Association of Fish Intake with Low-density Lipoprotein Cholesterol/high-density Lipoprotein Cholesterol Ratio in Apparently Healthy Males in Japanese

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ABSTRACT

Background: Higher fish consumption has been reported to be associated with a lower incidence of coronary artery disease (CAD). We hypothesized that higher fish intake may be associated with lower serum low-density lipoprotein cholesterol/high-density lipoprotein cholesterol (L/H) ratios, an atherogenic marker, and healthier lifestyle behaviors.

Methods: This cross-sectional study was conducted in 2,768 apparently healthy males receiving no lipid-modifying therapy and visiting the Health Planning Center of Nihon University Hospital between April and August 2019.

Results: The average number of days of fish intake per week was 2.32 ± 1.31. The L/H ratio decreased significantly as the weekly frequency of fish intake (0-2 days, 3-4 days, or 5-7 days) increased (p < 0.0001). Multivariable regression analysis after adjustment for age, subject background factors, and cardio-metabolic risk revealed that increased weekly frequency of fish intake was a weak, but significantly independent determinant of a decreased L/H ratio (β = −0.064, p = 0.0008). Furthermore, as the frequency of fish intake per week increased, the proportion of subjects with cigarette smoking decreased (p = 0.003), the proportion of subjects engaging in habitual aerobic exercises increased (p < 0.0001), and the sleep duration and alcohol intake of the subjects increased (p < 0.0001).

Conclusions: These results suggest that a high weekly frequency of fish intake was associated with lower L/H ratios, as well as healthier lifestyle behaviors; thus, it may represent a component of a healthy lifestyle associated with a lower risk of CAD in Japanese males.

Key words coronary artery disease, atherosclerotic cardiovascular disease, fish intake, lifestyle behavior, L/H ratio

Introduction

Epidemiological studies have demonstrated the existence of an inverse correlation between the weekly frequency of fish consumption and the risk of onset of coronary artery disease (CAD)1-12. Japan Arteriosclerosis Society Guidelines for Prevention of Atherosclerotic Cardiovascular Disease 2017 also recommend frequent intake of fish rich in n-3 unsaturated fatty acids (n-3 PUFAs), which have been reported to exert cardiovascular protective effects9,10. In regard to the mechanism underlying the reduced risk of CAD associated with a high frequency of fish intake, numerous studies have shown that it may be attributable to the diverse cardioprotective effects of the n-3 polyunsaturated fatty acids (n-3 PUFAs: eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) that are contained in abundance in fish oils, such as sardine and saury fish oils1-8. Improvement of the serum lipid profile, especially serum levels of triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C), is reported as one of the representative cardioprotective effects of the n-3 PUFAs7-11.

Elevated serum low density lipoprotein cholesterol (LDL-C)/ HDL-C (L/H) ratio reflects the overall metabolic abnormalities of LDL-C and HDL-C, and is widely recognized as a risk factor for atherosclerotic cardiovascular disease (ASCVD) in western countries13. The Framingham Heart Study, a long-term, ongoing cardiovascular cohort study of residents of the city of Framingham, Massachusetts, in the United States of America demonstrated that the serum L/H ratio was a better predictor of cardiovascular events than the serum LDL-C or HDL-C level alone14. According to a meta-analysis of studies in which coronary plaques were evaluated using intravascular ultrasound in coronary artery disease patients receiving statins, the lower the serum L/H ratio, the greater was the inhibitory effect on the progression of coronary atherosclerosis, and coronary plaque regression was observed in patients with serum L/H ratios of less than 1.515. In the Japan Arteriosclerosis Society Guidelines, the serum L/H ratio is recognized as a marker of atherosclerosis disease
(Evidence level E -1 a), and most of these data are derived from studies in western countries, and the management goal of the serum L/H ratio in Japan is not clarified11. However, our previous pilot cross-sectional study also reported a lower prevalence of CAD in subjects with higher serum levels of n-3 PUFAs, as well as the existence of a relationship between the serum levels of EPA and the serum L/H ratio in Japanese subjects16). Therefore, the serum L/H ratio may be lower in the Japanese population as a result of the frequency of fish intake. Thus, Intake of fish containing n-3 PUFAs may be related to the serum L/H ratio, and the relationship between the serum L/H ratio and the frequency of fish intake may play an important role in nutritional guidance provided as a part of specific health guidance.

It is well-known that improved lifestyle behaviors, including the dietary habit, are closely associated with a reduced risk of onset of ASCVD. In particular, diets focused on a high frequency of fish intake, such as the Japanese diet, which lays emphasis on fish intake, are known to be useful for reducing the risk of onset of ASCVD17). Thus, the high frequency of fish intake as part of the Japanese diet may contribute to the lower prevalence of hypercholesterolemia and lower risk of CAD in the Japanese population. Until date, however, few reports have been published concerning the relationship between the frequency of fish intake per se (rather than the intake of purified n-3 PUFAs) and the serum L/H ratio or overall lifestyle behaviors.

We hypothesized that a high average weekly frequency of fish intake may be associated with lower serum L/H ratios and healthy lifestyle behaviors. The purpose of the present study, conducted using a cross-sectional design, was to investigate the association of the frequency of fish intake (average number of days of fish intake per week) and the serum L/H ratio and lifestyle behaviors in apparently healthy males in the Japanese population. Among 4,796 Japanese subjects who had undergone their annual health checkups between April 2019 and August 2019 at the Health Planning Center of Nihon University Hospital, 2,767 healthy males were recruited for this study. The exclusion criteria included refusal to provide consent for participation in the study, female subjects, current intake of lipid-modifying therapy, positive history of ASCVD, and serum TG ≥ 400 mg/dL. The flow diagram of the study participants is shown in Figure 1. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The study design and objective were approved by the Nihon University Hospital Ethics Committee (approved number: 20200405) and written informed consent was obtained from all the study participants. The rationale and study design of this study have already been published18).

Questionnaire to Determine the Health Behaviors

Health behavior surveys at our institute were conducted in face-to-face interviews of the subjects by trained interviewers. The surveys consisted of comprehensive questions designed to assess the demographic and socioeconomic characteristics of the subjects: age, occupation, marital status, previous and current medical history, medication history, family history, and lifestyle behaviors. The subjects undergoing health checkups were given the fol-
lowing lifestyle questionnaire and asked to provide appropriate responses.

1) Cigarette smoking habit: Do you smoke habitually?: No/Yes/I have quit smoking/I quit smoking ( ) years ago.

2) Alcohol intake habit: Please indicate the frequency at which you drink: Every day/sometimes/I used to drink previously, but have stopped drinking/I stopped drinking ( ) years ago/I drink rarely/I cannot drink; How much do you drink per day when you drink? (ethanol equivalent [g/day]): < 20 g/20 to < 40 g/40 to < 60 g/60 g; How many days per week do you drink?:

3) Aerobic exercise habit: Have you engaged in exercise that makes you sweat slightly for ≥30 minutes a day, at least twice a week for ≥1 year?

4) Sleep habit: How many hours a day, on average, do you sleep? Do you feel sufficiently rested by sleep (presence/absence of a sensation of deep sleep)?

5) Fish intake: How many days, on average, per week have you eaten fish in the past 1 month? Fish consumption was assessed in weekly frequencies in the questionnaire (0, 1, 2, 3, 4, 5, 6, and approximately every day).

The questionnaire was a modified version of the Questionnaire on Specific Health Examination, which is used for specific health guidance after health checkups under the jurisdiction of the Ministry of Health, Labour and Welfare in Japan[9, 20].

Health Examinations and Blood Samples

Anthropometric variables (height, weight and waist circumference [WC]) were measured with the subjects in a standing position. Height and weight data were obtained using standardized techniques and equipment. Body mass index (BMI) is calculated as the body weight (measured in kilograms) divided by height (measured in meters) squared (kg/m²). WC was measured at the level of the umbilicus with a non-stretchable tape measure during the late exhalation phase with the subject in a standing position[21]. Blood pressure (BP) was measured twice, with a 3-minute interval between the two measurements, using a standard mercury sphygmomanometer following a 5-minute rest period. The average of the 1st and 2nd measurements was used for the analysis in this study. Fasting blood samples were collected in the early morning after the subjects had fasted for 8 hours. At the central clinical laboratory of our institute, serum total cholesterol (TC) and TG levels were measured using enzymatic methods. The serum HDL-C level was also measured using an enzymatic method following heparin and calcium precipitation. The Friedewald formula was used to calculate the serum LDL-C level[22]. The hemoglobin A1c (HbA1c) value was measured using high-performance liquid chromatography. The serum uric acid level was determined using an enzymatic method based on the uricase-peroxidase system. The estimated glomerular filtration rate (e-GFR) was calculated using the abbreviated MDRD (Modification of Diet in Renal Disease) study formula modified by a Japanese coefficient[23].

Statistical Analysis

Data are expressed as the means ± standard deviation (SD) for continuous variables and as percentages for discrete variables. We performed a subset analysis by one-way analysis of variance (ANOVA) using the fish intake frequency as a categorical variable (0-1 day, 2-3 days, 4-5 days, or 6-7 days per week), followed by Tukey-Kramer’s adjustment for covariates if differences were detected in the patient characteristics or cardio-metabolic risk markers. To investigate the relationship between the average weekly frequency of fish intake, the serum L/H ratio, and lifestyle behaviors we performed univariate linear regression analysis using serum L/H ratio as the dependent variable, and age, WC, HbA1c, serum UA level, eGFR, systolic BP (sBP), diastolic BP (dBP), and lifestyle behaviors (cigarette smoking habit, aerobic exercise habit, sleep duration, and alcohol intake [days/week]) as independent variables. Factors identified as significant with p < 0.05 by the univariate linear regression analysis were entered into a multivariate linear regression analysis model. Since a strong correlation is known to exist between the WC and the BMI, the WC, which is a better indicator of visceral obesity than the BMI and also serves as an indicator of energy intake, was entered into the multivariate linear regression analysis model in this study as a cardio-metabolic risk factor. Furthermore, the sBP and dBP are also known to be strongly correlated with each other, and the sBP, which varies more widely and is therefore a more sensitive indicator of the BP, was entered into the multivariate linear regression analysis model as an indicator of the BP. All statistical analyses were performed with the SPSS software (SPSS Inc., Chicago, Illinois, USA) for Windows (version 24).

Results

Patients

The average weekly frequency (in days) of fish intake in the subjects was 2.3 ± 1.3. The weekly frequencies of fish intake are shown in Figure 2. The serum L/H ratio ranged from 0.39 to 6.55 (mean ± SD: 2.21 ± 0.77).

Comparison of the Subject Characteristics and Cardio-metabolic Risk Factors According to the Weekly Frequency of Fish Intake

The subject characteristics and cardio-metabolic risk factors

![Average Frequency of Fish Intake per Week (days)](image_url)

Fig. 2 The Frequencies of Fish Intake per Week

n = 2,767
were compared according to the weekly frequency of fish intake as a categorical variable (0-2 days, 3-4 days, or 5-7 days per week). Higher age was associated with a higher weekly frequency of fish intake \( (p < 0.0001) \). There were no significant associations between the weekly frequency of fish intake and the WC or BMI. The estimated GFR decreased significantly as the weekly frequency of fish intake increased \( (p = 0.0007) \). Although the serum levels of TC and TG decreased as the weekly frequency of fish intake increased, the associations did not reach statistical significance \( (p = 0.055 \) and \( 0.093 \)). As the weekly frequency of fish intake increased, the serum LDL-C decreased \( (p = 0.004) \), serum HDL-C increased \( (p = 0.0004) \), blood HbA1c level increased \( (p < 0.0001) \), sBP increased \( (p < 0.0001) \), and dBP increased \( (p = 0.0001) \). There were no significant associations between the weekly frequency of fish intake and the serum uric acid level (Table 1).

**Comparison of the Serum L/H Ratios According to the Weekly Frequency of Fish Intake**

The serum L/H ratios in the subjects were also compared according to the weekly frequency of fish intake as a categorical variable (0-2 days, 3-4 days, or 5-7 days). The serum L/H ratio decreased significantly as the weekly frequency of fish intake increased \( (p < 0.0001) \). The serum L/H ratios classified according to the weekly frequency of fish intake of 0-2 days, 3-4 days, and 5-7 days were as follows: 2.25 ± 0.79, 2.17 ± 0.74, and 2.01 ± 0.70, respectively (Figure 3).

**Univariate and Multivariate Linear Regression Analysis to Identify Factors Influencing the Serum L/H Ratio**

Univariate and multivariate linear regression analysis identified the weekly frequency of fish intake as an independent determinant of the serum L/H ratio \( (\beta = -0.064, p = 0.0008) \). The serum L/H ratio decreased as the weekly frequency of fish intake increased. Habitual aerobic exercises \( (\beta = -0.067, p = 0.0003) \) and alcohol intake \( (\beta = -0.245, p = 0.0008) \) were negative independent determinants, while the cigarette smoking habit was a positive independent determinant of the serum L/H ratio \( (\beta = 0.048, p = 0.012) \). (Table 2).

**Comparison of Lifestyle Behaviors according to the Weekly Frequency of Fish Intake**

As the weekly frequency of fish intake increased, the proportion of subjects with the cigarette smoking habit decreased \( (p = \)
The main findings of the present study were as follows: as the weekly frequency of fish intake increased, the serum L/H ratio, a marker of atherosclerosis, decreased. Accordingly, a higher weekly frequency of fish intake may be associated with an improvement of both LDL and HDL metabolism, suggesting, as a noteworthy finding, that it may be a marker of a healthy lifestyle. This study may be viewed as a novel one in the following aspects. First, using a cross-sectional study design, the study revealed relationships among three factors (fish intake-centered dietary style, favorable lifestyle associated with such a dietary style, and the serum L/H ratio, a marker of atherosclerosis). Second, while we could not arrive at definitive conclusions because of the cross-sectional design of the study, this study has established the basis for prospective cohort studies to analyze the causal relationships among fish intake frequency, lifestyle behaviors and ASCVD.

Increased fish consumption has been shown in many previous studies to be associated with lower serum TG levels and higher serum HDL-C levels. In the present study, as the weekly frequency of fish intake increased, the serum TG level decreased or significant increase of the serum HDL-C level. It is quite reasonable to assume that if the weekly frequency of fish intake increases, the serum L/H ratio would inevitably decrease because of a favorable change in the serum lipid profile (reduction of the serum LDL-C level and elevation of the serum HDL-C level, among others). Regarding the favorable effects of n-3 PUFAs (contained in fish) on lipid metabolism, DHA has definitely been shown to elevate the serum LDL-C level, but there is no consensus about the effects of EPA. If we consider the small impact of n-3 PUFAs on serum LDL-C reduction, the lower serum LDL-C level observed in our subjects may be associated with the fact that a dietary lifestyle focused on fish intake is a part of a healthier lifestyle, in general. In addition, in individuals with a dietary lifestyle characterized by a high intake of fish as a protein source, consumption of meat rich in saturated fatty acids is inevitably small. This is very likely to be the reason for the reduced serum LDL-C level.

In addition, in the NILS-LSA (National Institute for Longevity Science - Longitudinal Study of Aging), investigators studied fish and meat consumption by age group. The results showed that as age increased, seafood intake increased, but the intake of meat, which is high in saturated fatty acids and causes an increase of serum LDL-C levels, decreased. The same phenomenon may occur in individual cases, not only by age group, but also in each individual case. However, since either fish or meat are representative protein sources, this phenomenon may support our results. Interestingly, in the Nordic Nutrition Recommendations based on the evidence extracted from 607 high-quality randomized controlled trials, prospective cohort studies and case-control studies, partial replacement of saturated fat with polyunsaturated fat or monounsaturated fat is recommended, based on evidence, described as convincing, that it is associated with a lowering of the serum total and LDL-C levels.

The present study additionally showed that frequent fish intake was associated with a lower frequency of the smoking habit, higher frequency of the aerobic exercise habit and increase of sleep duration. This finding suggests that a dietary lifestyle involving a high frequency of fish intake is associated with a better lifestyle. Heavy smoking has been reported to be associated with elevation of the serum LDL-C and reduction of the serum HDL-C levels.
HDL-C level\(^{22}\). Habitual aerobic exercises are useful for improving the serum lipid profile, especially for increasing the serum HDL-C level\(^{22}\). In the present study, the frequency of alcohol consumption rose as the weekly frequency of fish intake increased, suggesting that alcohol intake may also have contributed to the elevation of the serum HDL-C level observed in subjects with an increased weekly frequency of fish intake\(^{34}\). This possibility, however, requires careful interpretation, because excessive alcohol consumption can be hazardous to health. We propose to conduct a prospective cohort study in the near future to analyze the causal relationships among fish intake, serum HDL-C level, health hazards associated with alcohol consumption, and ASCVD.

An inappropriately short sleep duration is known to adversely affect the overall serum lipid profile\(^{35}\). Interestingly enough, fishes containing n-3 PUFAs stimulate the secretion of serotonin and play an important role in the regulation of high-quality sleep\(^{36,37}\). These previous reports lend support to the results of the present study. However, the cross-sectional design of this study does not allow a definitive conclusion to be arrived at as to the exact causal relationships among the frequency of fish intake, serum lipid levels and lifestyle behaviors.

Female subjects were not included in the study sample. We concluded that it would be difficult to obtain results with sufficient statistical power comparing the results between males and
females due to the fact that, as shown in Figure 1, the number of females was smaller than that of males in the recruitment of study subjects. In future studies, we intend approach the subject from different perspectives by extending the observation period and increasing the number of female subjects.

In our preceding study involving Japanese males aged 50 years old or over whose fish intake levels were higher than those of the subjects of the present study, we demonstrated that higher frequencies of fish intake were associated with lower serum non-HDL-C levels (an indicator of lipid metabolism in general) and more favorable lifestyle behaviors. In the present study, the evidence for the relationships among the fish intake frequency, markers of atherosclerotic risk, and lifestyle behaviors, revealed in our preceding study, was markedly reinforced by the inclusion also of younger individuals with lower frequencies of fish intake as the subjects in the study. Japan ranks highest in the world in terms of the average fish consumption. On the basis of the evidence accumulated until date, the American Heart Association (AHA) Scientific Statement recommends fish intake at least once or twice a week for the prevention of atherosclerotic cardiovascular disease. The mean weekly frequency of fish intake in the present study of 2.3 ± 1.3 times/week was higher than the AHA-recommended frequency. However, based on the evidence accumulated until date, it remains unclear if the relationships observed between fish intake and improvement in the serum lipid profile (including the relationship between lifestyle behaviors and fish intake) are specific to the Japanese population or are applicable more extensively also to other ethnic groups.

As shown in Table 1, the blood HbA1c level and blood pressure increased and the e-GFR decreased as the weekly frequency of fish intake increases. Age is a confounding factor in this phenomenon. In other words, it is assumed that the weekly frequency of fish intake increases with increasing age, and advancing age is well known to lead to an increase in the prevalence of diabetes mellitus and hypertension and decrease in the e-GFR. Furthermore, we performed multiple stepwise regression analyses using the above as dependent variables and age and weekly frequency of fish intake as independent variables. While the weekly frequency of fish intake was not identified as a significant independent determinant, age was identified as a significant independent determinant of the above-mentioned variables.

Study limitations

First, information on the total intake of calories, especially intake of saturated fatty acids, which is a major cause of lipid metabolism disorder, was not available for this study. Second, it is known that the content of polyunsaturated fatty acids varies depending on the type of fish. The type and amount of fish, especially oily fish, was not considered in the analysis in this study. Thirdly, females were not enrolled to this study because the number of female candidates not falling under any of the exclusion criteria was small, raising the possibility that inclusion of females, as compared to enrollment of males alone, could lead to a reduction of the statistical power of the study. Forth, since this study was designed as a cross-sectional study, therefore, analysis to endorse the causality for each outcome is difficult to perform. If it is possible to quantify the overall lifestyle in some way with the use of a prospective cohort study design and to attempt correlating the quantified lifestyle to the quantity of fish consumed, we may expect that the significance of the standard partial regression coefficient (β) of the frequency of fish intake (an independent variable of multivariate analysis) as a factor affecting the serum L/H ratio will be larger through combination with the high quality dietary lifestyle focused on fish intake. Finally, we should have prepared a detailed, internationally standardized questionnaire or used standard variables for international comparisons (lifestyle, dietary habits, and physical activity).

Clinical Implications

Most previous studies conducted to determine the association of fish intake with the risk factors for ASCVD focused primarily on the amount of fish intake. The present study, on the other hand, demonstrated that it was the improvement in the lifestyle behaviors triggered by frequent fish intake that was associated with the reduction in the L/H ratio (a risk factor for ASCVD). Numerous large-scale clinical studies reported until date have demonstrated the effects of n-3PUFAs in suppressing cardiovascular events. Now, we speculate that a high frequency of fish intake may lead to the suppression of cardiovascular events through a combination of two factors, i.e., (1) the beneficial effects of the n-3PUFAs contained in fish and (2) the beneficial effect of improvement of the lifestyle of individuals (a basic aspect of prevention of ASCVD) through the adoption of a fish intake-centered dietary style.

Conclusion

These results suggest that higher frequency of fish intake may be one of several components constituting a healthy lifestyle associated with lower L/H ratio and a lower risk of CAD in Japanese males without lipid-modifying therapy.

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The authors state that they have no Conflict of Interest (COI).

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