# Influence of dietary intake of fish oil, magnesium and zinc on metabolic parameters among individuals tested for diabetes

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**Abstract**

**Background and Aims:** The present study aimed to assess the significance and degree of correlation between intake of fish oil, magnesium (Mg), and zinc (Zn) and metabolic parameters.

**Methods:** Correlation coefficients among nutrient intake and physical and laboratory parameters were determined using Spearman's rho (ρ) test or a multiple regression model among Japanese individuals (male:female, 37:66; median age, 55 years) who completed a semi-quantitative food questionnaire and underwent diabetes tests. Individuals with diabetes were excluded.

**Results:** Spearman's test revealed several weak but significant correlations between intake of fish oil including ω-3 polyunsaturated fatty acids (PUFA) and various metabolic parameters. The test showed that Zn intake in women significantly correlated with reduced systolic blood pressure (SBP), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), and homeostasis model assessment-insulin resistance (HOMA-R). Multivariate analysis revealed that intake of fish oil, eicosapentaenoic acid (EPA), and Zn was significantly associated with increased serum levels of high-density lipoprotein cholesterol (HDL-C) (fish oil vs. HDL-C, p/95%CI = 0.0438/0.0055 to 0.3724; EPA vs. HDL-C, 0.0439/0.0053 to 0.3724; Zn vs. HDL-C, 0.0041/0.0890 to 0.4609). Multivariate analysis revealed that ω-3 PUFA was associated with decreased serum ALT levels (0.0240/-5.000 to -0.0367) and that Zn correlated with SBP (0.0239/-0.5149 to -0.0377) in women.

**Conclusion:** Intake of fish oil, Mg, and Zn was associated with some metabolic parameters. Abundant intake of fish oil including ω-3 PUFA and Zn can exert anti-arteriosclerotic effects through increasing serum levels of HDL-C. Omega-3 PUFA can reduce liver inflammation and Zn reduced systolic blood pressure in women.

**Key Words**: docosahexaenoic; eicosapentaenoic; polyunsaturated fatty acids; insulin resistance; renal function.

**Abbreviations:** ADA, American Diabetes Association; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Cr, creatinine; CI, confidence interval; CVD, cardiovascular disease; DBP, diastolic blood pressure; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FBG, fasting blood glucose; γ-GTP, γ-glutamyl transpeptidase; HbA1c, glycated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-β; homeostasis model assessment-β; HOMA-R, homeostasis model assessment-insulin resistance; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; IRI, immunoreactive insulin; JNK, c-Jun N-terminal kinase; LDL-C, low-density lipoprotein cholesterol; MCP-1, monocyte chemoattractant protein-1; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OGTT, oral glucose tolerance test; %FAT, percentage of body fat; PUFA, polyunsaturated fatty acids; ROS, reactive oxidative stress; SBP, systolic blood pressure; SFA, saturated fatty acids; T2DM, type 2 diabetes mellitus; T-Chol, total cholesterol; TG, triglycerides; TNF-α, tumor necrosis factor-α; UA, uric acid; WHR, waist-hip ratio; Zn, zinc.

## Introduction

Nutrition affects various metabolic aspects, and fatty acids are involved in lipid and glucose metabolism. Fatty acids from animal sources, such as meat and poultry, consist mainly of saturated fatty acids, or ω-6 (n-6) polyunsaturated fatty acids (PUFA), whereas oils derived from certain types of fish consist mainly of ω-3 PUFA. Eicosanoids derived from ω-6 PUFA have proinflammatory and immunoactive functions, whereas eicosanoids derived from ω-3 PUFA have anti-inflammatory properties [1]. Randomized controlled trials have identified beneficial effects of ω-3 PUFA on cardiovascular disease (CVD) in patients with preexisting CVD as well as in healthy individuals [2], although the beneficial effects of ω-3 PUFA remain controversial. The results of a recent meta-analysis indicated that ω-3 PUFA protects against vascular diseases, but the evidence was not clear-cut [3]. A recent systematic review [4] states that partial replacement of saturated fatty acids with PUFA or monounsaturated fat lowers serum levels of total and low-density lipoprotein (LDL) cholesterol, whereas an association between dietary ω-3 PUFA and type 2 diabetes mellitus (T2DM) remained unclear. Eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) are long-chain ω-3 PUFA that are abundant in oil from blue-backed fish (blue-fish) that are popular components of Japanese cuisine.

Magnesium (Mg) is a trace element that affects many cellular functions, including the transport of potassium and calcium ions, as well as the modulation of signal transduction, energy metabolism, and cell proliferation [5]. A study of middle-aged

North Americans has shown that Mg intake reduces the risk of impaired glucose and insulin metabolism and of progression from pre-diabetes to diabetes [6], and a large-scale study has shown that magnesium intake reduces risk for T2DM [7].

Zinc (Zn) is a trace element that is essential for cell differentiation and apoptosis. The ZNT zinc transporter isoform, ZNT8, is expressed mainly in β-cells and it delivers zinc for insulin maturation and secretion [8]. Increased zinc intake might be associated with a lower risk of T2DM in women [9].

The influence of the nutrients described above on various metabolic parameters has been studied in detail. However, the significance and closeness of the correlation between nutrient intake and metabolic parameters have not been fully evaluated. We therefore aimed to investigate relationships between nutrient intake and metabolic parameters among Japanese community dwellers who presented for diabetes testing. Since all of them resided in a town facing the Pacific Ocean, we focused on their intake of fish oil including ω-3 PUFA and investigated the effects of Mg or Zn on various metabolic parameters.

**Participants and methods**

One hundred and forty residents (male, n = 64; female, n = 77; median age, 57 y) who had never been diagnosed with diabetes attended a health center in the town of Mie, Japan for health checks including diabetes tests between 2011 and 2012. All of them underwent a 75-g oral glucose tolerance test (OGTT) after an overnight fast of at least 12 hours. Six who had diabetes, defined according to the American Diabetes Association (ADA) [10] as fasting blood glucose (FBG) ≥ 126 mg/dL and/or blood sugar concentration at two hours after 75 g OGTT ≥ 200 mg/dL and/or HbA1c ≥ 6.5%, were excluded from the study. Seven, 20, and 6 individuals who were under medication to treat hypertension, dyslipidemia, and gout, respectively, and eight who consumed > 210 g/week (male) or 140 g/week (female) of ethanol were excluded from the study. The cut-off value of significant ethanol consumption was defined according to the guidelines for diagnosing non-alcoholic steatohepatitis (NASH) [11] and 10 individuals overlapped. Finally, 37 (27 men and 10 women) were excluded and 103 residents (male, n = 37; female, n = 66; median age, 55 y) were enrolled in the present study. The local residents consume large quantities of Pacific saury, a blue-backed fish that is caught and then landed in the town harbor.

The diets of the participants were surveyed using a semi-quantitative food questionnaire [12] that addressed the consumption of staple foods, side dishes, deep-fried foods, fruits, sweets, alcohol, and supplements. Public health nurses interviewed the participants to obtain precise information and ensure that the questionnaire was understood and completed. Daily intake of each nutrient was calculated using software developed by

Tokudome et al. [13]. None of the participants used Zn, Mg, or ω-3 PUFA supplements or antioxidants.

We evaluated nutrition and metabolic status by measuring body weight, body mass index (BMI), percentage of body fat (%FAT), waist/hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and serum levels of alanine aminotransferase (ALT), aspartate amino transferase (AST), γ-glutamyl transpeptidase (γ-GTP), total cholesterol (T-Chol), HDL-C, TG, creatinine (Cr), uric acid (UA), FBG, and glycated hemoglobin A1c (HbA1c). We measured %FAT by analyzing bioelectrical impedance using a DC-320® body composition analyzer (TANITA Corporation, Tokyo, Japan). Blood pressure was measured using an HBP-9020® digital manometer (OMRON Healthcare Co. Ltd, Kyoto Japan) after resting for more than 10 minutes. High-sensitivity CRP (hsCRP) concentrations were determined using a modification of the Behring latex-enhanced CRP assay. Serum levels of inflammatory cytokines TNF-α, Interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1), which might be associated with insulin resistance [14-16], were measured using enzyme-linked immunosorbent assays (ELISA). Individuals with BMI $\leq 18.5$, 25.0-30.0, and $> 30.0$ kg/m$^2$ were defined as underweight, overweight, and obese, respectively. According to the guideline of the Japan Society for the Study of Obesity [17], BMI $\geq 25.0$ kg/m$^2$ was defined as obese.

The homeostasis model assessment of insulin resistance (HOMA-R) was calculated according to the formula [18]: [fasting serum insulin level ($IRI_0$, μU/mL) × FBG (mg/dL)] / 405

Homeostasis model assessment beta (HOMA-β) was calculated according to the formula [19]: [$IRI_0$ (μU/mL) × 360] / [FBG (mg/dL) − 63]

Data are expressed as medians with ranges. Before analyzing correlations among parameters, we assessed whether the continuous variables were normally distributed. The Shapiro-Wilk test revealed that the distribution of many parameters (BMI, hsCRP, AST, ALT, γ-GTP, T-Chol, TG, CRE, UA, FBG, HbA1c, HOMA-R, HOMA-β, TNF-α, IL-6, MCP-1) and intake of fish oil, ω-3 PUFA, EPA, DHA, and Mg was not normal. We therefore used non-parametric tests to evaluate correlations among parameters. Correlation coefficients between variables in univariate analyses were calculated using Spearman's rho (ρ) corrected for ties. Values were replaced to rank each parameter in multiple linear regression models for multivariate analysis. Values from two groups were compared using the Mann-Whitney U test. All tests were two-tailed, and *p* values $< 0.05$ were considered statistically significant. Data were statistically analyzed using Multivariable Analysis Ver. 2.0 for Mac software (Esumi Co. Ltd., Tokyo, Japan). All those enrolled provided written informed consent to participate in the study, which was approved by the Ethics Committee at Mie University.

## Results

### 1. Basic and physical characteristics, and survey of dietary intake.

**Table 1. Basic and physical characteristics and diet survey.**

| Basic and physical characteristics | Total | Male | Female | p* |
|---|---|---|---|---|
| No. of participants | 103 | 37 | 66 | |
| Age (years) | 55 (40-68) | 54 (40-66) | 56 (40-68) | 0.310 |
| BMI | 21.6 (16.8-32.1) | 23.6 (17.3-32.1) | 20.6 (16.8-27.6) | <0.001 |
| %FAT | 27 (15-38) | 22 (15-29) | 29 (21-38) | <0.001 |
| WHR | 0.85 (0.70-1.06) | 0.92 (0.82-1.06) | 0.81 (0.70-0.98) | <0.001 |
| SBP (mm Hg) | 116 (84-170) | 120 (88-170) | 113 (84-148) | 0.018 |
| DBP (mmHg) | 72 (52-98) | 78 (52-98) | 70 (56-86) | 0.008 |
| **Dietary intake** | | | | |
| Caloric (kcal/day) | 1696 (588-4360) | 1947 (609-3640) | 1671 (588-4360) | 0.468 |
| Fish oil (g/day) | 5.1 (0.1-18.3) | 3.7 (0.1-18.3) | 5.6 (0.2-13.8) | 0.101 |
| PUFA (g/day) | 13.2 (0.3-26.1) | 13.1 (0.3-22.6) | 12.7 (2.3-26.1) | 0.277 |
| ω-3 PUFA (mg/day) | 2528.0 (11.3-5408.0) | 2242.0 (11.3-5408.0) | 2607.8 (387.7-5334.8) | 0.075 |
| α-Linolenic acid (mg/day) | 1558.2 (11.3-3854.8) | 1565.6 (11.3-2974.9) | 1535.8 (271.5-3854.8) | 0.293 |
| EPA (mg/day) | 289.5 (2.2-1090.0) | 194.9 (2.2-1090.0) | 301.4 (10.7-888.3) | 0.078 |
| DHA (mg/day) | 514.9 (0.1-1909.1) | 339.6 (0.1-1909.1) | 543.4 (0.1-1383.7) | 0.111 |
| Mg (mg/day) | 220.2 (4.3-613.2) | 206.6 (19.7-545.8) | 231.4 (4.3-613.2) | 0.356 |
| Zn (mg/day) | 7.2 (0.6-15.8) | 6.7 (1.2-15.8) | 7.3 (0.6-15.7) | 0.224 |

*Male vs. female. BMI, body mass index; DBP, diastolic blood pressure; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; %FAT, ratio of body fat; Mg, magnesium; PUFA, polyunsaturated fatty acids; SBP, systolic blood pressure; WHR, waist-hip ratio; Zn, zinc.

Table 1 summarizes the basic and physical characteristics and dietary intake of the 103 participants. The women had significantly lower BMI and blood pressure but a higher %FAT content and WHR than the men. Daily intake of calories, fish oil, ω-3 PUFA, Mg, and Zn did not significantly differ between the two groups.

### 2. Correlation among dietary intake, physical parameters, and laboratory data

Table 2 shows that physical parameters and laboratory data significantly correlated with the intake of fish oil, Mg, and Zn. Daily intake of fish oil including ω-3 PUFA, α linolenic acid, EPA and DHA, Mg, and Zn significantly correlated with various metabolic parameters in Spearman's test. Multivariate regression analysis adjusted for confounding factors (BMI, %FAT, WHR, SBP, DBP, hsCRP, AST, ALT, γ-GTP, HDL-C, TG, Cr, UA, and HOMA-R for analysis of fish oil; BMI, %FAT, WHR, hsCRP, ALT, γ-GTP, HDL-C, TG, Cr and UA for analysis of EPA; WHR, ALT, γ-GTP, HDL-C, TG, and HOMA-R for Zn) revealed that fish oil, EPA, and Zn were significantly associated with elevated HDL-C (Table 3).

**Table 2. Univariate analysis: physical parameters and laboratory data correlating with fish oil, Mg and Zn.**

| Parameter | Correlation coefficient | p |
|---|---|---|
| **Fish oil vs.** | | |
| BMI | -0.42 | <0.001 |
| %FAT | 0.391 | <0.001 |
| WHR | -0.477 | <0.001 |
| SBP | -0.26 | 0.012 |
| DBP | -0.312 | 0.003 |
| hsCRP | -0.24 | 0.02 |
| AST | -0.28 | 0.007 |
| ALT | -0.385 | <0.001 |
| γ-GTP | -0.35 | 0.001 |
| HDL-C | 0.365 | 0.001 |
| TG | -0.267 | 0.01 |
| Cr | -0.507 | <0.001 |
| UA | -0.437 | <0.001 |
| HOMA-R | -0.223 | 0.031 |
| **ω-3 PUFA vs.** | | |
| BMI | -0.462 | <0.001 |
| %FAT | 0.44 | <0.001 |
| WHR | -0.514 | <0.001 |
| SBP | -0.231 | 0.025 |
| DBP | -0.289 | 0.005 |
| hsCRP | -0.247 | 0.017 |
| TNF-α | -0.28 | 0.007 |
| IL-6 | -0.258 | 0.013 |
| AST | -0.317 | 0.002 |
| ALT | -0.41 | <0.001 |
| γ-GTP | -0.367 | <0.001 |
| HDL-C | 0.392 | <0.001 |
| TG | -0.315 | 0.002 |
| Cr | -0.559 | <0.001 |
| UA | -0.558 | <0.001 |
| HOMA-R | -0.207 | 0.045 |
| **α Linolenic acid vs.** | | |
| BMI | 0.409 | <0.001 |
| %FAT | -0.552 | <0.001 |
| WHR | 0.439 | <0.001 |
| DBP | 0.257 | 0.013 |
| TNF-α | 0.316 | 0.002 |
| IL-6 | 0.31 | 0.003 |
| ALT | 0.269 | 0.009 |
| γ-GTP | 0.218 | 0.034 |
| **α Linolenic acid vs. (contined)** | | |
| HDL-C | -0.282 | 0.006 |
| Cr | 0.656 | <0.001 |
| UA | 0.511 | <0.001 |
| **EPA vs.** | | |
| BMI | -0.307 | 0.003 |
| %FAT | 0.27 | 0.009 |
| WHR | -0.401 | <0.001 |
| hsCRP | -0.22 | 0.033 |
| ALT | -0.233 | 0.024 |
| γ-GTP | -0.339 | 0.001 |
| HDL-C | 0.308 | 0.003 |
| TG | -0.279 | 0.007 |
| Cr | -0.343 | 0.001 |
| UA | -0.224 | 0.029 |
| **DHA vs.** | | |
| %FAT | 0.278 | 0.007 |
| TNF-α | -0.253 | 0.014 |
| IL-6 | -0.26 | 0.012 |
| Cr | -0.325 | 0.002 |
| UA | -0.224 | 0.03 |
| **Mg vs.** | | |
| BMI | 0.362 | <0.001 |
| %FAT | -0.589 | <0.001 |
| WHR | 0.422 | <0.001 |
| DBP | 0.231 | 0.025 |
| TNF-α | 0.305 | 0.003 |
| IL-6 | 0.354 | <0.001 |
| HDL-C | -0.238 | 0.021 |
| Cr | 0.665 | <0.001 |
| UA | 0.448 | <0.001 |
| HOMA-R | -0.232 | 0.024 |
| **Zn vs.** | | |
| WHR | -0.219 | 0.034 |
| ALT | -0.258 | 0.012 |
| γ-GTP | -0.232 | 0.024 |
| HDL-C | 0.226 | 0.029 |
| TG | -0.241 | 0.019 |
| HOMA-R | -0.247 | 0.017 |

**ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRE, creatinine; DBP, diastolic blood pressure; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; %FAT, ratio of body fat; γ-GTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; HOMA-R, homeostasis model assessment-insulin resistance; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; Mg, magnesium; PUFA, polyunsaturated fatty acids; SBP, systolic blood pressure; TG, triglycerides; TNF-α, tumor necrosis factor-α, UA, uric acid; WHR, waist-hip ratio; Zn, zinc.**

**Table 3. Multivariate analysis: physical parameters and laboratory data associated with fish oil, EPA and Zn.**

| | Regression coefficient | 95% CI | p |
|---|---|---|---|
| **HDL-C vs.** | | | |
| Fish oil | 0.1899 | 0.0054 to 0.3724 | 0.0438 |
| EPA | 0.1897 | 0.0054 to 0.3724 | 0.0439 |
| Zn | 0.275 | 0.0890 to 0.4609 | 0.0041 |

**Parameters (BMI, %FAT, WHR, SBP, DBP, hsCRP, AST, ALT, γ-GTP, HDL-C, TG, Cr, UA and HOMA-R for fish oil; BMI, %FAT, WHR, hsCRP, ALT, γ-GTP, HDL-C, TG, Cr and UA for EPA; WHR, ALT, γ-GTP, HDL-C, TG and HOMA-R for Zn) were adjusted for multivariate regression analysis.**
**ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Cr, creatinine; CI, confidence interval; DBP, diastolic blood pressure; EPA, eicosapentaenoic acid; γ-GTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; HOMA-R, homeostasis model assessment-insulin resistance; %FAT, percentage of body fat; SBP, systolic blood pressure; TG, triglycerides; UA, uric acid; WHR, waist-hip ratio; Zn, zinc.**

### 3. Correlations between intake of fish oil, Mg, and Zn and physical parameters and laboratory findings in women

We separated the participants into groups of males and females. No correlations were notable among the men, whereas ω-3 PUFA values correlated in the women with reduced serum levels of AST ($\rho = -0.329$, $p = 0.009$), ALT ($\rho = -0.328$, $p = 0.009$), TG ($\rho = -0.263$, $p = 0.032$), and reduced HbA1c ($\rho = -0.282$, $p = 0.024$). Zinc correlated with reduced SBP, serum ALT, γ-GTP, and HOMA-IR (Figure 1A, B, C, and D, respectively). Multivariate analysis adjusted for confounding factors (AST, ALT, TG, and HbA1c for analysis of ω-3 PUFA; SBP, hsCRP, AST, ALT, γ-GTP, and HOMA-R for analysis of Zn) showed that ω-3 PUFA intake was associated with reduced serum

ALT levels and that Zn correlated with reduced SBP (Table 4).

**Table 4. Multivariate analysis: physical parameters and laboratory data associated with ω-3 PUFA and Zn in women.**

| | | Regression coefficient | 95% CI | p |
|---|---|---|---|---|
| **ω-3 PUFA vs.** | | | | |
| | **ALT** | **-0.2684** | **-0.5000 to -0.0367** | **0.024** |
| **Zn vs.** | | | | |
| | **SBP** | **-0.2778** | **-0.5149 to -0.0377** | **0.0239** |

**Parameters (AST, ALT, TG, HbA1c for ω-3 PUFA; SBP, hsCRP, AST, ALT, γ-GTP and HOMA-R for Zn) were adjusted for multivariate analysis.**
**AST, aspartate aminotransferase; ALT, alanine aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HbA1c, glycated hemoglobin A1c; HOMA-R, homeostasis model assessment-insulin resistance; hsCRP, high-sensitivity C-reactive protein; PUFA, polyunsaturated fatty acids; SBP, systolic blood pressure; TG, triglycerides; Zn, zinc.**

**Discussion**

We determined correlation coefficients between nutrient intake and several metabolic factors. The daily intake of calories and animal lipid were lower by 140 kcal and 5 g, respectively, than the values reported in part one of the National Health and Nutrition Examination Survey report for fiscal year 2011 (in Japanese) published by the Ministry of Health, Labour and Welfare, Japan (http://www.mhlw.go.jp/bunya/kenkou/eiyou/h23-houkoku.html). The daily intake of ω-3 PUFA, α-linolenic acid, EPA, and DHA was greater by 460, 240, 85, and 135 mg, respectively, than the reference Japanese dietary intake reported by the Ministry of Health, Labour and Welfare, Japan (Reference Japanese dietary intake in fiscal year 2010 [in Japanese]; http://www.mhlw.go.jp/bunya/kenkou/sessyu-kijun.html). The daily intake of Mg and Zn was 20 and 5 mg lower, respectively, than the values in part one of the National Health and Nutrition Examination Survey report for fiscal year 2011 (in Japanese) published by the Ministry of Health, Labour and Welfare. The results of the food survey showed that the study participants consumed less animal lipid and more ω-3 PUFA including linoleic acid, EPA, and DHA than the average Japanese individual. This finding seems consistent with the geographic location of the participants, namely a community that faces the Pacific Ocean where blue-backed fish comprise an important part of the local diet.

The median BMI of the 103 participants was 21.6. Nine (7.8%) of them were overweight, 14 (13.5%) were underweight, and 3 (2.9%) were obese. According to the guidelines of the Japan Society for the Study of Obesity [17], 12 (11.7%) were obese.

This BMI distribution and the low rate of obesity should be considered when interpreting the data.

The intake of certain nutrients reduced or increased some specific parameters in the present study. Table 2 shows a univariate analysis of the intake of fish oil, ω-3 PUFA reduced BMI, WHR, and blood pressure. Fish oil and ω-3 PUFA increased serum levels of HDL-C and reduced those of hsCRP, liver enzymes, TG, Cr, and UA. The results suggest that fish oil including ω-3 PUFA can improve adiposity, liver and renal function, lipid profiles, and insulin sensitivity. Reports indicate that ω-3 PUFA including EPA and DHA provide beneficial effects on several disorders including dyslipidemia, cardiovascular diseases, hypertension, and T2DM in humans and in animal models [19-21], but their beneficial roles remain controversial. Treating rat models of diabetes with EPA improved lipid metabolism, but the effect on insulin sensitivity has remained unclear [22]. A recent study found that short-term dietary therapy with both ω-3 and ω-6 PUFA significantly reduces body weight and BMI and significantly improves lipoprotein lipid profiles and insulin sensitivity in patients with T2DM [23], but the absence of a control group in that study restricts the conclusion that these fatty acids reduce body weight. The present study found that ω-3 PUFA seemed to be associated with reduced BMI and WHR, improved lipid profiles, and improved insulin sensitivity in individuals without diabetes, which is somewhat consistent with the findings of previous studies. However these effects were not found in multivariate analysis, except for increased serum HDL-C levels.

Long-term supplementation with fish oil lowered the ω-6/ω-3 ratio in skeletal muscle and improved insulin sensitivity in association with increased insulin-stimulated p38 activation in a rat model [24]. However, one meta-analysis found that fish oil, which contains both ω-3 and ω-6 PUFA, does not alter HbA1c levels and tends to increase FBS in patients with T2DM [25]. Another meta-analysis found that fish oil significantly reduces serum triglycerides but increases low-density lipoprotein cholesterol (LDL-C) without significantly affecting FBG, HbA1c, and HDL-C [26]. Table 2 shows that the consumption of more fish oil, ω-3 PUFA, and EPA increased serum HDL-C and that the intake of fish oil and ω-3 PUFA reduced HOMA-R. These results suggested that fish oil potentially improves glucose metabolism by reducing insulin resistance. In contrast to EPA or DHA, the present study found that excessive intake of the ω-3 PUFA α-linolenic acid might have increased serum inflammatory cytokines such as TNF-α and IL-6, which could cause insulin resistance to deteriorate. The advantages and disadvantages of consuming fish oil should therefore be considered.

Reactive oxidative stress (ROS), TNF-α, and IL-6 cause insulin resistance, hepatocyte fat accumulation, and cellular injury, which are involved in the pathological processes of NAFLD by directly activating JNK [27]. Table 2 shows that ω-3 PUFA reduced AST, ALT, γ-GTP, inflammatory cytokines IL-6 and TNF-α, and HOMA-R. Although IL-6 and TNF-α are not oxidative stress markers, they directly mediate fibrosis to induce inflammation of the liver and steatohepatitis [28, 29]. Reducing these inflammatory cytokines might ameliorate liver inflammation and steatosis that can associate with reduced ROS production. These results therefore suggest that ω-3 PUFA can improve insulin resistance by reducing hepatic inflammation and ROS production.

Magnesium intake significantly correlated with reduced HOMA-R, suggesting that Mg can help to reduce insulin resistance. Many reports have described the effects of Mg intake on glucose metabolism in patients with T2DM [30-35]. Although the populations in these studies differed from ours, the present findings support the notion that increased Mg intake significantly protects against the development of T2DM [36]. In addition, the present study showed that Zn and Mg similarly affect insulin sensitivity. This is potentially in line with the findings of a recent Australian study, in which higher levels of serum, but not dietary zinc, are associated with increased serum insulin sensitivity [37].

The present study showed that Mg was positively correlated with BMI and WHR, but negatively correlated with %FAT. In contrast, Zn intake was negatively correlated with WHR. These findings suggest that Mg and Zn play different modulatory roles in controlling adiposity. Seki et al. recently reported that calcium combined with Mg and lactulose could reduce body fat mass in middle-aged Japanese women [38]. They describe that increased levels of Mg in the bloodstream might affect the intracellular concentration of calcium ions in adipocytes. However, further investigation is required to clarify the mechanism of how Mg and Zn affect adiposity.

Because the physical background significantly differed between men and women, we analyzed them separately and found that Zn intake reduced SBP, ameliorated liver inflammation, and reduced insulin resistance in women. A mild hepatocyte-protective effect of Zn was also identified in women. These findings suggest that Zn can reduce blood pressure and ameliorate liver inflammation by improving metabolic status and glucose metabolism. Hepatoprotective effects and possible mechanisms of Zn in some animal models have been proposed. Yu et al. reported that Zn (II)-curcumin protects rats from ethanol-induced liver injury and hematological abnormalities [39]. Zhong et al. showed that a dietary zinc deficiency exaggerates ethanol-induced liver injury in mice [40] and worsened the ethanol-induced imbalance between hepatic pro-oxidant and antioxidant enzymes and the hepatic expression of cell death receptors. They also described that a dietary Zn deficiency enhances ethanol-induced gut permeability, leading to plasma endotoxin elevation. Thus, repressed oxidative stress and plasma endotoxin levels might be involved in the hepatocyte-protective effect of Zn, but the precise mechanism requires further clarification.

The present study found some associations in women, but not in men. This might be due to the small sample size particularly of men since 27 (42.2%) of 64 men who applied to participate were excluded, which might have created some bias. Another limitation of the present study was that a semi-quantitative food questionnaire was not the best method for assessing micronutrients.

In conclusion, some mild correlations were identified between intake of fish oil, Mg, and Zn and various metabolic parameters. Abundant intake of fish oil, including ω-3 PUFA and Zn, can exert anti-arteriosclerotic effects through increasing serum levels of HDL-C. Fish oil, ω-3 PUFA, Mg, and Zn might improve insulin resistance. Omega-3 PUFA reduced liver inflammation in all participants and Zn reduced blood pressure in women. The optimal intake of these nutrients probably confers beneficial effects on lipid and glucose metabolism, but this should become clearer after long-term follow up of the participants.

## Statement of authorship

This is an original article that is not currently under consideration or in press elsewhere.

The authors' contributions are as follows:

H.T.: study concept and design, acquisition of data, sample analyses, statistical analysis, data interpretation, and drafting the article.

Y.K.: study concept and design, statistical analysis, data analysis, data interpretation, and critically revising the article for important intellectual content.

O.T.: study concept and design, data interpretation, and critically revising the article for important intellectual content.

Y.T.: study concept and design, data interpretation, and critically revising the article for important intellectual content.

Y.S.: study concept, design and coordination, data interpretation, and critically revising the article for important intellectual content.

All authors have read and approved this submitted version.

## Conflict of interest

None of the authors have any potential conflicts of interest that could inappropriately influence this work.

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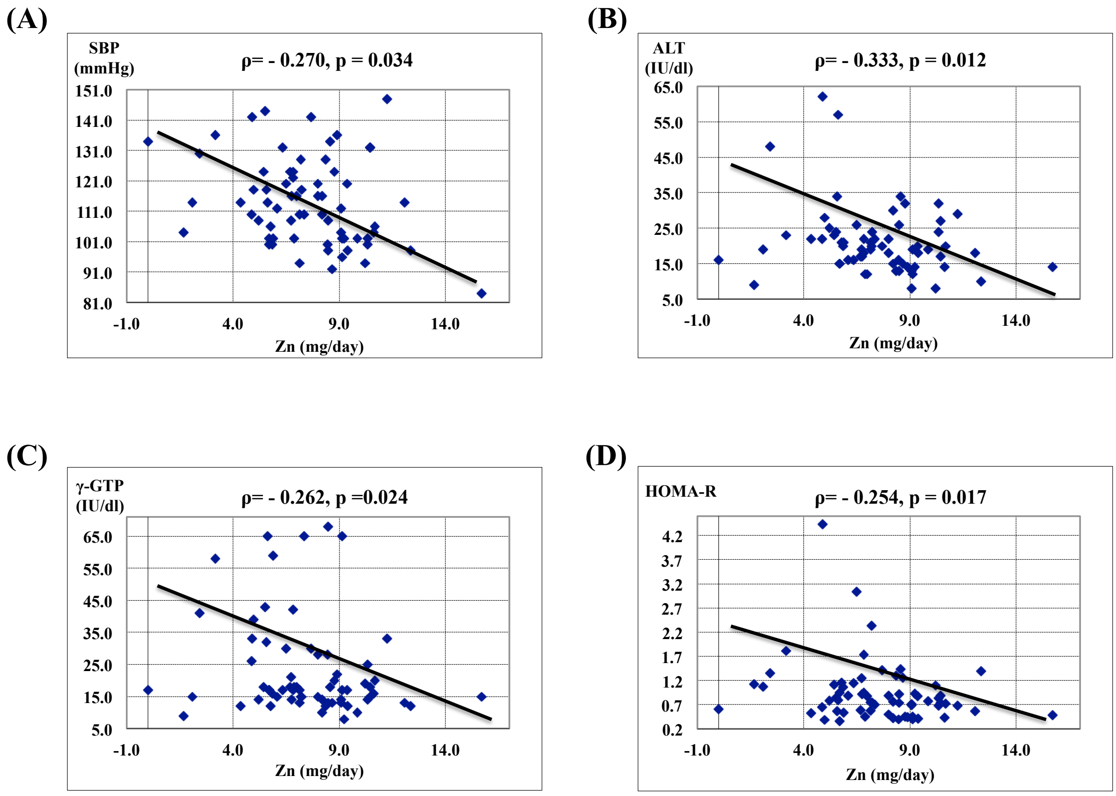
**Figure 1.**




## Figure legend

Figure 1. Anti-arteriosclerotic and hepatocyte-protective effects of zinc (Zn) in women.

Dietary intake of Zn reduces SBP (A), serum levels of ALT (B), and γ-GTP (C), as well as HOMA-R (D). ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HOMA-R, homeostasis model assessment-insulin resistance; SBP, systolic blood pressure.