Associations Among Daily Fish Intake, Monocyte/HDL-C Ratio, and Lifestyle in Japanese Males Aged >50: Risk Stratification of Atherosclerotic Cardiovascular Disease

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Research Article

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Abstract

Background: The monocyte/high-density lipoprotein cholesterol (HDL-C) ratio (MHR) may be a novel inflammatory marker of the developing atherosclerotic cardiovascular disease (ASCVD). We investigated the relationship between the average number of days of fish intake per week and the MHR and lifestyle behaviors and to explore the validity of stratifying the risk of ASCVD using the combination of MHR and the serum HDL-C level.

Methods: This cross-sectional study was conducted in a population of 2485 males aged over 50 years at the Health Planning Center of Nihon University Hospital between April 2018 and March 2019.

Results: The average frequency of fish intake was 2.32 ± 1.31 per week. Multiple stepwise regression analysis identified increased weekly fish frequency intake as an independent determinant of a decreased MHR ($\beta = -0.072$, $p < 0.0001$). Healthier lifestyle behaviors were also significantly associated with decreased MHR. As the fish intake frequency increased, the proportion of subjects with the cigarette smoking habit decreased ($p = 0.014$), that of subjects with aerobic exercise habit increased ($p < 0.0001$), and that of subjects with alcohol drinking habit increased ($p < 0.0001$). A risk stratification or ASCVD by combining the HDL-C level and fish intake frequency with the MHR could be developed, indicating that even with similar HDL-C levels, higher HMR and fish frequency are associated with higher risk ASCVD.

Conclusion: A high fish intake frequency may be associated with healthier lifestyle behaviors as well as a lower MHR, and may thus represent a component of a healthy lifestyle associated with a lower risk of ASCVD in Japanese males aged over 50 years. These associations may be related to being the preventive effect of fish intake on ASCVD.

Clinical Trial Registration: UMIN (http://www.umin.ac.jp/) Study ID: UMIN 000041368 registered 10/08/2020

Background

Several prospective cohort studies have demonstrated a negative correlation between the amount of fish intake and the incidence of coronary artery disease (CAD) [1, 2]. A recent science advisory review of the American Heart Association recommended fish intake, especially oily fish, at least once or twice weekly for the prevention of CAD, ischemic cerebrovascular disease, and sudden cardiac death [3].

High-density lipoprotein cholesterol (HDL-C) exerts its anti-atherosclerotic effects by neutralizing the pro-inflammatory and pro-oxidant effects of monocytes. Specifically, HDL-C inhibits the migration of macrophages and low-density lipoprotein (LDL) oxidation, in addition to causing an efflux of cholesterol from atherosclerotic plaques on the arterial wall. Thus, the accumulation of monocytes and reduction in HDL-C levels may be involved in the onset of atherosclerotic cardiovascular diseases (ASCVD) [4]. The existence of a relationship between a high number of monocytes and low HDL-C levels has been reported in inflammatory disorders leading to the development of ASCVD [5–7]. Therefore, the monocyte-to-HDL-C ratio (MHR) may be a more convenient-to-measure marker to accurately predict the risk of developing ASCVD compared to the monocyte count or the serum HDL-C level alone. Indeed, several epidemiologic studies have observed a positive correlation between the MHR, a marker of systemic inflammation, and the incidence of ASCVD [8].

Furthermore, improved lifestyle behaviors, including healthy dietary habits, are closely associated with a reduced risk of developing ASCVD [9]. In particular, diets focused on frequent fish intake, such as the Japanese dietary style (i.e., the JAPAN DIET), which emphasizes frequent fish intake, are useful for reducing the risk of developing ASCVD [10]. However, to date, few studies have assessed the relationship between the frequency of fish intake and the MHR, which is a putative predictor of the development of ASCVD.

We hypothesized that a higher frequency of fish intake along with healthier lifestyle behaviors may be associated with lower MHR, and that measuring the MHR plus serum HDL-C levels may allow a more detailed assessment of the risk of ASCVD than measuring serum HDL-C levels alone. Therefore, the purpose of the present cross-sectional study was to investigate the relationship between the average number of days of fish intake per week, the MHR, and lifestyle behaviors as well as to explore the validity of stratifying the risk of ASCVD using a combination of the MHR and serum HDL-C levels in Japanese males aged over 50 years.

Methods

Study Design and Study Populations

Among 11 379 Japanese subjects who had undergone annual health check-ups between April 2018 and March 2019 at the Health Planning Center of Nihon University Hospital, we recruited for this study 2485 males.

The primary endpoints were the associations of the frequency of fish intake with MHR and lifestyle behaviors. The secondary endpoint was the risk of ASCVD stratified by combining the MHR with serum HDL-C levels and fish intake frequency.

This study was conducted following the ethical principles of the Declaration of Helsinki for Medical Research Involving Human Subjects. The study protocol was approved by the Nihon University Hospital Ethics Committee (approved number: 20200405), and written informed consent was obtained from all study participants.

Questionnaire of Health Behaviors

We obtained data on subjects' health behaviors using the same self-reported questionnaire as in previous studies [11, 12]. The surveys also consisted of overall questions designed to assess subjects' socioeconomic characteristics (i.e., age, occupation, marital status, previous and current medical history, and family history). We were, therefore, able to receive appropriate responses to the following questions from the subjects.

1) Cigarette smoking habit Do you smoke habitually?: No/Yes

2) Alcohol drinking habit: Please enter the frequency of your drinking alcohol: Every day/sometimes/I cannot drink; On how many days of the week do you drink alcohol? Please answer the volume of alcohol consumed on a typical drinking day (ethanol equivalent [g/day]): <20 g/20 to <40 g/40 to <60 g/≥60 g

3) Aerobic exercise habit: Have you been exercising (for 30 minutes or more, until you sweat lightly) on two days or more of the week for one year or longer?: No/Yes

4) Fish intake habit: How many days, on average, per week have you eaten fish in the past one month? Fish intake was evaluated in weekly frequencies in the questionnaire (0, 1, 2, 3, 4, 5, 6, and 7 days).

The questionnaires were modified versions of the questionnaire on specified health examinations [13] used for specific health guidance following health examinations under the Ministry of Health, Labour and Welfare (MHLW) in Japan. The questionnaire is an excerpt from the actual questionnaire (Supplement 1) of our institution that includes questions related to this study.

We also verified the consistency of the relationship between the frequency of fish intake as determined from the responses to the questionnaire and the amount of fish intake estimated by the National Nutrition Survey conducted by the MHLW of Japan among our study subjects **(Supplemental Table 2 and Supplemental Figure 1)**.

Examinations of Health Check and Laboratory Profiles

We measured anthropometric variables (height, weight, and waist circumference) with the subjects in the standing position. Waist circumference was measured at the umbilicus level using a non-stretchable tape measure during the late exhalation phase with the subject in the standing position [14]. Fasting blood samples were collected in the early morning after the subjects had fasted for 8 hours. Total serum cholesterol (TC) and triglyceride (TG) levels were measured using enzymatic methods. Serum HDL-C level was measured using an enzymatic method following heparin and calcium precipitation. Serum LDL-C level was calculated by the Friedewald formula [15]. The white blood cell and differential white blood cell counts were determined using a Beckman Coulter STKS (Beckman Coulter Inc, Fullerton, California, USA).

Statistical Analysis

Data are expressed as the means ± standard deviation (SD) for continuous variables and as percentages for discrete variables. A subset analysis for comparison between continuous variables was performed using a one-way analysis of variance (ANOVA) or the Kruskal–Wallis analysis of variance when assumptions of normality of the distribution or homogeneity of variances were not verified. Tukey–Kramer's adjustment and Bonferroni correction were applied as post-hoc analysis for covariates if differences were detected in patient characteristics or laboratory profiles, and the chi-square test was used to compare categorical variables. A multiple stepwise regression analysis was performed with the MHR as the dependent variable and age, waist circumference, history of treatment for hypertension, diabetes mellitus, and/or hyperuricemia, and lifestyle behaviors (frequency of fish intake [average days of fish intake per week], cigarette smoking habit [yes/no], aerobic exercise habit [yes/no], and alcohol intake habit [g/week]) (model 1). Waist circumference, hypertension, diabetes mellitus, and hyperuricemia were involved in developing ASCVD associated with MHR. Therefore, we performed a multiple stepwise analysis using the above factors as independent variables. Similarly, multiple stepwise regression analyses were performed using blood monocyte count (model 2) and serum HDL-C level (model 3) as dependent variables. A two-way ANOVA was used to confirm the effect of the interaction between the fish intake frequency and lifestyle behaviors on the MHR, monocyte count, and serum HDL-C level. A regression analysis was performed using a linear regression model, estimating Pearson's correlation coefficients. We conducted all statistical analyses using the SPSS software (SPSS Inc., Chicago, Illinois, USA) for Windows (version 24).

Results

Subjects

The average frequency of fish intake was 2.32 ± 1.31 days per week. Figure 2 shows the weekly frequencies of fish intake. The subject characteristics and laboratory profiles according to the MHR tertiles are shown in **Table 1**.

Comparison of the MHR and Lifestyle Behaviors According to the Weekly Frequency of Fish Intake

The subjects' MHR values were compared according to the weekly frequency of fish intake as categorical variables (0–1 day, 2–3 days, 4–5 days, or 6–7 days per week). The MHR was decreased significantly as the weekly frequency of fish intake was increased **(Figure 3-1)**.

As the weekly frequency of fish intake was increased, the proportion of subjects with habitual cigarette smoking was decreased significantly and the proportion of those with an aerobic exercise habit was increased significantly. The weekly frequency of alcohol intake was also increased significantly with an increased weekly frequency of fish intake **(Figure 3-2)** (Figures with *p*-values are included).

Comparison of the Subject Characteristics with Cardiometabolic Risk According to the MHR

The body mass index, waist circumference, and proportion of subjects with metabolic syndrome was increased significantly as the MHR tertile was increased. The blood HbA1c levels were also increased significantly as the MHR tertile was increased. The serum LDL-C, TG, and non-HDL-C levels were increased significantly as the MHR tertile was increased. The serum HDL-C level was decreased significantly as the MHR tertile was increased. As the MHR tertile was increased, the proportions of subjects receiving treatment for hypertension and diabetes mellitus were increased significantly, while the proportions of those receiving treatment for hyperuricemia remained unchanged. Thus, these findings suggest that a higher MHR was associated with a worse cardiometabolic risk **(Table 1)**. As the MHR tertile was increased, the WBC count, neutrophil count, monocyte count, and CRP level were increased significantly, while the blood lymphocyte count showed no significant change. The weekly frequency of fish intake was decreased significantly as the MHR tertile was increased. As the MHR tertile was increased, the proportion of subjects with the cigarette smoking habit was also increased significantly, while that of subjects with an aerobic exercise habit and alcohol intake habit was decreased significantly (Table 1 with *p*-values included).

Multiple Stepwise Regression Analysis to Identify Factors Influencing the MHR, Monocyte Count, and Serum HDL-C level

As shown in **Table 2**, in model 1, the multiple stepwise regression analysis identified the weekly frequency of fish intake as a negative independent determinant of the MHR. Age, waist circumference, and history of treatment for diabetes mellitus were significant positive independent determinants of the MHR. On the other hand, cigarette smoking habit, aerobic exercise habit, and alcohol intake habit were also significant independent determinants of the MHR.

However, as shown in **Figure 3-2**, there was a significant correlation between the frequency of fish intake and lifestyle behaviors (cigarette smoking habit, aerobic exercise habit, and alcohol intake habit); therefore, they could confound the association between the frequency of fish intake and the MHR ratio. A two-way ANOVA revealed the absence of an interaction between the weekly frequency of fish intake and the above-mentioned lifestyle behaviors in regard to the relationship with the MHR ratio (p-value for interaction = 0.332, 0.072, and 0.185). These analyses indicated that lifestyle behaviors, as well as a higher frequency of fish intake, were independent determinants of the MHR. This result verified the primary hypothesis of this study.

In model 2, the weekly frequency of fish intake was identified as a negative independent determinant of the MHR. Age, waist circumference, cigarette smoking habit, and history of treatment for diabetes mellitus were found to be significant positive independent determinants of the blood monocyte count. In model 3, the weekly frequency of fish intake was identified as an independent positive determinant of the serum HDL-C level. Waist circumference and cigarette smoking habit were negative independent determinants of the serum HDL-C level. Both aerobic exercise habit and alcohol intake habit were independent positive determinants of the serum HDL-C level (Table 2 with β-coefficient and p-values included).

These results suggest that a high frequency of fish intake may be an independent predictor of a lower blood monocyte count and higher serum HDL-C, and therefore, a lower MHR. **Figure 4** shows the relationship between fish intake frequency and monocyte count, serum HDL-C level, and MHR derived from these analyses.

A Risk Stratification for ASCVD by Combining the Serum HDL-C Level with the MHR and Fish Intake Frequency

Figure 5 shows the relationship between the MHR and serum HDL-C levels (left). The vertical axis represents the MHR; the higher the MHR was, the worse the cardiometabolic risk became, as shown in Table 1, and the lower the MHR was, the lower the cardiometabolic risk became. Individual cases with the same serum HDL-C levels had different MHR values (right), and thus the risk of ASCVD could not be precisely determined from the absolute serum HDL-C level alone. The functions of HDL may be approximately deduced from this model. Figure 5 shows that there are different HMRs depending on the number of monocytes in cases with the same serum HDL-C level. Therefore, the HMR provides a risk stratification for ASCVD based on serum HDL-C levels.

Discussion

This study yielded the following results: 1) Aggressive fish consumption, per se, as well as better lifestyle behaviors noted in individuals with fish-based eating habits, were associated with lower MHR values; the two may act synergistically to reduce the risk of developing ASCVD; and 2) Combining MHR values and serum HDL-C levels along with fish intake frequency may allow for stratification of the risk of ASCVD.

Several epidemiological studies have reported that the preventive effect of fish intake on CAD development was due to the cardioprotective effect of the n-3 PUFAs that are abundant in oily fishes [16]. However, fish-eating habits is also associated with healthier lifestyle behaviors [17, 18]. This study showed that lifestyle behaviors such as habitual aerobic exercise, non-smoking, and habitual alcohol intake (in this case, the amount of alcohol consumed was moderate, and it should be noted that excessive alcohol drinking is hazardous to health), were associated with lower MHR values. Habitual aerobic exercise and habitual alcohol intake have been reported to be associated with increased serum HDL-C levels [19–21]. Furthermore, non-smokers have a lower risk of developing atherosclerosis and higher serum HDL-C levels than smokers [21]. Non-smokers are also known to have lower MHR values than smokers [22]. Therefore, the ability of daily fish intake to inhibit the development of atherosclerosis and reduce the risk of developing ASCVD may be synergistically mediated by the physiological activities of n-3 PUFAs abundantly found in fish and the healthy lifestyle behaviors associated with high fish intake.

The relationship between serum HDL-C levels and MHR is not one-to-one, but multiple MHRs correspond to individual serum HDL-C levels, indicating a heterogeneous combinations of MHRs with a higher risk of ASCVD and MHRs with a lower risk of ASCVD. These results suggest that individuals with the same serum HDL-C level may have different MHR values and therefore different risk levels for developing ASCVD. Therefore, the combination of monocyte counts and serum HDL-C levels may be useful for predicting HDL function (i.e., prediction of functional or dysfunctional HDL) [23–25]. Furthermore, adding fish intake frequency to this risk stratification model revealed an association between lower ASCVD risk and higher fish intake (Fig. 5).

The "HDL-C hypothesis", which suggests that an increase in serum HDL-C levels is associated with an attenuated risk of CAD, has long gained traction [26]. However, a recent study reported that serum HDL-C levels did not contribute to the development of CAD using a Mendelian randomization analysis [27]. Furthermore, the suppression of CAD by HDL-C has been associated, to a greater degree, with the cholesterol efflux capacity of HDL than with the absolute serum HDL-C level [28]. Therefore, more recent efforts to reduce the risk of developing atherosclerosis have focused on improving HDL functions rather than increasing absolute serum HDL-C levels [29]. In recent observational and interventional studies, we have demonstrated that n-3 PUFAs may alter HDL heterogeneity leading to improved HDL function [30–32]. Therefore, we speculate that a higher frequency of fish intake improves HDL functions due to the high content of n-3 PUFAs in fish.

As shown in Table 2, a higher frequency of fish intake was also an independent determinant of a higher serum HDL-C level and lower blood monocyte count. Consistent with this observation, several epidemiological studies have reported both as being prognostic factors of CAD [8]. Given the association of serum HDL-C levels and monocyte count with the development of atherosclerosis, we thought that the ratio between the two parameters might be a better predictor of the development of ASCVD. Therefore, we suggest that the MHR, which can be easily calculated in clinical practice, may be a useful index for assessing the risk of developing ASCVD.

Unfortunately, there were no data on the serum n-3 PUFA levels in this study. If we had measured the serum levels of n-3 PUFAs, more detailed results could have been obtained. We previously showed that a dietary habit with a higher frequency of fish intake might involve a healthier lifestyle and a better serum lipid profile [11, 12]. We consider that the improvement of the serum lipid profile with a high frequency of fish intake is mediated by the effect of n-3 PUFAs that are abundantly found in fish, especially oily fish, and a better lifestyle. Hence, a positive correlation may exist between the frequency of fish intake and the serum n-3 PUFAs level. Indeed, Wennberg et al. reported that the correlation coefficient between the frequency of fish intake estimated by the Food Frequency Questionnaire and the fatty acid level in the erythrocyte membrane was ranging between 0.42–0.51 [33]. Thus, we assumed that the index of frequency of fish intake used in this study, even though not precise, was an approximate true reflection of the frequency of fish intake by the study subjects. We carried out our investigations based on the above-described rationales.

This study showed that alcohol intake was a statistically negative independent determinant of the MHR. Moderate alcohol drinking habits (especially red wine consumption) have been reported to inhibit atherosclerosis progression [34]. However, excessive alcohol drinking causes health problems. When the correlation curve between alcohol intake and MHR was examined in detail, the correlation curve flattened when alcohol intake increased to a certain threshold and then showed a gradual positive slope in the group that consumed large amounts of alcohol **(Supplemental Fig. 2)**. Thus, excessive alcohol consumption may cause an increase in MHR and a decrease in HDL function. Importantly, an increase in the monocyte number is associated with promoting alcoholic liver disease [35], and this phenomenon may increase MHR.

Study limitations and clinical Implications

First, since this was a cross-sectional study, no causal associations could be established. Second, the contents of PUFAs in fish vary depending on the type of fish. The types and amounts of fish included in the diet, especially the amount of oily fish intake known to be high in PUFA content, were not considered in this study. Third, we should evaluate HDL efflux capacity and HDL fraction to support the hypothesis that MHR is an index of HDL-C function. Finally, the results of this study suggest that it might be possible to use the combination of serum HDL-C plus MHR to assess the risk of developing ASCVD. Therefore, we propose using this risk assessment tool in a future prospective cohort study to establish appropriate cutoff values for HMR and serum HDL-C levels to determine the risk of developing ASCVD. Moreover, the accuracy of the prediction model of CAD may be improved by incorporating HMR into the Suita Score [36].

Conclusions

The results of this study revealed that beneficial lifestyle behaviors, including habitual aerobic exercise and non-smoking as well as a higher frequency of fish intake, were associated with a lower MHR, which is known to be a potential systemic inflammatory marker of the risk of ASCVD. This association may be partially related to the preventive effects of a higher frequency of fish intake on ASCVD events in Japanese males aged over 50 years. Furthermore, the combination of HMR and serum HDL-C levels may be useful for risk assessment of ASCVD, focusing on serum HDL-C levels and fish intake frequency.

Abbreviations

ASCVD, atherosclerotic cardiovascular disease; ANOVA, analysis of variance; CAD, coronary artery disease; CRP, C-reactive protein; Hb, hemoglobin; HDL, high-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; MHR, monocyte/HDL-C ratio; MHLW, Ministry of Health, Labour and Welfare; PUFA, polyunsaturated fatty acid;

SD, standard deviation, TC, total cholesterol; TG, triglyceride.

Declarations

- **Ethical approval and consent to participate**

This study was approved by the Institutional Ethics Committees of Nihon University Hospital. All participants were consentient to participate the study and signed the informed consent.

- **Consent for publication**

Not Applicable

- **Availability of data and materials**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

- **Competing interests**

The authors declare no competing interests.

- **Funding**

No funding was obtained for this study

- **Authors' contributions**

ST has designed this study as a whole and drafted this manuscript. WA, TY, KI, and YS have contributed to collect data. ST and NM have contributed to statistical analyses in this study. ST and YO have contributed to providing advice on the interpretation of the results. ST revised this manuscript critically for important intellectual content and approved finally the manuscript submitted. All authors read and approved the final manuscript.

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Tables

Due to technical limitations, table 1-2 is only available as a download in the Supplemental Files section.

Figures

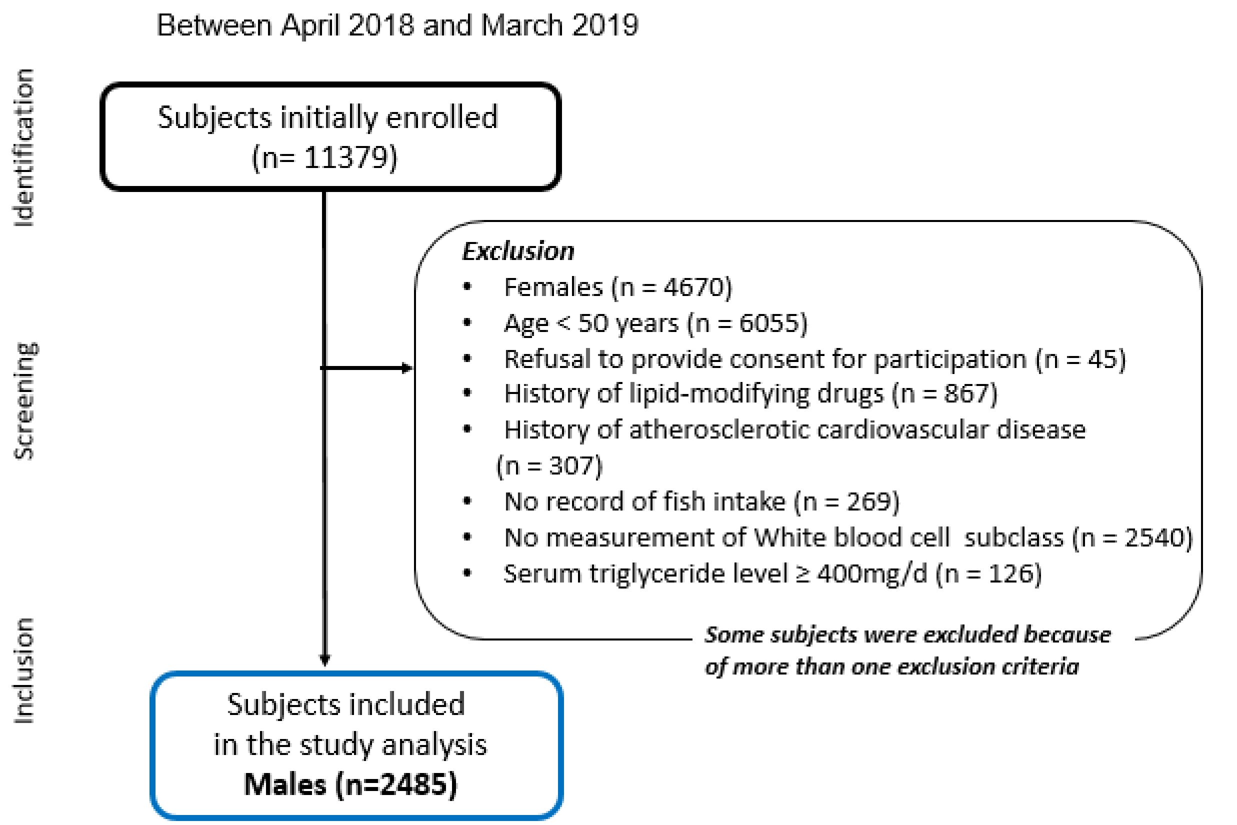

Figure 1

The Flow Diagram of the Study Participants

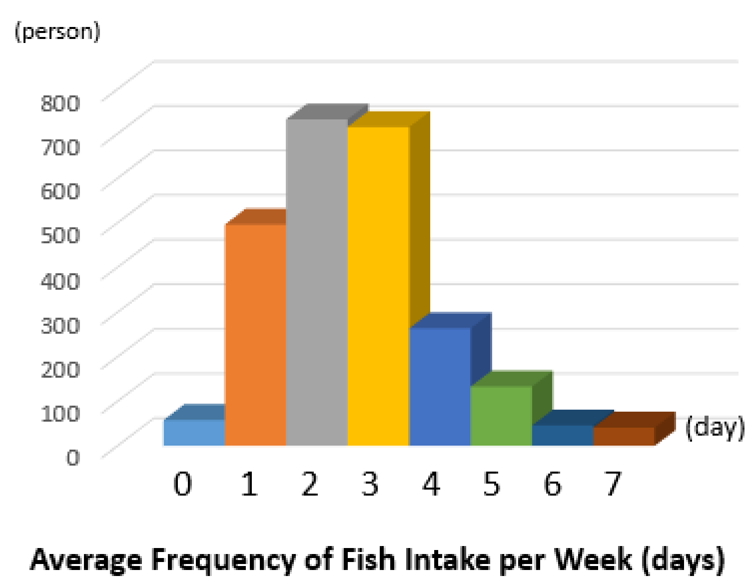

Figure 2

Frequency of Daily Fish Consumption

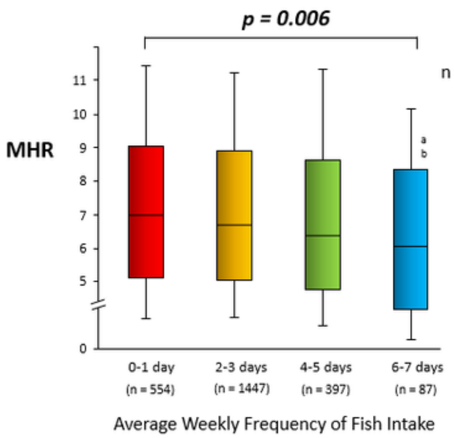

Figure 3-1

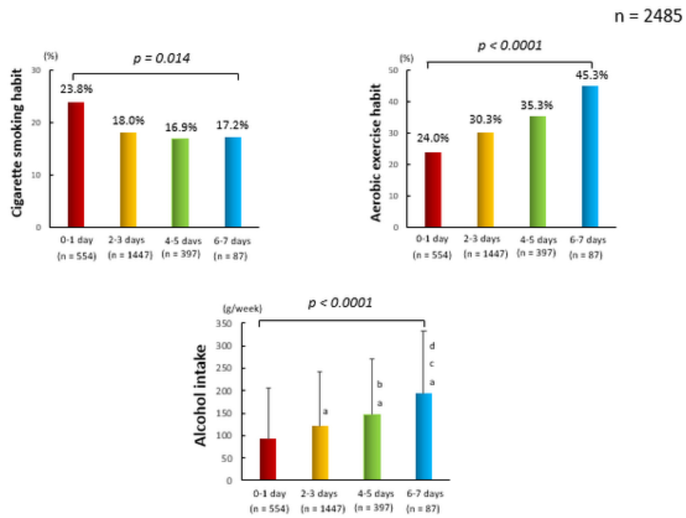

Figure 3-2

Figure 3

(1) Comparison of the MHR According to the Weekly Frequency of Fish Intake Caption: MHR = monocyte /HDL-C ratio Kruskal-Wallis test and post hoc tests with Bonferroni correction were performed to test between-group differences. a $p < 0.05$ vs. 0-1 day b $p < 0.05$ vs 2-3 days (2) Lifestyle Behaviors According to the Weekly Frequency of Fish Intake Caption: ANOVA and post hoc tests with Turkey-Kramer correction were performed to test between-group differences. a $p < 0.0001$ vs. 0-1 day b $p < 0.01$, c $p < 0.0001$ vs. 2-3 days d $p < 0.05$ vs. 4-5 days

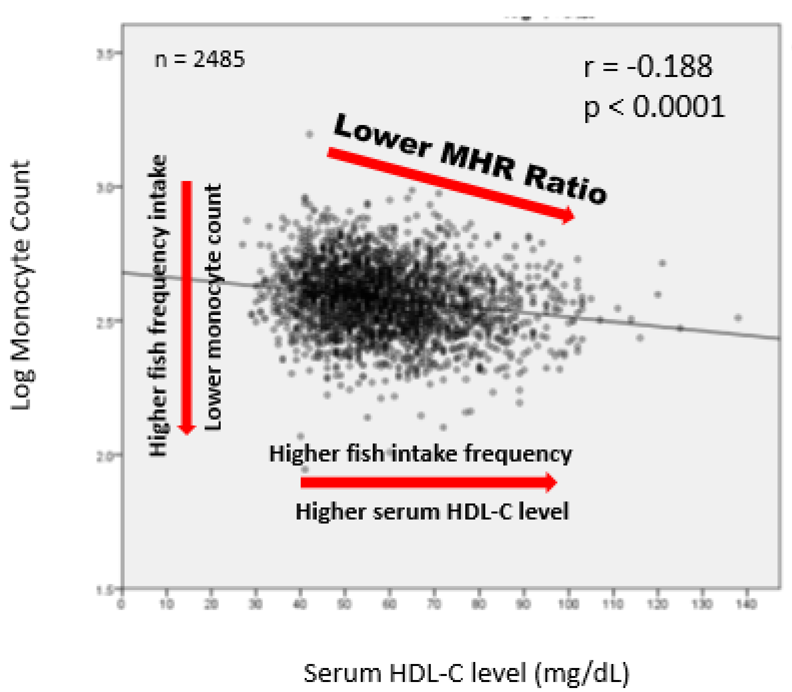

Figure 4

Association between MHR, Serum HDL-C Level, and Fish Intake Frequency Caption: HDL = high-density lipoprotein; MHR = monocyte/HDL-C ratio

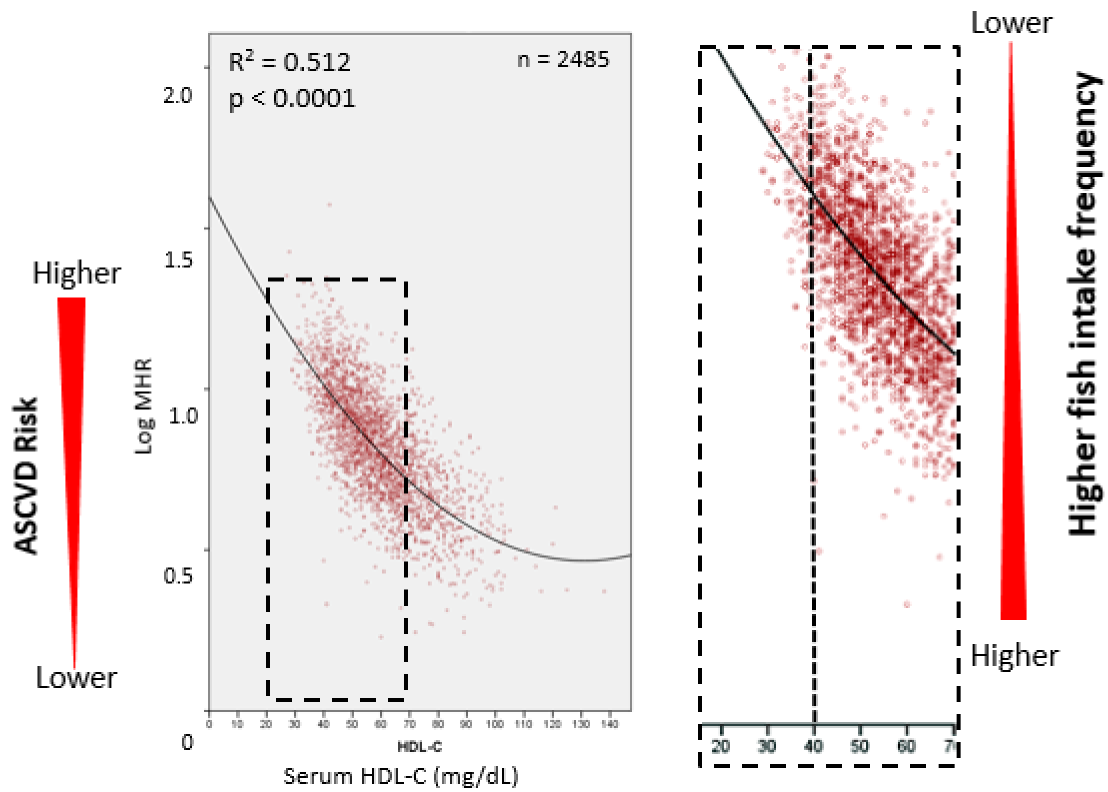

Figure 5

A Risk Stratification for ASCVD by Combining the Serum HDL-C Level with the MHR and Fish Intake Frequency Caption: The relationship between log MHR and serum HDL-C level was shown by a quadratic curve.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalFigure.docx
- SupplementalFigure.docx
- SupplementalTable1.pdf
- SupplementalTable2.pdf
- Tables.pdf