, and serum concentrations of EPA+DHA and n-6 PUFA were included in Model 2.

All P values were two-tailed, and the significance level was set at $P < 0.05$. The statistical package SPSS 20.0J for Windows (SPSS, Tokyo, Japan) was used to perform the analyses.

RESULTS

In all participants, the mean (SD) concentration of serum PUFA was 1457.3 (239.0) μg/mL total PUFA, 286.7 (95.8) μg/mL n-3 PUFA, 260.6 (92.5) μg/mL long-chain n-3 PUFA, and 1170.6 (198.6) μg/mL n-6 PUFA. The mean $eGFR_{cre}$ was 73 (13) mL/min/1.73 m^2, and the mean $eGFR_{cys}$ was 89 (16) mL/min/1.73 m^2. Eighty-one individuals had chronic kidney disease (CKD) defined by $eGFR_{cre}$ <60 mL/min/1.73 m^2, and 12 individuals had CKD defined by $eGFR_{cys}$ <60 mL/min/1.73 m^2.

Table 1 shows the characteristics of the participants according to serum (EPA+DHA):AA tertile in all participants. Age, BMI, and prevalence of hypertension and diabetes were higher in the higher (EPA+DHA):AA tertile. The percentage of medication for dyslipidemia was lower in the higher (EPA+DHA):AA tertile. eTables 1 and 2 show the sex-specific characteristics of the participants. In men, prevalence of smoking was higher in the lowest tertile compared to other groups (43.6% in T_1, 25.0% in T_2, and 29.1% in T_3). Figure shows the relationships between serum (EPA+DHA):AA tertile and geometric mean of fish intake (g/week). The higher (EPA+DHA):AA tertile was significantly associated with higher fish intake (trend $P < 0.001$, $P < 0.001$ between T_1 and T_2, and $P < 0.05$ between T_2 and T_3). In sex-specific analysis, the results were similar.

eTable 3 shows the relationships between eGFRs and n-3 and n-6 PUFA profiles in multivariate-adjusted linear regression models. All long-chain n-3 PUFA concentrations and ratios of long-chain n-3 PUFA to AA showed significant relationships with $eGFR_{cys}$. These concentrations and ratios did not show significant relationship with $eGFR_{cre}$; however, n-3 PUFA:AA ratios showed higher coefficients for $eGFR_{cre}$ than n-3 PUFA concentrations.

Table 2 shows the association between serum (EPA+DHA):AA tertiles and $eGFR_{cre}$ in ANCOVA after adjusting for the confounders. In men, higher (EPA+DHA):AA tertiles were associated with higher $eGFR_{cre}$ without statistical significance. In women, adjusted means of $eGFR_{cre}$ were the same among (EPA+DHA):AA tertiles. In all participants, higher (EPA+DHA):AA tertiles were associated with higher $eGFR_{cre}$ without statistical significance. Table 3 shows the association between (EPA+DHA):AA tertiles and $eGFR_{cys}$ in ANCOVA after adjusting for the confounders. In men, higher (EPA+DHA):AA tertiles were significantly associated with higher $eGFR_{cys}$. In women, higher (EPA+DHA):AA tertiles were associated with higher $eGFR_{cys}$ without statistical significance. In all participants, higher (EPA+DHA):AA tertiles were significantly associated with higher $eGFR_{cys}$, and Bonferroni's correction for multiple post-hoc comparisons showed significant differences between T_1 and T_2 ($P < 0.05$) and between T_1 and T_3 ($P < 0.05$). In addition, as shown in Table 4, $eGFR_{cre}$ was higher in T_2+T_3 than in T_1 without statistical significance, and $eGFR_{cys}$ was significantly higher in T_2+T_3 than in T_1 in all participants.

In addition, eGFRs were also compared among tertiles of fish intake (T_1, T_2, and T_3) in all participants by ANCOVA after adjusting for the confounders described as Model 1. Mean $eGFR_{cre}$ was 72 mL/min/1.73 m^2 in T_1, 75 mL/min/1.73 m^2 in T_2, and 73 mL/min/1.73 m^2 in T_3 (P for ANCOVA = 0.204), and mean $eGFR_{cys}$ was 88 mL/min/1.73 m^2 in T_1, 90 mL/min/1.73 m^2 in T_2, and 90 mL/min/1.73 m^2 in T_3 (P for ANCOVA = 0.163) (data not shown).

Table 1. Characteristics of study participants according to serum (EPA+DHA):AA tertile in the Sasayama study, 2012–2013

	Tertile of (EPA+DHA):AA		
	T_1 (0.338–0.925)	T_2 (0.928–1.301)	T_3 (1.302–3.188)
Number of participants	184	181	184
Sex, % males	44.0	38.1	47.3
Age, years	53 (8)	58 (6)	59 (5)
BMI, kg/m^2	22.7 (2.9)	23.0 (3.5)	23.2 (3.4)
Systolic blood pressure, mm Hg	121 (16)	128 (19)	130 (19)
Diastolic blood pressure, mm Hg	73 (11)	76 (11)	78 (11)
Hypertension, %	20.7	39.2	39.7
Glucose, mg/dL	95	98	99
Diabetes, %	7.6	7.7	9.8
Total cholesterol, mg/dL	210 (34)	221 (35)	220 (38)
LDL cholesterol, mg/dL	127 (31)	134 (33)	133 (34)
HDL cholesterol, mg/dL	63 (14)	64 (15)	63 (17)
Medication for dyslipidemia, %	19.6	14.9	13.6
Current smoking, %	24.5	13.3	15.2
Current drinking, %	52.2	51.9	55.4
Past or present history of CVD, %	3.3	5.0	3.3
C-reactive protein, mg/L	0.3	0.4	0.4
Fish intake, g/week	114 (5)	256 (2)	344 (2)
Serum N-3 PUFA,[a] μg/mL	203.3 (48.3)	278.9 (57.9)	377.8 (80.3)
Serum Long chain n-3 PUFA,[b] μg/mL	180.7 (45.0)	252.0 (56.1)	348.9 (78.8)
Serum EPA, μg/mL	42.2 (15.7)	68.0 (24.9)	111.2 (42.0)
Serum DHA, μg/mL	120.6 (28.8)	162.1 (34.0)	210.5 (42.5)
Serum α-linolenic acid, μg/mL	22.6 (7.8)	26.9 (10.6)	29.0 (10.6)
Serum n-6 PUFA,[c] μg/mL	1190.3 (190.0)	1181.5 (203.5)	1140.1 (199.6)
Serum AA, μg/mL	224.9 (47.2)	207.7 (45.0)	197.2 (42.1)
Serum linoleic acid, μg/mL	907.1 (163.1)	918.2 (183.0)	894.5 (178.3)
Serum creatinine, mg/dL	0.76 (0.21)	0.74 (0.15)	0.76 (0.17)
$eGFR_{cre}$, mL/min/1.73 m^2	74 (13)	73 (12)	73 (14)
Serum cystatin C, mg/L	0.85 (0.18)	0.83 (0.12)	0.85 (0.13)
$eGFR_{cys}$, mL/min/1.73 m^2	91 (18)	89 (15)	88 (15)

AA, arachidonic acid; BMI, body mass index; CVD, cerebral and cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PUFA, polyunsaturated fatty acid.
Values are means (standard deviations), except glucose and high-sensitivity C-reactive protein levels, which are presented as medians.
Fish intake is presented as geometric mean (SD).
[a]Serum n-3 PUFA: sum of α-linolenic acid, EPA, DHA, and docosapentaenoic acid.
[b]Serum n-6 PUFA: sum of linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, and AA.
[c]Long-chain n-3 PUFA: sum of EPA, DHA, and docosapentaenoic acid.

Table 5 shows the standardized coefficients of EPA+DHA and other PUFA concentrations in relation to $eGFR_{cre}$ and $eGFR_{cys}$ in multivariate-adjusted linear regression analysis in all participants. None of the presented PUFA concentrations, including EPA+DHA, were significantly associated with $eGFR_{cre}$; however, EPA+DHA concentration was significantly associated with $eGFR_{cys}$.

DISCUSSION

In the present study, the higher serum (EPA+DHA):AA tertile was significantly associated with higher fish intake in Japanese community-dwelling men and women. Furthermore, especially in men, higher serum (EPA+DHA):AA was significantly associated with higher $eGFR_{cys}$. In all participants, $eGFR_{cre}$ non-significantly increased according to an increase of (EPA+DHA):AA.

To our knowledge, the present study is the first to investigate the relationships between serum long-chain n-3 and n-6 PUFA profile and $eGFR_{cys}$. Because the ratios of n-3 PUFA to AA are considered to be markers for balance of anti-inflammatory and proinflammatory action by n-3 PUFAs and AA,[5] previous studies among Japanese patients have investigated the relationships between cardiac events and the ratio of n-3 PUFAs to AA. Among patients undergoing coronary angioplasty or hemodialysis, lower EPA:AA ratios were associated with higher risk of acute coronary syndrome,[16] and lower (EPA+DHA):AA ratios were associated with higher incidence of cardiovascular disease.[5] However, the relationships between serum long-chain n-3 and n-6 PUFA profiles and GFR estimated by Japanese-specific equations have not been investigated in community-dwellers.

In Western populations, only a few previous studies have investigated the relationships between PUFAs and renal function in community-dwellers. Gopinath et al showed that dietary intake of long-chain n-3 PUFA was inversely associated with the prevalence of CKD in a cross-sectional study of 2600 community-dwellers in Australia.[15] Lauretani

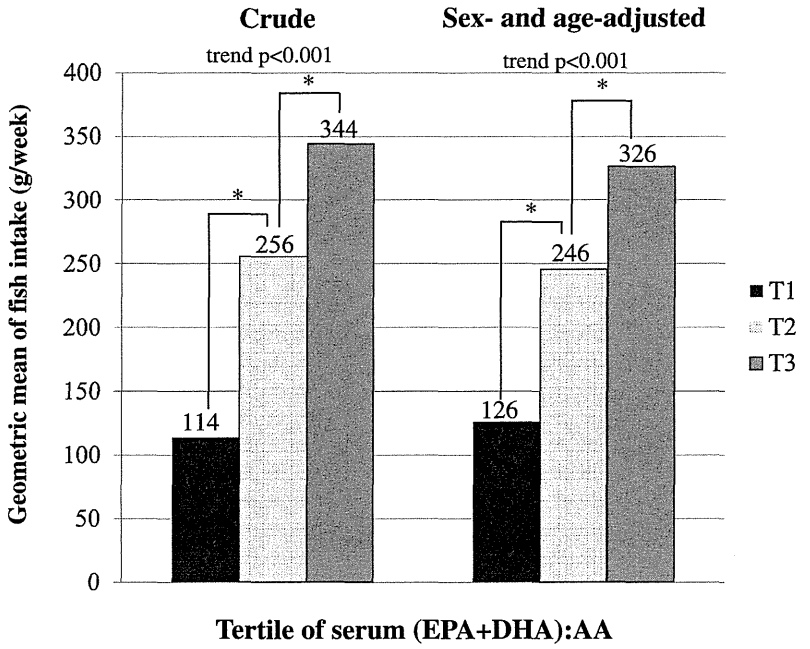

Figure. Tertile of serum (EPA+DHA):AA and geometric mean of fish intake. *Bonferroni correction for multiple post-hoc comparisons. Significance between the presented tertiles: $P < 0.05$.

Table 2. Multivariate-adjusted $eGFR_{cre}$ according to tertile of serum (EPA+DHA):AA ratio in the Sasayama study, 2012–2013

	Tertile of serum (EPA+DHA):AA			*P* value[a]
	T_1	T_2	T_3	
Men				
Number of participants	78	80	79	
Range of (EPA+DHA):AA ratio	0.409–0.904	0.908–1.318	1.323–3.188	
Sex- and age-adjusted mean $eGFR_{cre}$ (95% CI)	72.1 (68.8–75.4)	72.2 (69.1–75.4)	75.2 (72.0–78.5)	0.322
Multivariate-adjusted mean $eGFR_{cre}$ (95% CI) (Model 1)[b]	71.3 (68.0–74.6)	72.5 (69.4–75.6)	75.8 (72.6–79.0)	0.139
Multivariate-adjusted mean $eGFR_{cre}$ (95% CI) (Model 2)[c]	71.3 (68.0–74.6)	72.5 (69.4–75.6)	75.8 (72.6–79.0)	0.147
Women				
Number of participants	103	104	105	
Range of (EPA+DHA):AA ratio	0.338–0.925	0.929–1.282	1.283–2.777	
Sex- and age-adjusted mean $eGFR_{cre}$ (95% CI)	73.2 (70.7–75.7)	73.2 (70.9–75.5)	73.0 (70.7–75.3)	0.989
Multivariate-adjusted mean $eGFR_{cre}$ (95% CI) (Model 1)[b]	73.2 (70.6–75.7)	73.2 (70.9–75.5)	73.1 (70.7–75.4)	0.998
Multivariate-adjusted mean $eGFR_{cre}$ (95% CI) (Model 2)[c]	73.2 (70.6–75.7)	73.1 (70.8–75.5)	73.1 (70.8–75.5)	1.000
Men and women combined				
Number of participants	184	181	184	
Range of (EPA+DHA):AA ratio	0.338–0.925	0.928–1.301	1.301–3.188	
Sex- and age-adjusted mean $eGFR_{cre}$ (95% CI)	72.7 (70.7–74.7)	73.1 (71.2–75.0)	73.7 (71.8–75.6)	0.776
Multivariate-adjusted mean $eGFR_{cre}$ (95% CI) (Model 1)[b]	72.3 (70.3–74.2)	73.3 (71.4–75.2)	74.0 (72.1–75.9)	0.507
Multivariate-adjusted mean $eGFR_{cre}$ (95% CI) (Model 2)[c]	72.2 (70.2–74.2)	73.3 (71.4–75.1)	74.0 (72.1–75.9)	0.476

AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GFR, glomerular filtration rate.
[a]*P* value for ANCOVA.
[b]Multivariate-adjusted (Model 1): eGFR adjusted for age, sex, BMI, hypertension, diabetes, HDL-C, LDL-C, medication for dyslipidemia, current smoking, and current drinking.
[c]Multivariate-adjusted (Model 2): eGFR adjusted for variables in model 1 plus log-transformed high-sensitivity C-reactive protein.

et al showed that participants with higher plasma n-3 PUFA concentration had a significantly lower risk of developing CKD and mortality in a cohort study of 931 community-dwellers.[1] The results of these previous studies are consistent with those of the present study.

On the other hand, there has been a series of conflicting reports regarding the benefit of fish oil preparations containing n-3 PUFA given to patients with a variety of disease. Hsu et al showed that frequent intake of fish and vegetables correlated significantly with decreased creatinine and marginally with increased GFR estimated by Cre in a cohort study of patients with type 2 diabetes in Taiwan.[17] According to a meta-analysis of clinical trials by Miller III et al, the decline of GFR was slower in participants with n-3 PUFA supplementation

Table 3. Multivariate-adjusted $eGFR_{cys}$ according to tertile of serum (EPA+DHA):AA ratio in the Sasayama study, 2012–2013

	Tertile of serum (EPA+DHA):AA			*P* value[a]
	T_1	T_2	T_3	
Men				
Number of participants	78	80	79	
Range of (EPA+DHA):AA ratio	0.409–0.904	0.908–1.318	1.323–3.188	
Sex- and age-adjusted mean $eGFR_{cys}$ (95% CI)	82.9 (79.5–86.3)	89.5 (86.2–92.7)	90.7 (87.4–94.1)	0.003
Multivariate-adjusted mean $eGFR_{cys}$ (95% CI) (Model 1)[b]	83.9 (80.5–87.2)	88.2 (85.0–91.3)	91.0 (87.8–94.3)	0.015
Multivariate-adjusted mean $eGFR_{cys}$ (95% CI) (Model 2)[c]	84.0 (80.6–87.3)	88.2 (85.1–91.4)	90.9 (87.7–94.1)	0.017
Women				
Number of participants	103	104	105	
Range of (EPA+DHA):AA ratio	0.338–0.925	0.929–1.282	1.283–2.777	
Sex- and age-adjusted mean $eGFR_{cys}$ (95% CI)	89.6 (86.8–92.3)	91.2 (88.7–93.8)	91.2 (88.6–93.8)	0.654
Multivariate-adjusted mean $eGFR_{cys}$ (95% CI) (Model 1)[b]	89.3 (86.6–91.9)	91.5 (89.1–93.9)	91.2 (88.8–93.7)	0.466
Multivariate-adjusted mean $eGFR_{cys}$ (95% CI) (Model 2)[c]	89.2 (86.6–91.8)	91.4 (89.0–93.8)	91.4 (89.0–93.7)	0.454
Men and women combined				
Number of participants	184	181	184	
Range of (EPA+DHA):AA ratio	0.338–0.925	0.928–1.301	1.301–3.188	
Sex- and age-adjusted mean $eGFR_{cys}$ (95% CI)	86.9 (84.7–89.0)	90.7 (88.6–92.8)	90.7 (88.5–92.7)	0.023
Multivariate-adjusted mean $eGFR_{cys}$ (95% CI) (Model 1)[b]	86.9 (84.9–89.0)	90.6 (88.7–92.6)	90.6 (88.7–92.6)	0.021
Multivariate-adjusted mean $eGFR_{cys}$ (95% CI) (Model 2)[c]	86.9 (84.9–88.9)	90.5 (88.6–92.4)	90.8 (88.9–92.7)	0.016

AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GFR, glomerular filtration rate.
[a]*P* value for ANCOVA.
[b]Multivariate-adjusted (Model 1): eGFR adjusted for age, sex, BMI, hypertension, diabetes, HDL-C, LDL-C, medication for dyslipidemia, current smoking, and current drinking.
[c]Multivariate-adjusted (Model 2): eGFR adjusted for variables in model 1 plus log-transformed high-sensitivity C-reactive protein.

Table 4. Multivariate-adjusted eGFR in T_1 and T_2+T_3 of serum (EPA+DHA):AA ratio in the Sasayama study, 2012–2013

	Tertile of serum (EPA+DHA):AA		*P* value[a]
	T_1 (0.338–0.925)	T_2+T_3 (0.928–3.188)	
Number of participants	184	365	
Mean $eGFR_{cre}$ (ml/min/1.73 m^2)			
Sex- and age-adjusted	72.7 (70.8–74.7)	73.4 (72.0–74.8)	0.598
Multivariate-adjusted (Model 1)[b]	72.3 (70.3–74.3)	73.6 (72.3–75.0)	0.291
Multivariate-adjusted (Model 2)[c]	72.3 (70.3–74.2)	73.6 (72.3–75.0)	0.282
Mean $eGFR_{cys}$ (ml/min/1.73 m^2)			
Sex- and age-adjusted	86.9 (84.7–89.0)	90.7 (89.2–92.1)	0.006
Multivariate-adjusted (Model 1)[b]	86.9 (84.9–89.0)	90.6 (89.2–92.0)	0.005
Multivariate-adjusted (Model 2)[c]	86.9 (84.9–88.9)	90.6 (89.3–92.0)	0.004

AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GFR, glomerular filtration rate.
[a]*P* value for ANCOVA.
[b]Multivariate-adjusted (Model 1): eGFR adjusted for age, sex, BMI, hypertension, diabetes, HDL-C, LDL-C, medication for dyslipidemia, current smoking, and current drinking.
[c]Multivariate-adjusted (Model 2): eGFR adjusted for variables in model 1 plus log-transformed high-sensitivity C-reactive protein.
Multivariate-adjusted (Model 2): eGFR adjusted for variables in model 1 plus log-transformed high-sensitivity C-reactive protein.

Table 5. Multivariate-adjusted linear regression models[a] between eGFR and serum PUFAs, including EPA+DHA, in the Sasayama study, 2012–2013

	Independent variables			
	$eGFR_{cre}$		$eGFR_{cys}$	
	Standardized coefficients	*P* value	Standardized coefficients	*P* value
Model 1				
Serum EPA+DHA	−0.012	0.815	0.097	0.025
Serum α-linolenic acid	0.088	0.191	0.015	0.797
Serum AA	0.008	0.862	−0.025	0.541
Serum linoleic acid	−0.144	0.064	−0.016	0.803
Model 2				
Serum EPA+DHA	0.014	0.770	0.096	0.015
Serum n-6 PUFA[b]	−0.055	0.313	−0.022	0.622

AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GFR, glomerular filtration rate; PUFA, polyunsaturated fatty acids.
[a]Relationships between presented fatty acids and eGFR were evaluated by linear regression model after adjusting for age, sex, BMI, presence of hypertension and diabetes mellitus, serum HDL- and LDL- cholesterol level, medication for dyslipidemia, current smoking and drinking, and log-transformed C-reactive protein.
[b]Serum n-6 PUFA: sum of linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, and arachidonic acid.

than in control participants, but this effect was not significant, and they concluded that n-3 PUFA supplementation did not ameliorate the decline in GFR. However, they also noted that differences in methods of assessing GFR, such as GFR measured or estimated by serum Cre or 24-h urine Cre clearance, limited the ability to draw conclusions.[18] Furthermore, serum Cre level is affected by various factors, such as muscle mass and diet.[10]

In the present study, the difference between $eGFR_{cys}$ and $eGFR_{cre}$ in relation to EPA+DHA:AA was especially apparent in men. Thus, muscle mass could be an important factor influencing the relationship between serum EPA+DHA:AA and $eGFR_{cre}$, and $eGFR_{cys}$ might be more useful than $eGFR_{cre}$ when investigating the relationship between eGFR and PUFA profiles. In addition, in the previous studies,[1,15,17,18] ratios of long-chain n-3 and n-6 PUFAs, such as (EPA+DHA):AA,

were not investigated in relation to eGFR. As shown in eTable 3, all long-chain n-3 PUFA concentrations and ratios of long-chain n-3 PUFAs to AA showed significant associations with $eGFR_{cys}$. And although both the concentrations and the ratios did not show significant relationship with $eGFR_{cre}$, the ratio of long-chain n-3 PUFAs to AA showed higher coefficients with $eGFR_{cre}$ than n-3 PUFA concentrations. Accordingly, the present study suggests that ratios of serum long-chain n-3 PUFAs to AA could be useful when investigating the relationship between PUFA profiles and renal function.

The mechanisms by which higher (EPA+DHA):AA ratios protect renal function are still not clear. However, dietary fish oil supplementation has been shown to slow renal disease progression in patients with IgA nephropathy[19] and to suppress mesangial cell activation and proliferation in animal models.[20] Therefore, long-chain n-3 PUFAs are considered to attenuate inflammation through several pathways, including those involved in reduction of nitric oxide, downregulation of tumor necrosis factor-α, and modulation of protein kinases.[21–23] Furthermore, Minuz et al demonstrated that alterations in cytochrome P450 (CYP)-dependent AA metabolism are associated with the development of vascular and tubular abnormalities in patients with renovascular disease.[24] Arnold et al showed that EPA and DHA are efficient alternative substrates of AA-metabolizing CYP enzymes, and that dietary EPA/DHA supplementation causes a profound shift in the CYP-eicosanoid profile.[25] In addition, Cicero et al showed that long-term n-3 PUFA supplementation was associated with significant reduction in blood pressure.[26]

The hypocholesterolemic effects of fish intake have also been reported.[27] The association between (EPA+DHA):AA ratio and eGFR was independent of the presence of hypertension and cholesterol level; however, these mechanisms could be also associated with the results in the present study. Furthermore, the difference in eGFR between T_1 and T_3 was higher in men than in women. According to sex-specific characteristics of the participants (eTable 1), prevalence of smoking in men was high in T_1. Therefore, unfavorable lifestyles, such as smoking and low fish intake, might be one possible reason for poorer renal function.

Gopinath et al also showed that linolenic acid (18:3n-3; α-linolenic acid) intake was positively associated with the odds of CKD.[15] In contrast, Lauretani et al showed that a higher plasma concentration of α-linolenic acid, n-6 PUFAs, linoleic acid, and AA were associated with lower decline in Cre clearance.[1] In the present study, serum concentration of α-linolenic acid, n-6 PUFAs, linoleic acid, and AA did not show significant relationships with eGFR. A previous study reported that serum concentrations of EPA and DHA were generally higher in the Japanese population than in Western populations.[2] Therefore, in the present study, the relatively higher concentration of EPA+DHA might mask the relationship between renal function and other PUFAs, such as α-linolenic acid, n-6 PUFAs, linoleic acid, and AA.

The significant relationships between the tertile of (EPA+DHA):AA and fish intake in the present study suggest that higher fish intake could be related to higher eGFR. Indeed, the participants with higher fish intake tended to have higher $eGFR_{cys}$ in the present study. According to previous studies, fish consumption differs by country (mean daily fish intake: 71–125 g in Japan, 32 g among Caucasians in the United States [U.S.], and 85–110 g in Norway).[2,28,29] Hallen et al performed an international comparison of CKD prevalence between the participants in the third National Health and Nutrition Examination Survey (NHANES III) in the U.S. and those of a survey (HUNT II) in Norway.[30] After age standardization, the prevalence of CKD in HUNT II was 9.3%, and the prevalence in NHANES III was 11.0%. Although they did not consider fish consumption in the analyses, the difference in CKD prevalence between the two populations might be partly due to the difference in fish consumption, as well as due to the lower prevalence of diabetes and obesity in Norway. However, the prevalence of CKD is not low in the general Japanese population (12.9%), despite high fish consumption.[8] This discrepancy is most likely because average life expectancy has been extended[31]; blood pressure is relatively high[4]; and the prevalence of diabetes, hypercholesterolemia, and obesity has increased in the Japanese population.[32]

The present study had several limitations. First, because the study was cross-sectional, causality could not be determined. Second, information about corticosteroid use was not available. Third, sex-specific analyses were difficult due to the small number of participants. Fourth, although Cre clearance and insulin clearance are better markers of renal function, these data were not available in the present study. Finally, body muscle mass might be related to the results of the present study; however, these data were also not available.

In conclusion, serum long-chain n-3 and n-6 PUFA profiles, especially the (EPA+DHA):AA ratio, were significantly associated with GFR estimated by an equation using serum Cys C in Japanese community-dwellers. The results of the present study suggest that increased fish intake or supplementation with long-chain n-3 PUFAs might prevent renal dysfunction in the general population.

ONLINE ONLY MATERIALS

eTable 1. Characteristics of study participants according to serum (EPA+DHA):AA tertile in men: the Sasayama study, 2012–2013.

eTable 2. Characteristics of study participants according to serum (EPA+DHA):AA tertile in women: the Sasayama study, 2012–2013.

eTable 3. Multivariate-adjusted linear regression models between serum n-3 and n-6 PUFA profiles and estimated GFRs by 2 different equations.

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Conflicts of interest: None declared.

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Cholesterol Absorption Inhibitor Ezetimibe: Risk–Benefits and Role in Treating Dyslipidemias

28

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Introduction

Niemann-Pick C1-Like 1 (NPC1L1) is a 13-transmembrane domain cell surface cholesterol-sensing receptor. It is localized on the apical membrane or brush border of small intestines (especially jejunum) and has recently been reported to play an important role in dietary cholesterol absorption and biliary cholesterol reabsorption by enterocytes [1–2]. Genetic inactivation of *NPC1L1* gene decreases cholesterol levels and atherosclerotic lesions in mice with diet-induced hyperlipidemia [3] and in hyperlipidemic apolipoprotein (apo) E-knockout mice fed a Western diet [4]. Ezetimibe, a novel lipid-lowering compound, selectively inhibits intestinal cholesterol absorption by binding to NPC1L1 [5] and inhibiting the internalization of NPC1L1 [6]. NPC1L1 has three large loops that protrude into the extracellular space, several smaller cytoplasmic loops, and a C-terminal cytoplasmic tail [7]. Studies using in vitro ezetimibe-binding assays, demonstrated that ezetimibe directly binds to the second extracellular loop of NPC1L1 [8–9].

Ezetimibe reduces the hepatic influx of cholesterol via chylomicrons (CM) remnants, which enhances the hepatic expression of low-density lipoproteins (LDL) receptor, and thus reducing LDL-cholesterol (LDL-C) levels. Ezetimibe is also reported to reduce the development of atherosclerosis in apoE-knockout mice [10]. Clinically, the administration of ezetimibe has been shown to decrease the fasting levels of total cholesterol and LDL-C in patients with primary hypercholesterolemia [11] and plant sterols (sitosterol and campesterol) in patients with sitosterolemia [12–13]. A meta-analysis demonstrated that a significantly greater percentage reduction in LDL-C levels was achieved in patients treated with ezetimibe–statin combination compared with statin monotherapy [14]. Since ezetimibe is an inhibitor of intestinal cholesterol absorption, the pharmacological effects of ezetimibe have been focused primarily on the metabolism of sterols, including cholesterol, plant sterols, and oxidized cholesterol rather than triglycerides (TG) or TG-rich lipoproteins (TRL).

Ezetimibe has been reported to significantly decrease fasting TG levels in patients with combined hyperlipidemia [15] and those with hypertriglyceridemia (TG≥150 mg/dl); however, its underlying mechanism of action on TRL metabolism has not yet been elucidated. We have recently reported the effects of ezetimibe in patients with

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type IIb hyperlipidemia with a special reference to postprandial TRL and remnant metabolism. We reported that ezetimibe administration could attenuate postprandial hyperlipidemia in oral fat-loading tests [16]. We also evaluated the mechanisms for the attenuation of postprandial hyperlipidemia in mouse models and reported that ezetimibe can reduce the production of CM from the small intestines and decrease the absorption of free fatty acids (FFA) [17].

More recently, ezetimibe has been reported to attenuate nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH). This chapter highlights the effects of ezetimibe on postprandial hyperlipidemia, hepatic lipid depositions in NAFLD or NASH, and insulin resistance. The potential and comprehensive mechanisms for the improvement by ezetimibe of these conditions usually associated with metabolic syndrome are presented.

History of Ezetimibe

Although cholesterol was supposed to be absorbed in the small intestine, especially the jejunum, the detailed mechanisms for cholesterol absorption were not understood. From the initial screening of an acyl-CoA to cholesterol acyltransferase 2 (ACAT2) inhibitor, ezetimibe was discovered by Davis HR and colleagues at the Research Institute of Schering-Plough as an inhibitor of cholesterol absorption in the small intestine [18–19]. It was demonstrated that ezetimibe is a potent and selective inhibitor of intestinal cholesterol uptake and absorption in animal models and humans. It was launched into the market before its molecular target was finally identified. Extensive studies were performed to identify the molecular target of ezetimibe. Since ezetimibe was shown localized in the brush border of enterocytes in the jejunum, it was speculated that the target of ezetimibe was localized there. Several candidate genes which are localized in the brush border of the small intestine were investigated, including scavenger receptor class B type I (SR-BI), ATP-binding cassette transporter A1 (ABCA1), and CD36, a transporter of long-chain fatty acids. However, the knockout mice of these genes did not show any changes in the absorption of radio-labeled cholesterol [20]. Thus, it had been very difficult to discover the molecular target of ezetimibe.

Genomic bioinformatics approach was then applied to identify genes involved in the absorption of cholesterol in the small intestine. Altmann et al. [1] hypothesized that intestinal cholesterol transporter should be localized mainly in the luminal surface and brush border of jejunum and possess sequence motifs known to interact with sterols. They generated a complementary deoxyribonucleic acid (cDNA) library of rat intestine, sequenced ~16,500 genes and examined these data in comparison with the database of mice and human genes. They analyzed the sequence database of all transcripts containing transmembrane domains, extracellular signal peptides, N-linked glycosylation sites, and sterol-sensing domain. They finally identified a candidate gene and it was a rat homologue of NPC1L1.

Structure, Function, and Regulation of NPC1L1

NPC1L1 possesses a secretion signal, 13 transmembrane domains, extensive N-linked glycosylation sites located in the extracellular loops, and a sterol-sensing domain. NPC1L1 was shown highly and exclusively expressed in the jejunum of mice. It was localized on the luminal surface of jejunal enterocytes. Altmann et al. [1] generated NPC1L1-knockout mice and showed that cholesterol absorption in these mice was reduced by more than 70%. Furthermore, the low levels of cholesterol absorption in NPC1L1-knockout mice were not affected by administration of ezetimibe. Acute cholesterol absorption was decreased by ~90% in the NPC1L1-knockout mice, which was similar to the inhibition of cholesterol absorption in mice, hamsters, and rats treated with ezetimibe. Thus, NPC1L1 is involved in the uptake and absorption of cholesterol from the lumen of jejunum at the brush border membrane of the enterocytes [21]. The uptake of TG by the intestine and its absorption were not altered in the NPC1L1-knockout mice and animals treated with ezetimibe.

NPC1L1 messenger ribonucleic acid (mRNA) levels in the liver and small intestines are upregulated in animals deprived of cholesterol [22, 23]. Intestinal NPC1L1 mRNA levels are downregulated in cholesterol/cholate-fed mice [24] or ACAT2-deficient and phospholipid transfer protein (PLTP)-deficient mice in which free cholesterol is accumulated [25, 26]. The regulation of NPC1L1 expression by sterol is mediated by the binding of sterol regulatory element-binding protein (SREBP)-2 to 2 sterol regulatory elements within the promoter region of *NPC1L1* gene. Statins are known to increase the expression of intestinal NPC1L1 mRNA [27], leading to an increase in cholesterol absorption. Atorvastatin increased intestinal NPC1L1 mRNA levels by 19%, while it decreased mRNA levels of both ATP-binding cassette transporter G5 (ABCG5) and ATP-binding cassette transporter G8 (ABCG8) by 14% in hyperlipidemic men [27]. These effects were most likely mediated by upregulation of the transcription factors SREBP-2 and hepatocyte nuclear factor-4α (HNF-4α) [27]. Statins that are more potent in lowering LDL-C levels increase NPC1L1 expression in the small intestine more than regular statins [28]. In streptozotocin-induced diabetic rats, and in Zucker diabetic fatty *fa/fa* rats the expression of NPC1L1 in the small intestine and thus cholesterol absorption are enhanced [29]. In mice, the expression of NPC1L1 increases with aging [30]. In humans, 45 single nucleotide polymorphisms (SNPs) of nonsynonymous sequence variants in the NPC1L1 gene have been reported [31, 32]. Some of these SNPs influence the sterol absorption and plasma LDL-C levels [33, 34].

NPC1L1 is abundantly expressed in the small intestine of all species, but not expressed in the liver of mice [1]. In contrast to mice, the expression level of NPC1L1 mRNA is similarly high in the liver of humans, monkeys, pigs, and dogs. NPC1L1 is localized in the bile canalicular membrane in the human liver [35, 36]. Therefore, its function may be the reabsorption of cholesterol excreted into the bile, while ABCG5/G8 excretes cholesterol and phytosterol into bile. Overexpression of NPC1L1 in the transgenic mice liver reduced biliary cholesterol and increased plasma cholesterol level, suggesting that bile canalicular NPC1L1 is involved in the absorption of cholesterol from bile and its reuptake into the hepatocyte [36]. Ezetimibe may also inhibit reabsorption of cholesterol from bile.

Mechanisms of Intestinal Cholesterol Absorption and Chylomicron Synthesis

Plasma TG is mainly found in TRL, including CM, very low density lipoproteins (VLDL), and their remnants. TRL constitute a population of particles of heterogeneous size, origin, and apolipoprotein and lipid content. The cholesterol and plant sterols absorbed from the intestinal lumen via NPC1L1 are esterified by ACAT2, forming cholesteryl esters or plant sterol esters (Fig. 28.1) [37]. These cholesteryl esters are assembled with TG, phospholipids, and apoB-48 by microsomal TG transfer protein (MTP) to form CM, which are secreted into the intestinal lymph [37]. CM enter thoracic lymph, from which they flow into the systemic circulation [37]. CM particles undergo partial hydrolysis predominantly by lipoprotein lipase (LPL) into smaller and denser particles known as CM remnants, which are believed to be more atherogenic than the larger CM [38]. LPL hydrolyses the TG moiety of CM to FFA, and residual particles become CM remnants which are taken up by the liver via remnant receptors.

After the uptake of CM remnants by hepatocytes, VLDL are assembled from endogenous hepatic TG, cholesterol, and apoB-100 and are secreted directly into the blood stream. Thereafter, the TG moiety of VLDL is hydrolyzed to FFA by LPL, becoming VLDL remnants, intermediate-density lipoproteins (IDL). The liver takes up VLDL remnants and LDL via LDL receptors, while these particles are supplying energy and lipids to peripheral tissues. In the postprandial state, the serum levels of CM and CM remnants rise quickly to reflect the increased exogenous lipid supply [39]. The increased hepatic lipid inflow leads to an augmented hepatic production of VLDL.

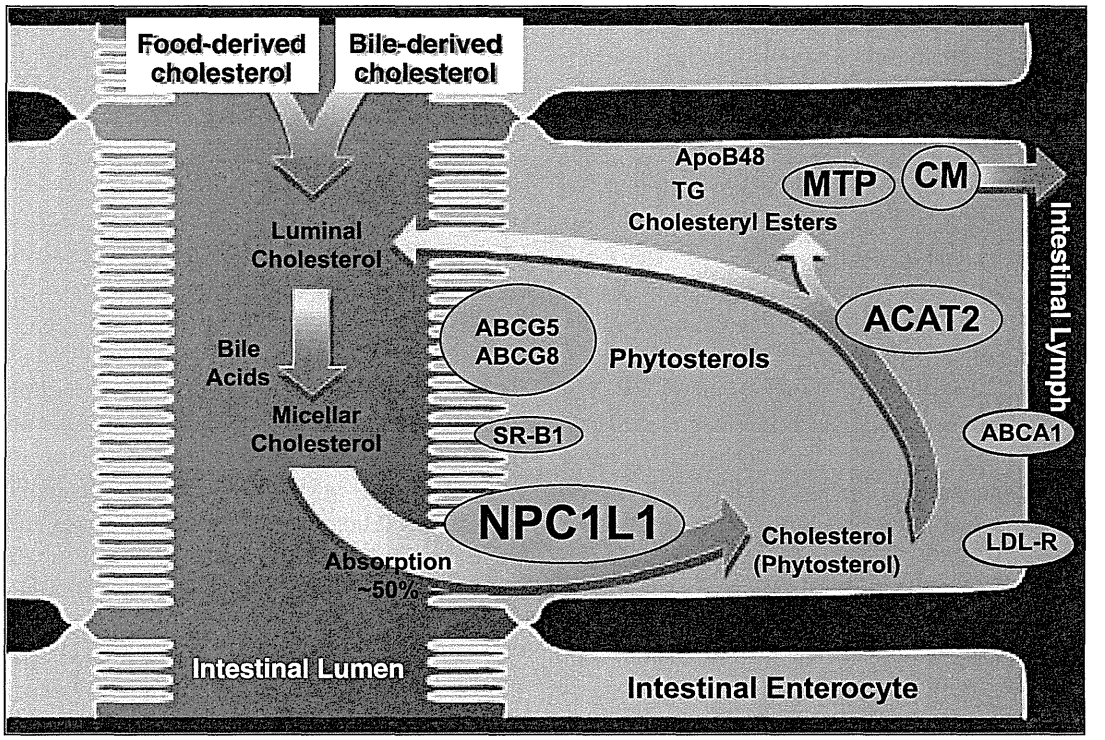

Fig. 28.1 Molecular mechanisms of cholesterol absorption, chylomicron synthesis, and secretion in the small intestines

Absorption, Metabolism, and Pharmacodynamics of Ezetimibe

Ezetimibe (SCH58235; 1-(fluorophenyl)-(3R)-[3-(4-fluorophenyl)-(3S)-hydroxypropyl]-(4S)-(4-hydroxyphenyl)-2-azetidinone) was first discovered by utilizing in vivo models of cholesterol absorption [18]. Its chemical structure is illustrated in Fig. 28.2. It was found by the characterization of the active biliary metabolites of its predecessor, SCH48461, and analysis of structure–activity relationship based upon cholesterol feeding of hamsters. Ezetimibe inhibited diet-induced hypercholesterolemia in hamsters and its ED_{50} was 0.04 mg/kg. In rats, ezetimibe inhibited the absorption and appearance of radiolabeled cholesterol into plasma with an ED_{50} of 0.0015 mg/kg [40]. Ezetimibe was also effective in cholesterol-fed rhesus monkeys with an ED_{50} of 0.0005 mg/kg/day [41].

The cholesterol in the lumen of small intestines derives from bile as well as foods. The cholesterol synthesized in the liver is approximately 400 mg/day; however, the food-derived cholesterol intake is 300–500 mg/day and reabsorption of bile-derived cholesterol is two- to fourfold (800–2000 mg/day) more than that from foods. Ezetimibe is a selective inhibitor of cholesterol absorption in the small intestines and does not influence the esterification of ACAT2, hydrolysis of cholesteryl ester by cholesterol esterase (CEase), and the absorption of fatty acids. Ezetimibe does not affect the activity of pancreatic lipase nor the absorption of TG, vitamins A and D, and taurocholic acid in rats. Most importantly, ezetimibe is completely different from resins such as cholestyramine, colestipol, or colestimide since it does not bind bile acids nor inhibit their absorption. Ezetimibe has no significant effect on fat-soluble vitamin levels.

Ezetimibe is rapidly glucuronidated by uridine 5-diphosphate (UDP)-glucuronosyl-transferase in the intestine, after which the glucuronidated ezetimibe is excreted into the bile. Gluc-