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Habitual fish consumption and masticatory difficulties in Japanese adults: a cross-sectional study of lifestyle characteristics and systemic inflammation

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Abstract

Masticatory difficulties reflect impaired oral function and may be associated with dietary habits, lifestyle characteristics, and systemic health. However, little is known about their relationship with habitual fish consumption. We examined the association between fish consumption frequency and masticatory difficulties in Japanese adults undergoing routine health examinations and explored accompanying lifestyle characteristics and the potential contribution of systemic inflammation. This cross-sectional study included 10,572 participants (mean age, 48.0 ± 13.0 years; 56.7% men) examined between April 2024 and March 2025. Fish consumption frequency was self-reported as days per week (0–7 days/week), and masticatory difficulties were assessed using a self-administered questionnaire. Associations were examined using multivariable logistic regression and restricted cubic spline analyses adjusted for demographic, metabolic, and lifestyle factors. The indirect effect through log-transformed white blood cell (WBC) count was assessed via exploratory mediation analysis. Each 1-day/week increase in fish consumption frequency was associated with lower odds of masticatory difficulties (adjusted odds ratio, 0.908; 95% confidence interval, 0.857–0.962; P = 0.001), with no evidence of non-linearity (P for non-linearity

= 0.673). Higher fish consumption frequency also clustered with regular aerobic exercise, longer sleep duration, and lower current smoking prevalence. The indirect effect through log-transformed WBC count was small (−0.0014; 95% bootstrap confidence interval, −0.0035 to 0.0000), providing limited evidence for an indirect contribution of systemic inflammation. More frequent fish consumption was associated with a lower prevalence of masticatory difficulties within a broader pattern of favorable lifestyle characteristics. Prospective studies are needed to clarify temporality and underlying mechanisms.

Keywords: masticatory difficulties; oral function; fish consumption; lifestyle characteristics; systemic inflammation

Introduction

Masticatory difficulties are an important manifestation of impaired oral function and may arise from diverse oral conditions, including tooth loss, periodontal disease, reduced occlusal support, and oral hypofunction [1–3]. Self-reported chewing difficulty is also an indicator of oral frailty [4, 5]. Impaired mastication may influence food selection and nutritional intake [6, 7], while periodontal disease is associated with systemic inflammation and metabolic disorders [8, 9]. As such, diet, oral health, systemic health, and masticatory function may have a bidirectional relationship.

Fish is an important dietary source of long-chain n-3 polyunsaturated fatty acids (PUFAs). Higher fish consumption has been associated with favorable cardiovascular health outcomes [10], and n-3 PUFAs may exert beneficial biological effects through modulation of inflammation, lipid metabolism [11], and endothelial function [12]. However, despite extensive research on the systemic health effects of fish consumption, relatively little is known about its relationship with oral functional status. In particular, whether habitual fish consumption frequency is associated with masticatory difficulties has not been well established.

Individuals who consume fish more frequently tend to exhibit favorable lifestyle characteristics, including greater physical activity and lower smoking prevalence [13, 14]. Because lifestyle behaviors are closely related to chronic low-grade systemic inflammation [15], an association between fish consumption and oral function may reflect not only dietary exposure itself but also accompanying lifestyle characteristics. Circulating white blood cell (WBC) count is a readily available marker of systemic inflammatory burden [16] and may provide insight into whether systemic inflammation contributes to the association between fish consumption and masticatory difficulties.

Accordingly, we investigated the association between habitual fish consumption frequency and masticatory difficulties in Japanese adults undergoing routine health examinations. We additionally examined the relationship between fish consumption frequency and major lifestyle characteristics and explored the potential indirect contribution of systemic inflammation assessed using WBC count. We hypothesized that more frequent fish consumption would be associated with a lower prevalence of masticatory difficulties within a broader pattern of favorable lifestyle characteristics, while any contribution of WBC-related systemic inflammation would be exploratory.

Materials and Methods

Study design and participants

This cross-sectional study included Japanese adults who underwent annual health examinations at the Health Planning Center of Nihon University Hospital between April 2024 and March 2025. Of the 11,724 individuals who underwent health examinations during the study period, 1,152 were excluded because of missing data for various items: 742 for masticatory difficulties; 291 for fish consumption frequency; 102 for prespecified covariates, and 17 for WBC count. The final analytic population comprised 10,572 participants with complete data for all variables required for the primary and exploratory analyses **(Figure 1)**.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Nihon University Hospital (Approval No. 20200405). The requirement for written informed consent was waived because of the retrospective use of anonymized health check-up data, and an opt-out approach was employed.

Assessment of fish consumption frequency

Fish consumption frequency was assessed using a self-administered questionnaire. Participants reported the number of days per week on which they habitually consumed fish, ranging from 0 to 7 days/week. For the primary analysis, fish consumption frequency was modeled as a continuous variable to estimate the association per 1-day/week increase. It was also analyzed as an eight-level categorical variable; the modeling approach is described under Statistical analysis.

Assessment of masticatory difficulties

Masticatory difficulties were assessed using the standardized self-administered questionnaire employed in the Japanese National Specific Health Checkups and Health Guidance Program developed by the Ministry of Health, Labour and Welfare (MHLW) [17], as described previously in our study [18]. Participants were asked to select the statement that best described their usual chewing ability from the following options: (1) "I can chew and eat any food," (2) "I can hardly chew food," or (3) "I sometimes have difficulty chewing food because of problems with the teeth, gums, occlusion, or similar issues." Participants selecting option (2) or (3) were classified as having self-reported masticatory difficulties, whereas those selecting option (1) were classified as not having

masticatory difficulties. The same questionnaire item has been used in previous population-based studies to assess self-reported chewing ability and oral functional status [19, 20]. Because this assessment reflects perceived chewing ability rather than objectively measured masticatory performance, masticatory difficulties in the present study should be interpreted as perceived oral functional impairment rather than objectively confirmed masticatory dysfunction [7, 18].

Assessment of lifestyle factors and covariates

Lifestyle characteristics were assessed using the same self-administered questionnaire, based on the Questionnaire on Specific Health Examination of the MHLW [17]. Smoking status was assessed by asking participants whether they currently smoked regularly, had never smoked, or had quit smoking; current smoking was treated as a binary variable (yes/no) in the present analysis. Alcohol consumption was assessed based on drinking frequency, the usual amount consumed per drinking occasion, and the number of drinking days per week, from which weekly ethanol intake (g/week) was estimated. Regular aerobic exercise was defined as engaging in exercise that induced slight sweating for at least 30 min per session, at least twice per week, and continuously for at least 1 year. Sleep duration was self-reported as the average number of hours slept

per day and was analyzed as a continuous variable.

Body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared. Diabetes mellitus was defined as fasting blood glucose ≥126 mg/dL, glycated hemoglobin ≥6.5%, or current use of antidiabetic medication.

White blood cell count

After an overnight fast of at least 8 h, venous blood samples were collected in the morning. WBC counts and leukocyte differential counts were determined using a Beckman Coulter STKS analyzer (Beckman Coulter Inc., Fullerton, CA, USA). Because WBC count was skewed, it was log-transformed before the regression and mediation analyses. Log-transformed WBC count was used as an indicator of systemic inflammatory burden.

Statistical analyses

No a priori sample size calculation was performed because this was an exploratory analysis of an existing large health-check-up cohort, and all participants meeting the eligibility criteria were included. Continuous variables are presented as means with

161 standard deviations or medians with interquartile ranges, as appropriate, and categorical
162 variables as numbers and percentages. For descriptive analyses of participant
163 characteristics, fish consumption frequency was categorized as 0, 1, 2–3, and 4–7
164 days/week. These categories were used to facilitate descriptive presentation while
165 avoiding unstable estimates in groups with relatively small numbers of participants.
166 Trends across increasing categories of fish consumption frequency were assessed using
167 the Jonckheere–Terpstra trend test for continuous variables and the Cochran–Armitage
168 trend test for categorical variables.
169
170 Multivariable logistic regression analysis was performed to examine the association
171 between fish consumption frequency and masticatory difficulties. In the primary
172 analysis, fish consumption frequency was modeled as a continuous variable (0–7
173 days/week) to estimate the association per 1-day/week increase. Adjusted odds ratios
174 (ORs) and 95% confidence intervals (CIs) were calculated after adjustment for age, sex,
175 BMI, diabetes mellitus, sleep duration, regular aerobic exercise, alcohol consumption,
176 and current smoking. Fish consumption frequency was additionally modeled as an
177 eight-level categorical variable (0, 1, 2, 3, 4, 5, 6, and 7 days/week), with 0 days/week
178 as the reference category, to examine associations across individual consumption

frequencies.

To examine the shape of the association between fish consumption frequency and masticatory difficulties and assess potential departure from linearity, restricted cubic spline analysis was performed with fish consumption frequency modeled continuously. The spline model was adjusted for the same covariates as the primary logistic regression model. Departure from linearity was assessed by comparing the spline model with the corresponding linear model. Three degrees of freedom were used for the spline function.

Associations between fish consumption frequency and selected lifestyle characteristics

Associations between fish consumption frequency and selected lifestyle characteristics were also examined. Unadjusted trends in regular aerobic exercise and current smoking across increasing fish consumption frequency were evaluated using logistic regression analysis, with fish consumption frequency entered as a continuous variable, whereas the unadjusted trend in sleep duration was evaluated using linear regression analysis. Predicted trends and their 95% CIs were graphically displayed together with the observed values at each fish consumption frequency.

197

198 ***Exploratory mediation analysis***

199 An exploratory mediation analysis [21] was conducted to examine whether systemic
200 inflammation, represented by log-transformed WBC count, indirectly contributed to the
201 association between fish consumption frequency and masticatory difficulties. Mediation
202 analysis was performed using PROCESS macro version 4.2 (Model 4) for IBM SPSS
203 Statistics. Fish consumption frequency was entered as the exposure (X), log-
204 transformed WBC count as the mediator (M), and masticatory difficulties as the binary
205 outcome (Y). The mediation model was adjusted for age, sex, BMI, diabetes mellitus,
206 sleep duration, regular aerobic exercise, alcohol consumption, and current smoking. The
207 indirect effect of fish consumption frequency on masticatory difficulties through log-
208 transformed WBC count was estimated using 5,000 bootstrap samples, and statistical
209 significance was determined when the 95% bootstrap CI did not include zero. Direct
210 and indirect effects are reported on the log-odds scale. Because of the cross-sectional
211 design, the mediation analysis was considered exploratory and was not interpreted as
212 evidence of causal mediation.

213

214 As a sensitivity analysis, the mediation analysis was repeated after excluding

participants with WBC counts ≥9,000/μL to reduce the potential influence of acute infection or other inflammatory conditions on circulating WBC counts.

All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant. Primary statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Restricted cubic spline analyses were performed using R version 4.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Participant characteristics

A total of 10,572 participants were included in the final analytic population (Figure 1). The mean age was 48.0 ± 13.0 years, and 5,997 participants (56.7%) were men. Masticatory difficulties were reported by 888 participants (8.4%). For descriptive analyses, participants were categorized according to fish consumption frequency as 0 days/week (n = 506), 1 day/week (n = 3,105), 2–3 days/week (n = 5,761), and 4–7 days/week (n = 1,200). Participant characteristics according to fish consumption frequency are summarized in **Table 1**.

Several lifestyle characteristics differed across fish consumption categories. Participants reporting more frequent fish consumption were more likely to engage in regular aerobic exercise, tended to report longer sleep duration, and were less likely to be current smokers. The numbers of participants reporting fish consumption frequencies of 0, 1, 2, 3, 4, 5, 6, and 7 days/week were 506, 3,105, 3,333, 2,428, 651, 356, 85, and 108, respectively.

Multivariable-adjusted association between fish consumption frequency and masticatory difficulties

Multivariable logistic regression analysis showed an inverse association between fish consumption frequency and self-reported masticatory difficulties (**Table 2**). In the primary analysis, each 1-day/week increase in fish consumption frequency was associated with 9.2% lower odds of masticatory difficulties after adjustment for age, sex, BMI, diabetes mellitus, sleep duration, regular aerobic exercise, alcohol consumption, and current smoking (adjusted OR, 0.908; 95% CI, 0.857–0.962; P = 0.001). Estimates for all covariates included in the fully adjusted model are presented in **Online Resource 1.**

When fish consumption frequency was additionally modeled as an eight-level categorical variable (0–7 days/week), the overall association with masticatory difficulties was significant (overall $P = 0.003$). Using 0 days/week as the reference, the adjusted ORs (95% CIs) were 0.709 (0.523–0.961) for 1 day/week, 0.562 (0.413–0.764) for 2 days/week, 0.654 (0.478–0.894) for 3 days/week, 0.548 (0.367–0.818) for 4 days/week, 0.553 (0.342–0.896) for 5 days/week, 0.358 (0.138–0.928) for 6 days/week, and 0.322 (0.134–0.775) for 7 days/week.

Restricted cubic spline analysis

The dose–response shape of the association between fish consumption frequency and masticatory difficulties was further examined using restricted cubic spline analysis. The estimated odds of masticatory difficulties generally decreased with increasing fish consumption frequency. No evidence of departure from linearity (P for non-linearity = 0.673) was observed, indicating that the observed association was compatible with an approximately linear inverse pattern across the range of fish consumption frequency **(Figure 2)**.

Fish consumption frequency and lifestyle characteristics

Increasing fish consumption frequency was associated with a higher prevalence of regular aerobic exercise and longer sleep duration, whereas the prevalence of current smoking tended to decrease with increasing fish consumption frequency. Overall, more frequent fish consumption clustered with a more favorable lifestyle profile **(Figure 3)**.

Exploratory mediation analysis through WBC count

An exploratory mediation analysis was performed to examine whether systemic inflammation, represented by log-transformed WBC count, might indirectly contribute to the association between fish consumption frequency and masticatory difficulties **(Figure 4)**. In the complete analytic population (n = 10,572), higher fish consumption frequency was associated with lower log-transformed WBC count (path a: $\beta = -0.0046$, SE = 0.0019, $P = 0.016$). Higher log-transformed WBC count was, in turn, associated with greater odds of masticatory difficulties after adjustment for fish consumption frequency and covariates (path b: $\beta = 0.3094$, SE = 0.1462, $P = 0.034$). The direct association between fish consumption frequency and masticatory difficulties remained significant after inclusion of log-transformed WBC count (direct effect $c' = -0.0951$, SE = 0.0295, $P = 0.001$). The estimated indirect effect through log-transformed WBC count was small (−0.0014; BootSE = 0.0009; 95% bootstrap CI, −0.0035 to 0.0000),

287 providing only limited evidence for an indirect contribution of WBC-related systemic
288 inflammation.
289
290 In a sensitivity analysis restricted to participants with WBC counts <9,000/μL (n =
291 10,413), higher fish consumption frequency remained associated with lower log-
292 transformed WBC count (path a: β = −0.0046, SE = 0.0018, *P* = 0.012), and higher log-
293 transformed WBC count remained associated with greater odds of masticatory
294 difficulties (path b: β = 0.4006, SE = 0.1554, *P* = 0.010). The direct effect of fish
295 consumption frequency remained significant (c′ = −0.0947, SE = 0.0297, *P* = 0.001). In
296 this restricted population, the indirect effect through log-transformed WBC count
297 remained small, but its bootstrap 95% CI excluded zero (−0.0018; BootSE = 0.0011;
298 95% bootstrap CI, −0.0044 to −0.0002) (**Online Resource 2**).
299
300 **Discussion**
301 In this cross-sectional study of Japanese adults undergoing routine health examinations,
302 more frequent fish consumption was associated with a lower prevalence of self-reported
303 masticatory difficulties. The association was approximately linear across the observed
304 range of fish consumption frequency and remained significant after adjustment for

305 demographic, metabolic, and lifestyle factors. More frequent fish consumption also
306 clustered with favorable lifestyle characteristics, including regular aerobic exercise,
307 longer sleep duration, and lower current smoking prevalence. Exploratory mediation
308 analysis provided no clear evidence for an indirect contribution of WBC-related
309 systemic inflammation to the association between frequent fish consumption and a
310 lower prevalence of self-reported masticatory difficulties. Taken together, these findings
311 suggest that the observed association between fish consumption and perceived oral
312 functional impairment may reflect a broader interplay among dietary habits, oral
313 function, lifestyle characteristics, and systemic health rather than a single biological
314 pathway.
315
316 The inverse association between fish consumption frequency and masticatory
317 difficulties should be interpreted within the close and potentially bidirectional
318 relationship between oral function and dietary intake. Masticatory difficulties may arise
319 from multiple oral conditions, including tooth loss, periodontal disease, reduced
320 occlusal support, and oral hypofunction [1–3], and impaired mastication may, in turn,
321 influence food choice and nutrient intake [6, 7]. Therefore, more frequent fish
322 consumption should not necessarily be interpreted as directly contributing to better

masticatory function. An alternative explanation is that individuals with preserved chewing ability may be better able to maintain a varied diet that includes fish, whereas those with perceived chewing difficulties may modify their food choices according to texture, ease of chewing, or underlying dental conditions. Further, dietary and systemic health characteristics associated with habitual fish consumption may also coexist with better oral health. Because of the cross-sectional design, the temporal direction of the observed association could not be determined.

Fish consumption was also closely associated with lifestyle behaviors. Participants reporting more frequent fish consumption were more likely to engage in regular aerobic exercise, tended to sleep longer, and were less likely to be current smokers. These findings are consistent with previous observations that fish consumption clusters with favorable lifestyle characteristics [13, 14] and suggest that fish intake may serve not only as a dietary exposure but also as a marker of a broader health-related behavioral pattern. The inverse association between fish consumption frequency and masticatory difficulties persisted after simultaneous adjustment for these measured lifestyle factors, suggesting that the association was not fully accounted for by exercise, sleep duration, alcohol consumption, or smoking. However, residual confounding by unmeasured

341 dietary patterns, socioeconomic factors, dental care behaviors, and oral health status
342 remains possible.
343
344 Systemic inflammation was explored as a potential pathway linking fish consumption to
345 masticatory difficulties. Long-chain n-3 PUFAs have anti-inflammatory properties [11],
346 and a systematic review and meta-analysis suggested that adjunctive n-3 fatty acid
347 supplementation may improve clinical periodontal outcomes [22]. However, such
348 evidence cannot be directly extrapolated to habitual fish consumption or self-reported
349 masticatory function. In the present study, higher fish consumption frequency was
350 associated with lower log-transformed WBC count, whereas higher WBC count was
351 associated with greater odds of masticatory difficulties. However, the estimated indirect
352 effect of the WBC count was very small, and the bootstrap CI in the overall population
353 reached zero. Although the indirect effect was statistically detectable after excluding
354 participants with WBC counts ≥9,000/μL, its magnitude remained small. Therefore,
355 these findings do not support WBC-related systemic inflammation as a dominant
356 explanation for the association between fish consumption and masticatory difficulties.
357 Rather, systemic inflammation may represent one component of a broader network
358 involving dietary, metabolic, behavioral, and oral-health factors. Because the mediation

analysis was cross-sectional, it should be considered exploratory and hypothesis-generating rather than establishing causal evidence.

From a clinical and public health perspective, self-reported masticatory difficulty may provide useful information beyond an isolated oral symptom. The questionnaire used in the present study assesses perceived chewing ability and may capture the functional consequences of several underlying oral conditions rather than a single dental disease. In routine health examinations of individuals reporting masticatory difficulties, dietary habits, lifestyle characteristics, and systemic health may be considered, in addition to dental assessment. Dietary patterns, such as habitual fish consumption, may contribute to a broader understanding of the behavioral context in which oral functional problems occur. These observations support an integrated medical–dental perspective in preventive healthcare, although prospective studies incorporating detailed oral examinations and comprehensive dietary assessment are required before clinical implications can be established.

Several limitations should be acknowledged. First, the cross-sectional design precludes causal and temporal inference. Reverse causation is plausible because masticatory

difficulties may alter food selection and reduce the consumption of difficult-to-chew foods, including some types of fish. Second, fish consumption frequency was self-reported, and information on portion size, type of fish, cooking method, n-3 PUFA intake, total energy intake, or overall dietary quality was not provided. Therefore, fish consumption frequency may partly represent broader dietary patterns not captured in the present study. Third, masticatory difficulties were assessed using a self-administered questionnaire and thus reflect perceived chewing ability rather than objectively measured masticatory performance. Detailed oral information, including the number of remaining teeth, periodontal status, denture use, occlusal support, oral pain, xerostomia, and objective measures of masticatory performance, was unavailable. Consequently, the specific oral conditions underlying the reported difficulties could not be determined. Fourth, although the multivariable models adjusted for several demographic, metabolic, and lifestyle factors, residual confounding by unmeasured socioeconomic, dietary, dental, and other health-related characteristics cannot be excluded. Fifth, WBC count is a nonspecific marker of systemic inflammatory burden and does not provide detailed information regarding individual inflammatory pathways. In addition, the mediation analysis was cross-sectional and should not be interpreted as evidence of causal mediation. Finally, participants were Japanese adults undergoing routine health

examinations at a single center, which may limit the generalizability of the findings to other populations.

In conclusion, more frequent fish consumption was associated with a lower prevalence of self-reported masticatory difficulties in Japanese adults and clustered with favorable lifestyle characteristics. Exploratory analyses revealed only a small indirect contribution of WBC-related systemic inflammation to this association. The observed relationship may therefore reflect a broader interplay among dietary habits, oral function, lifestyle characteristics, and systemic health rather than a single inflammation-related pathway. Longitudinal studies incorporating objective oral assessments and detailed dietary information are needed to clarify the temporal direction and mechanisms underlying this association.

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Author contributions

ST: Conceptualisation, Methodology, Formal analysis, Investigation, Data curation, Supervision, Writing – original draft.

YSi: Investigation, Data curation, Writing – review & editing.

TY: Investigation, Data curation, Writing – review & editing.

AT: Investigation, Writing – review & editing.

TN: Investigation, Data curation, Writing – review & editing.

All authors reviewed the manuscript.

Statements and Declarations

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Competing Interests

The authors declare none.

Ethics Approval

This study was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments and was approved by the Institutional Review Board of Nihon University Hospital (Approval No. 20200405).

Informed Consent

The requirement for written informed consent was waived because of the retrospective use of anonymized health check-up data, and an opt-out approach was employed.

Data Availability

The data supporting the findings of this study are not publicly available because they contain information that could compromise participant privacy. Data may be available from the corresponding author upon reasonable request and subject to institutional approval.

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517

Figure Captions

Fig. 1 Participant flowchart

In total, 10,572 participants with complete data on fish consumption frequency, masticatory difficulties, prespecified covariates, sleep duration, and white blood cell (WBC) count were included in the final analytic sample. Participants were subsequently classified according to the presence or absence of masticatory difficulties

Abbreviations: BMI, body mass index; DM, diabetes mellitus; WBC, white blood cell

Fig. 2 Restricted cubic spline analysis of the association between fish consumption frequency and masticatory difficulties

Restricted cubic spline analysis showing the association between fish consumption frequency and masticatory difficulties among 10,572 participants. The solid line represents adjusted odds ratios (ORs), and the shaded area represents 95% confidence intervals (CIs), with 0 days/week of fish consumption frequency as the reference. The model was adjusted for age, sex, body mass index, diabetes mellitus, sleep duration, regular aerobic exercise, alcohol consumption, and current smoking. Fish consumption frequency was modeled continuously using a restricted cubic spline with 3 degrees of freedom. The overall association in the spline model was significant ($P = 0.004$), with

no evidence of departure from linearity (*P* for non-linearity = 0.673)

Fig. 3 Associations between fish consumption frequency and lifestyle characteristics

Associations of fish consumption frequency with (a) regular aerobic exercise, (b) sleep duration, and (c) current smoking. Circles represent unadjusted observed values at each fish consumption frequency (0–7 days/week), with error bars indicating 95% confidence intervals (CIs). Solid lines represent unadjusted fitted trends, and shaded areas indicate 95% CIs. Regular aerobic exercise and current smoking are expressed as percentages, whereas sleep duration is expressed as mean hours per day. Trends were evaluated using univariable logistic regression for regular aerobic exercise and current smoking and univariable linear regression for sleep duration. Significant trends were observed for all three lifestyle characteristics (all *P* for trend < 0.001)

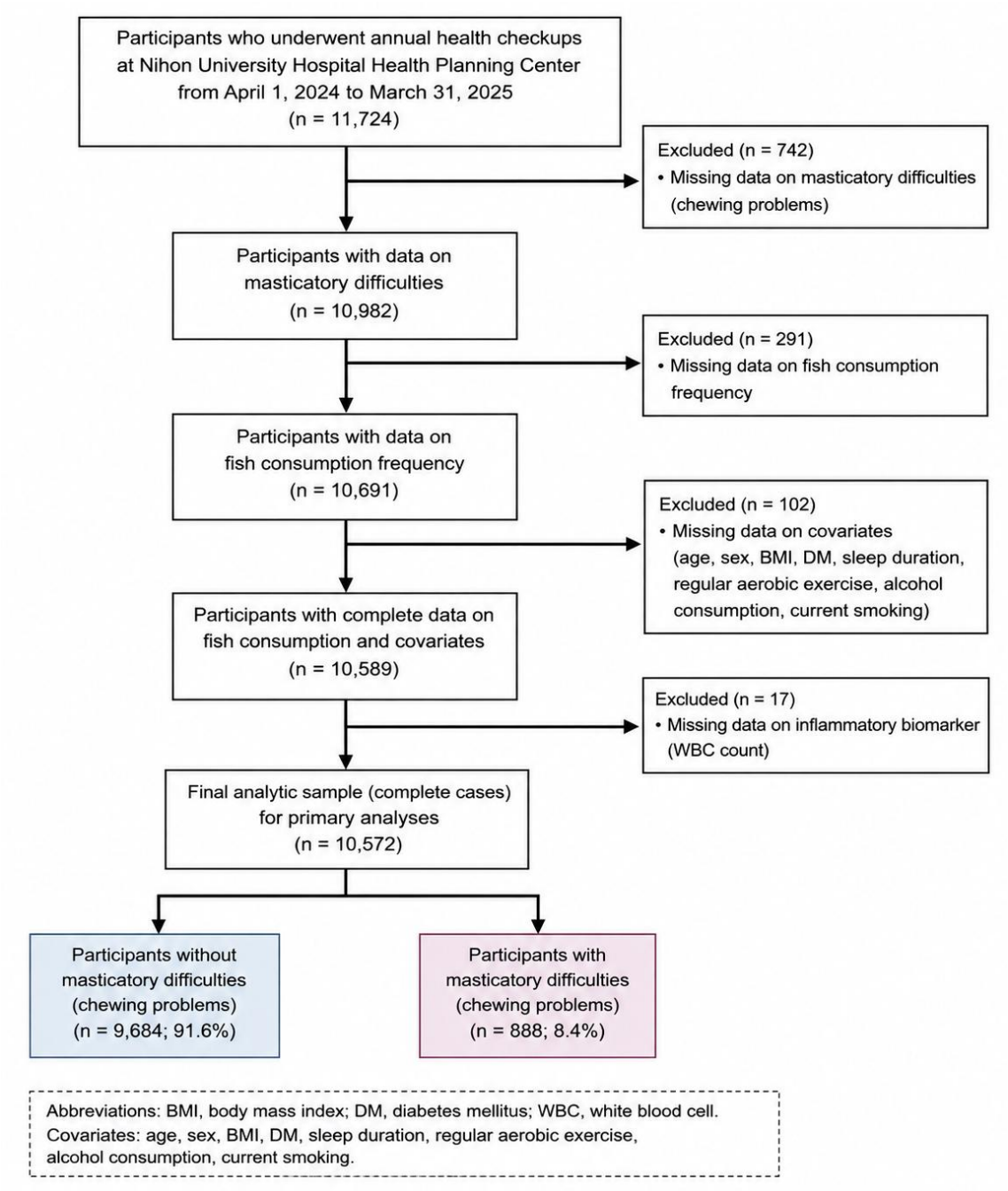
Fig. 4 Exploratory mediation analysis of the association between fish consumption frequency and masticatory difficulties through systemic inflammation

Fish consumption frequency was entered as the exposure, log-transformed white blood cell (WBC) count as the mediator, and masticatory difficulties as the binary outcome. The mediation model was adjusted for age, sex, body mass index, diabetes mellitus,

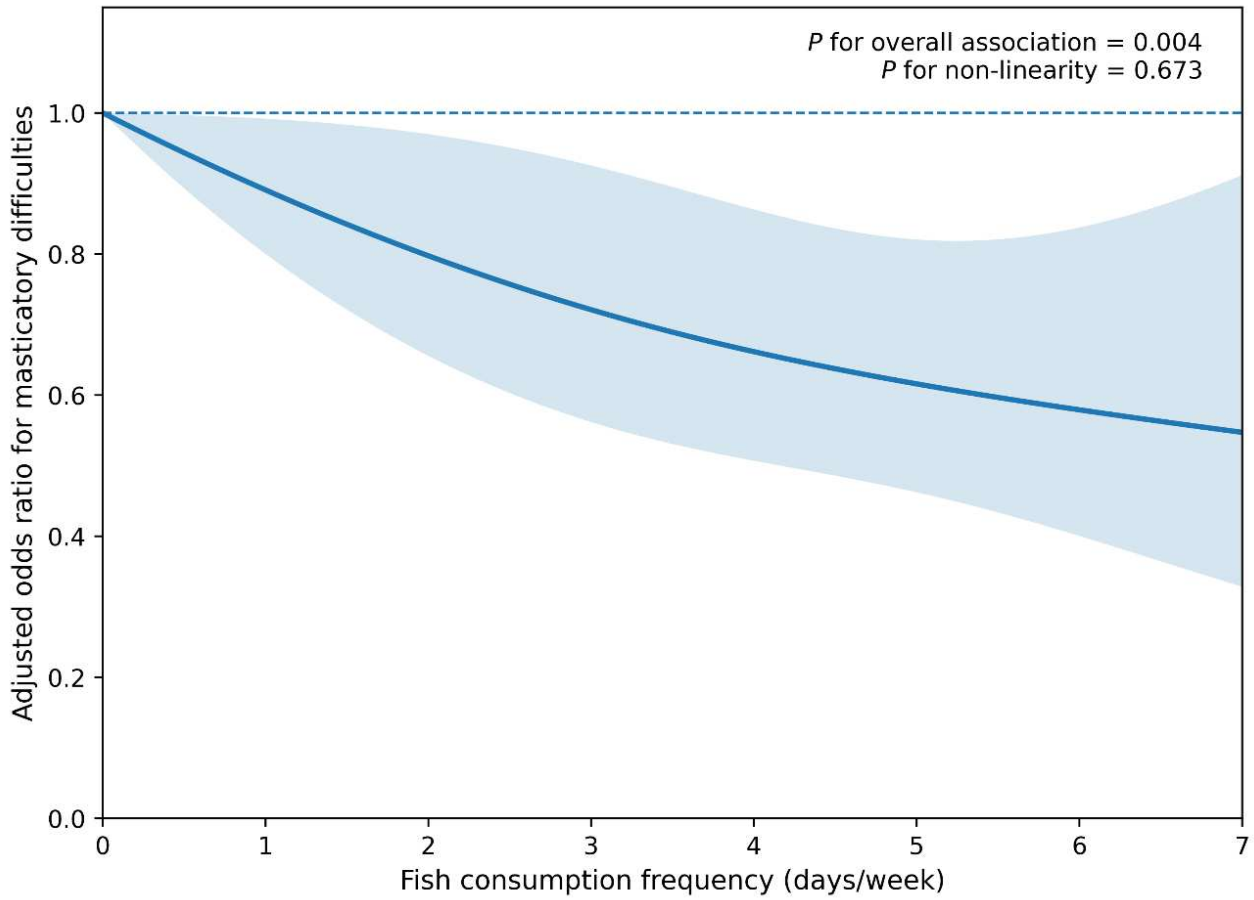
554 sleep duration, regular aerobic exercise, alcohol consumption, and current smoking. The
555 indirect effect through log-transformed WBC count was estimated using 5,000 bootstrap
556 samples. Regression coefficients (β) are shown for individual paths, and effects
557 involving the binary outcome are presented on the log-odds scale. The 95% bootstrap
558 confidence interval for the indirect effect included zero. *n* = 10,572. CI, confidence
559 interval; WBC, white blood cell
560

Figures

Figure 1



564 **Figure 2**



565

Figure 3

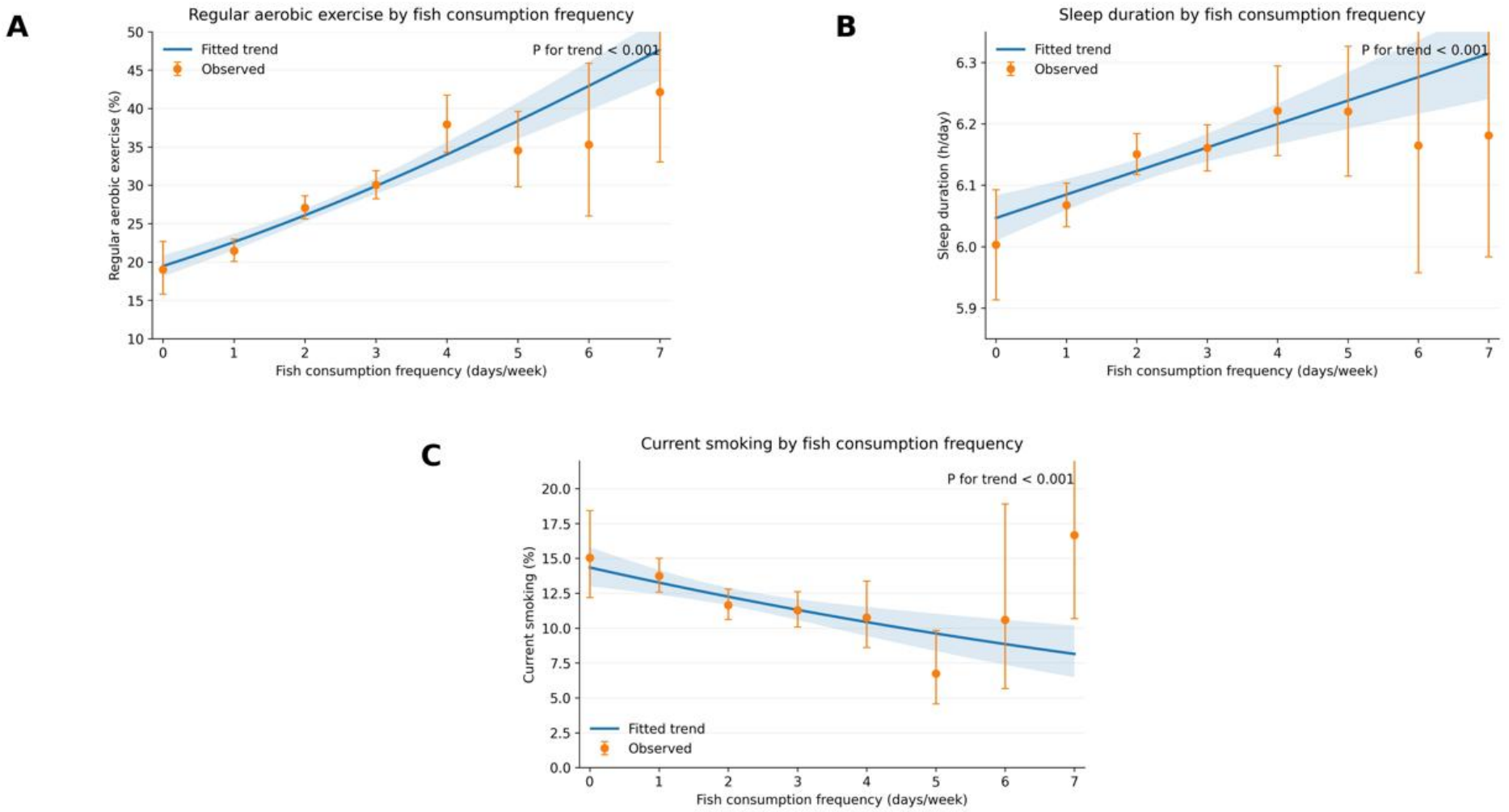
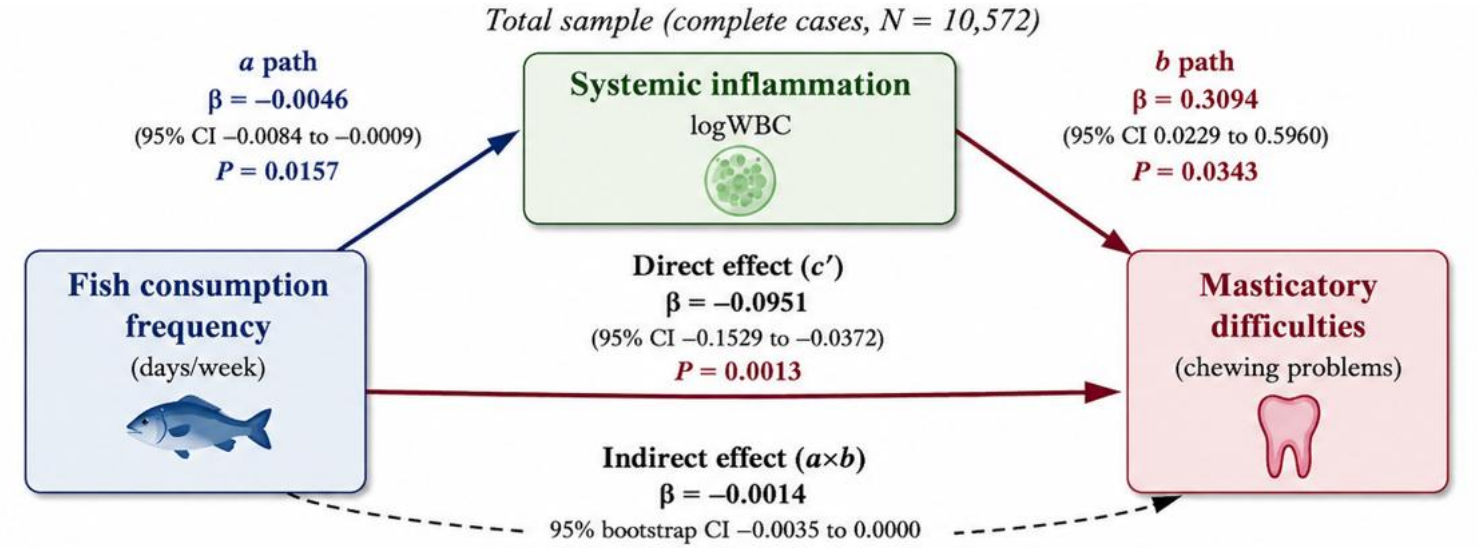

A
Regular aerobic exercise by fish consumption frequency
Fitted trend
Observed
P for trend < 0.001
Regular aerobic exercise (%)
Fish consumption frequency (days/week)
B
Sleep duration by fish consumption frequency
Fitted trend
Observed
P for trend < 0.001
Sleep duration (h/day)
Fish consumption frequency (days/week)
C
Current smoking by fish consumption frequency
P for trend < 0.001
Current smoking (%)
Fitted trend
Observed
Fish consumption frequency (days/week)

Figure 4



Effect	Effect (β)	SE / BootSE	*P* value	95% CI (Lower – Upper)
a path: Fish → logWBC	–0.0046	0.0019	0.0157	–0.0084 to –0.0009
b path: logWBC → Chewing difficulties	0.3094	0.1462	0.0343	0.0229 to 0.5960
Direct effect (*c′*): Fish → Chewing difficulties	–0.0951	0.0295	0.0013	–0.1529 to –0.0372
Indirect effect (*a*×*b*)	–0.0014	0.0009 (Bootstrap SE)	—	–0.0035 to 0.0000

Model: PROCESS macro Model 4 (SPSS), bootstrapping = 5,000 samples
Covariates: sex, age, BMI, diabetes mellitus, sleep duration, regular aerobic exercise, alcohol consumption, and current smoking
Abbreviation: logWBC, log-transformed white blood cell count

Values are expressed in a log-odds metric.

Table 1. Participant characteristics according to fish consumption frequency

Variable	All cases (n = 10,572)	0 day (n = 506)	1 day (n = 3,105)	2–3 days (n = 5,761)	4–7 days (n = 1,200)	*P* value for trend
Demographic and anthropometric characteristics						
Age (years)	48.0 ± 13.0	43.4 ± 13.5	45.0 ± 12.7	49.0 ± 12.8	53.3 ± 12.3	<0.001
Men, n (%)	5997 (56.7)	259 (51.2)	1673 (53.9)	3324 (57.7)	741 (61.8)	<0.001
BMI (kg/m²)	23.3 ± 3.9	23.1 ± 4.1	23.2 ± 3.9	23.3 ± 3.9	23.3 ± 3.8	0.012
Blood pressure and pulse						
Hypertension, n (%)	2127 (20.1)	68 (13.4)	495 (15.9)	1252 (21.7)	312 (26.0)	<0.001
Systolic blood pressure (mmHg)	116.3 ± 15.0	115.6 ± 14.1	114.8 ± 14.4	116.8 ± 15.2	117.8 ± 15.5	<0.001
Diastolic blood pressure (mmHg)	70.8 ± 11.7	70.3 ± 11.0	69.5 ± 11.3	71.3 ± 11.8	72.4 ± 11.8	<0.001
Pulse rate (bpm)	72.9 ± 11.0	73.7 ± 11.1	73.1 ± 11.0	72.9 ± 10.9	72.1 ± 11.2	0.003
Lipid profile						
Dyslipidemia, n (%)	4172 (39.5)	188 (37.2)	1138 (36.7)	2346 (40.7)	500 (41.7)	<0.001
TC (mg/dL)	201.3 ± 33.9	195.9 ± 33.1	199.1 ± 34.1	202.4 ± 33.5	203.8 ± 34.8	<0.001
LDL-C (mg/dL)	115.2 ± 29.6	113.2 ± 29.7	114.8 ± 30.0	115.7 ± 29.2	114.6 ± 30.1	0.079
HDL-C (mg/dL)	63.7 ± 16.3	62.1 ± 15.6	63.0 ± 15.9	63.8 ± 16.3	65.4 ± 17.0	<0.001
TG (mg/dL)	79 (56-117)	78 (56-113)	77 (55-114)	81 (57-118)	77 (54-116)	0.045
non-HDL-C (mg/dL)	137.3 ± 34.1	133.5 ± 33.5	136.0 ± 33.9	138.3 ± 33.9	137.7 ± 35.4	<0.001
Glycemic characteristics						
FBG (mg/dL)	98.1 ± 16.6	95.3 ± 14.6	96.6 ± 14.5	98.7 ± 17.2	100.5 ± 19.0	<0.001

HbA1c (%)	5.7 ± 0.6	5.6 ± 0.5	5.6 ± 0.5	5.7 ± 0.6	5.7 ± 0.6	<0.001
HOMA-IR	1.03 (0.70-1.59)	1.00 (0.73-1.52)	1.03 (0.71-1.60)	1.04 (0.70-1.60)	1.00 (0.68-1.58)	0.559
Diabetes mellitus, n (%)	579 (5.5)	15 (3.0)	143 (4.6)	339 (5.9)	82 (6.8)	<0.001
Inflammatory markers						
WBC (/μL)	5000 (4200-5900)	5200 (4300-6100)	5100 (4300-6100)	5000 (4200-5900)	4800 (4000-5700)	<0.001
CRP (mg/dL)	0.035 (0.016-0.088)	0.037 (0.018-0.106)	0.035 (0.016-0.092)	0.035 (0.016-0.085)	0.035 (0.016-0.093)	0.457
Lifestyle behaviors						
Regular aerobic exercise, n (%)	2839 (26.9)	96 (19.0)	667 (21.5)	1,633 (28.3)	443 (36.9)	<0.001
Current smoking, n (%)	1286 (12.2)	76 (15.0)	427 (13.8)	663 (11.5)	120 (10.0)	<0.001
Sleep duration (hours/day)	6.1 ± 1.0	6.0 ± 1.0	6.1 ± 1.0	6.2 ± 1.0	6.2 ± 1.0	<0.001
Alcohol intake (g/week)	72.5 ± 112.8	47.9 ± 89.2	61.3 ± 101.6	75.0 ± 114.6	100.1 ± 132.9	<0.001

Values are presented as mean ± standard deviation, median (interquartile range), or number (%), as appropriate. Trends across increasing categories of fish consumption frequency were assessed using the Jonckheere–Terpstra trend test for continuous variables and the Cochran–Armitage trend test for categorical variables.

Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or current use of antihypertensive medication. Dyslipidemia was defined as low-density lipoprotein cholesterol ≥140 mg/dL, high-density lipoprotein cholesterol <40 mg/dL, triglycerides ≥150 mg/dL, or use of lipid-lowering medication. Diabetes was defined as fasting blood glucose ≥126 mg/dL, glycated

hemoglobin ≥6.5%, or current use of antidiabetic medication.

Abbreviations: BMI, body mass index; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FBG, fasting blood glucose; Hb, hemoglobin; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; WBC, white blood cell; TG, triglycerides; non-HDL-C, non-high-density lipoprotein cholesterol; HbA1c, glycated hemoglobin; CRP, C-reactive protein.

Table 2. Multivariable-adjusted association between fish consumption frequency and masticatory difficulties

Fish consumption frequency	Adjusted OR	95% CI	P value
0 days/week (reference)	1.000	Reference	—
1 day/week	0.709	0.523–0.961	0.026
2 days/week	0.562	0.413–0.764	<0.001
3 days/week	0.654	0.478–0.894	0.008
4 days/week	0.548	0.367–0.818	0.003
5 days/week	0.553	0.342–0.896	0.016
6 days/week	0.358	0.138–0.928	0.035
7 days/week	0.322	0.134–0.775	0.011
Overall association of categorical fish variable			0.003
Continuous analysis			
Per 1-day/week increase	0.908	0.857–0.962	0.001

Adjusted for age, sex, body mass index, diabetes mellitus, sleep duration, regular aerobic exercise, alcohol consumption, and current smoking. n = 10,572. OR, odds ratio; CI, confidence interval.

Supplementary Files

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

- OnlineResource1.pdf
- OnlineResource2.pdf