



# Fish consumption, insulin sensitivity and beta-cell function in the Insulin Resistance Atherosclerosis Study (IRAS)

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## KEYWORDS

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**Abstract** *Background and aims:* Previous research on the association between fish consumption and incident type 2 diabetes has been inconclusive. In addition, few studies have investigated how fish consumption may be related to the metabolic abnormalities underlying diabetes. Therefore, we examined the association of fish consumption with measures of insulin sensitivity and beta-cell function in a multi-ethnic population.

*Methods and results:* We examined the cross-sectional association between fish consumption and measures of insulin sensitivity and secretion in 951 non-diabetic participants in the Insulin Resistance Atherosclerosis Study (IRAS). Fish consumption, categorized as <2 vs. ≥2 portions/week, was measured using a validated food frequency questionnaire. Insulin sensitivity ( $S_I$ ) and acute insulin response (AIR) were determined from frequently sampled intravenous glucose tolerance tests.

Higher fish consumption was independently associated with lower  $S_I$ -adjusted AIR ( $\beta = -0.13$  [−0.25, −0.016],  $p = 0.03$ , comparing ≥2 vs. <2 portions/week). Fish consumption was positively associated with intact and split proinsulin/C-peptide ratios, however, these associations were confounded by ethnicity (multivariable-adjusted  $\beta = 0.073$  [−0.014, 0.16] for intact proinsulin/C-peptide ratio,  $\beta = 0.031$  [−0.065, 0.13] for split proinsulin/C-peptide ratio). We also observed a significant positive association between fish consumption and fasting blood

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glucose (multivariable-adjusted  $\beta = 2.27$  [0.68, 3.86],  $p = 0.005$ ). We found no association between fish consumption and  $S_I$  (multivariable-adjusted  $\beta = -0.015$  [-0.083, 0.053]) or fasting insulin (multivariable-adjusted  $\beta = 0.016$  [-0.066, 0.10]).

**Conclusions:** Fish consumption was not associated with measures of insulin sensitivity in the multi-ethnic IRAS cohort. However, higher fish consumption may be associated with pancreatic beta-cell dysfunction.

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## Introduction

The global prevalence of type 2 diabetes is expected to rise from 6.4% in 2010 to 7.7% by 2030 [1]. Promoting diet and lifestyle modification remains a public health priority to slow the epidemic of type 2 diabetes. An ecological study of 41 countries on 5 continents showed that the prevalence of diabetes was lower in countries with higher fish consumption [2]. However, two recent meta-analyses of prospective cohort studies reported substantial geographical differences in the association between fish consumption and incident type 2 diabetes; studies from the US showed a higher risk whereas studies from Asian countries showed a lower risk [3,4].

Insulin resistance and beta-cell dysfunction are the two primary pathophysiological mechanisms underlying type 2 diabetes. Observational evidence on the association between fish consumption and these important metabolic traits is scarce. A single cross-sectional analysis of the ATTICA study has not provided evidence for an association of fish consumption with insulin sensitivity or beta-cell function measured by surrogate methods, including homeostasis model assessment–insulin resistance (HOMA-IR) and HOMA-beta cell function (HOMA-B) [5].

A number of clinical studies have examined the effects of fish or components of fish on insulin sensitivity or secretion in different populations. Dietary intake of oily fish, compared with dietary intake of red meat, improved insulin sensitivity in women with iron-deficiency [6]. A diet rich in cod protein, compared with that rich in beef, pork, veal, eggs, and milk products, improved insulin sensitivity in individuals with insulin resistance [7]. In a randomized controlled trial, treatment with omega-3-acid ethyl esters, compared with placebo, modestly reduced pancreatic beta-cell response in individuals with hypertriglyceridemia [8]. However, a meta-analysis of 11 randomized controlled trials showed that oral supplementation of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), the primary fatty acids found in fish, had no effects on insulin sensitivity [9].

Exploring how fish consumption is associated with insulin sensitivity and beta-cell function could provide insights into which aspect of diabetes may be impacted pathophysiologically by this modifiable exposure. Therefore, our objective was to examine the association between fish consumption and direct measures of insulin sensitivity ( $S_I$ ) and acute insulin response (AIR) from a frequently sampled intravenous glucose tolerance test (FSIGTT), as well as proinsulin to C-peptide ratios, in the multi-ethnic cohort of the Insulin Resistance Atherosclerosis Study (IRAS).

## Methods

### Study population

The study population comprised participants in the IRAS, a multi-center epidemiological study designed to explore the relationships between insulin resistance, atherosclerosis and its known risk factors across different ethnic groups and various states of glucose tolerance. A detailed description of the study design and methods has been published [10]. The IRAS recruited 1625 participants from four clinical centers located in San Antonio, TX, San Luis Valley, CO, Oakland, CA and Los Angeles, CA, in the USA between October 1992 and April 1994. The institutional review boards approved the study protocol and all participants provided written informed consent. For the current cross-sectional analysis, we excluded those with prevalent diabetes at baseline ( $n = 537$ ), as well as those with missing values of fish consumption,  $S_I$  and AIR ( $n = 137$ ), leaving a final sample size of 951 participants.

### Data collection

The IRAS protocol included two visits which occurred approximately one week apart. Participants were asked before each visit to fast for 12 h, to abstain from alcohol and heavy exercise for 24 h, and to abstain from smoking the morning of the examination. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated as weight (kg) divided by height ( $m^2$ ). Waist and hip circumferences were measured to the nearest 0.5 cm using a steel tape. Blood pressures were measured using a standard mercury sphygmomanometer. All anthropometric and blood pressure measurements were taken in duplicate and the averages of these measurements were used in the analyses. Demographics (age, sex, ethnicity, education, income) and lifestyle factors (smoking, alcohol consumption) were collected in questionnaires by self-report. Energy expenditure was estimated from information on physical activity in home, workplace and leisure environments collected from a validated questionnaire [10].

### Measurement of fish consumption

At baseline, participants completed a semi-quantitative food frequency questionnaire (FFQ), administered by centrally trained interviewers. The FFQ was designed to assess usual dietary intake for the past year. The 114-item FFQ was

adapted from the National Cancer Institute Health Habits and History Questionnaire (NCI-HHHQ) and the food items were expanded to reflect the diet of the diverse IRAS study populations [11]. Nutrient intakes from the FFQ were analyzed using the HHHQ-DIETSYS analysis software (version 3.0, 1993; National Cancer Institute, Bethesda, MD). The FFQ was validated against a series of eight 24-h dietary recalls [11]. The FFQ included nine frequency options, ranging from “never or less than once a month” to “six or more times per day”. Participants were asked to indicate how often they consume each item on the FFQ and to specify the portion size consumed, i.e., small, medium or large. To calculate the intake of each item, the frequency of intake was weighed by the portion size indicated using a factor of 0.5, 1 and 1.5 for small, medium and large, respectively.

The FFQ included three questions regarding the consumption of oily fish, lean fish and fried fish. Fish consumption was defined as the summation of average intake of oily fish, lean fish and fried fish. We divided fish consumption into two categories: <2 portions per week and  $\geq 2$  portions per week, based on the dietary recommendations of the American Heart Association [12] and the American Diabetes Association [13].

### Measurement of insulin sensitivity and beta-cell function

Insulin sensitivity and beta-cell function were measured using an FSIGTT, with two modifications from the original protocol. First, insulin, instead of tolbutamide, was injected to ensure adequate levels of plasma insulin to calculate insulin sensitivity accurately across a broad range of glucose tolerance. Second, a reduced sampling protocol, using 12 instead of 30 samples, was used for pragmatic reasons. Insulin resistance, expressed in  $S_I$ , was calculated using mathematical modeling methods (MINMOD version 3.0). A lower value of  $S_I$  suggests a decrease in insulin sensitivity. AIR was defined as the average increase in plasma insulin at time points 2 and 4 min after infusing glucose. A higher value of AIR indicates an increase in insulin secretion. Plasma insulin level was determined with the dextran-charcoal radioimmunoassay. Fasting serum intact proinsulin and split proinsulin were determined by means of highly specific two-site monoclonal antibody-based immunoradiometric assays. The values of intact proinsulin and split proinsulin were divided by fasting C-peptide to assess disproportionate hypoproinsulinemia [14]. Higher values of intact or split proinsulin/C-peptide ratios reflect a worsening of beta-cell function.

### Biochemical analysis

Plasma glucose was measured using the glucose oxidase technique on an auto-analyzer. Measurements of plasma lipids and lipoproteins were determined at the central IRAS laboratory using the Lipid Research Clinics methods [10].

### Statistical analysis

Characteristics of participants at baseline, including median and interquartile range for continuous variables

and percentages for categorical variables, were described. We used analysis of variance, Kruskal–Wallis tests and Chi-squared tests to test whether continuous and categorical variables differed across the distribution of fish consumption. We modeled measures of insulin sensitivity and beta-cell function, including  $S_I$ , fasting insulin,  $S_I$ -adjusted AIR, intact proinsulin/C-peptide ratio, split proinsulin/C-peptide ratio, as continuous outcome variables. The distribution of these outcome variables was skewed, therefore, we natural log-transformed them for normality. For  $S_I$ , we added a constant of 1 to all values before the log-transformation because of the presence of 0 values in the data. We used unadjusted and multivariable-adjusted linear regression to explore the association between fish consumption and each of the measures of insulin sensitivity and beta-cell function.

We included covariates in multivariable models if they were associated with both the exposure and the outcome, or if they were of *a priori* clinical relevance. Potential confounders included age, sex, ethnicity, education, income, smoking, alcohol consumption, energy expenditure, family history of diabetes, BMI, WHR, and dietary intakes of energy, protein, fat, fruits and vegetables. We examined the interaction between fish consumption and sex, ethnicity or median of BMI on all outcome variables. We tested whether the associations between fish consumption and measures of insulin sensitivity and beta-cell function differed by ethnicity using the  $\chi^2$  tests. In sensitivity analysis, we repeated the multivariable-adjusted regression analyses by modeling oily or lean or fried fish consumption as the exposure to assess whether the association between fish consumption and measures of insulin sensitivity and beta-cell function differed by the type of fish or the preparation method. Statistical analyses were performed using STATA 12.0 (StataCorp, College Station, TX).

### Results

In the IRAS cohort, the average age of the participants was 55 years (range 40–69 years); 55% were women; and the ethnicity distribution was 40% for Caucasians, 25% for African–Americans and 35% for Hispanics. The median fish consumption was 1.1 portions per week. Approximately 27% of the participants reported eating  $\geq 2$  portions of fish per week. The median intake of lean, oily and fried fish was 0.25, 0.38 and 0.13 portions per week. Substantial ethnic differences existed in the pattern of fish consumption. More African–Americans reported eating fish regularly (33% for African–Americans vs. 24% for Caucasians and 26% for Hispanics). Caucasians had higher lean fish consumption than Hispanics (9% vs. 4% for  $\geq 2$  portions/week). There was no significant difference in the measures of insulin sensitivity between participants with high or low intake of fish. Frequent consumption of fish was associated with higher values of intact proinsulin/C-peptide ratio and fasting blood glucose, and lower value of  $S_I$ -adjusted AIR. Among Caucasians, those who ate more fish reported to have higher income, were more likely to drink alcohol, and had higher dietary intake of fruits, vegetables, energy and fat (Table 1).

Unadjusted linear regression analysis revealed an inverse association of fish consumption with  $S_I$ -adjusted AIR

( $\beta = -0.13$  [ $-0.24, -0.021$ ],  $p = 0.02$ , comparing  $\geq 2$  vs.  $< 2$  portions/week). After adjusting for age, sex, education level, income, smoking, alcohol consumption, energy expenditure, family history of diabetes, dietary intake of fruits, vegetables, protein, fat and energy, ethnicity, BMI and waist-hip ratio (WHR), the association did not change materially ( $\beta = -0.13$  [ $-0.25, -0.016$ ],  $p = 0.03$ ). We observed a positive association between fish consumption and intact proinsulin/C-peptide ratio ( $\beta = 0.10$  [ $0.021, 0.19$ ],  $p = 0.01$ ) in unadjusted regression models. After adjusting for potential confounders, the associations remained statistically significant ( $\beta = 0.11$  [ $0.024, 0.20$ ],  $p = 0.01$ ). However, further adjustment of ethnicity attenuated the association to non-significance ( $\beta = 0.073$  [ $-0.014, 0.16$ ]). Fish consumption was positively associated with split proinsulin/C-peptide ratio in unadjusted ( $\beta = 0.10$  [ $0.0035, 0.20$ ],  $p = 0.04$ ), but not in multivariable-adjusted ( $\beta = 0.031$  [ $-0.065, 0.13$ ]) models. We also observed that higher fish consumption was associated with higher fasting blood glucose in unadjusted ( $\beta = 2.68$  [ $1.06, 4.29$ ],  $p = 0.001$ ) and multivariable-adjusted ( $\beta = 2.27$  [ $0.68, 3.86$ ],  $p = 0.005$ ) models. However, fish consumption was not associated with measures of insulin sensitivity ( $S_I$  and fasting insulin) or 2-h post-load blood glucose (Table 2).

There was no interaction between fish consumption and sex, ethnicity and BMI status on  $S_I$ -adjusted AIR, fasting insulin or  $S_I$ . However, we observed a significant interaction between fish consumption and ethnicity on the intact proinsulin/C-peptide ratio ( $p = 0.05$ ) and split proinsulin/C-peptide ratio ( $p = 0.02$ ). In stratified analysis, we observed significant ethnic differences in the association of fish consumption with measures of insulin secretion including  $S_I$ -adjusted AIR, intact and split proinsulin/C-peptide ratios, and fasting blood glucose. Specifically, higher fish consumption was significantly associated with lower  $S_I$ -adjusted AIR, higher intact and split proinsulin/C-peptide ratios and higher fasting blood glucose only among Caucasians. There were no significant associations between fish consumption and measures of insulin sensitivity and beta-cell function among African-Americans or Hispanics (Table 3).

In sensitivity analysis, lean fish consumption was positively associated with fasting blood glucose ( $\beta = 4.25$  [ $1.58, 6.92$ ]), whereas oily fish consumption was inversely associated with  $S_I$ -adjusted AIR ( $\beta = -0.20$  [ $-0.37, -0.034$ ]) and positively associated with fasting blood glucose ( $\beta = 3.30$  [ $0.97, 5.62$ ]). When stratified the regression analyses by ethnicity, higher lean fish consumption was associated with lower  $S_I$ -adjusted AIR

**Table 1** –Baseline characteristics of non-diabetic participants in the IRAS by distribution of fish consumption.

| Fish consumption                                                                      | Whole study population |                               | Caucasians       |                               |
|---------------------------------------------------------------------------------------|------------------------|-------------------------------|------------------|-------------------------------|
|                                                                                       | <2 portions/week       | $\geq 2$ portions/week        | <2 portions/week | $\geq 2$ portions/week        |
| N (%)                                                                                 | 695 (73.1)             | 256 (26.9)                    | 292 (76.0)       | 92 (24.0)                     |
| Age (years)                                                                           | 54.8 $\pm$ 8.5         | 54.6 $\pm$ 8.3                | 55.8 $\pm$ 8.5   | 55.0 $\pm$ 7.9                |
| Male (%)                                                                              | 43.7                   | 48.8                          | 47.3             | 53.3                          |
| Education > high school completed (%)                                                 | 57.7                   | 64.8 <sup>a</sup>             | 72.6             | 75.0                          |
| Income $\geq$ US\$40,000/year (%)                                                     | 43.0                   | 52.7 <sup>a</sup>             | 52.1             | 70.7 <sup>a</sup>             |
| Current smoker (%)                                                                    | 17.1                   | 14.5                          | 12.3             | 6.5                           |
| Current drinker <sup>b</sup> (%)                                                      | 72.0                   | 80.9 <sup>a</sup>             | 73.6             | 87.0 <sup>a</sup>             |
| Total energy expenditure (kcal/kg/day)                                                | 38.4 (35.4–44.0)       | 38.7 (35.8–42.2)              | 38.4 (35.2–43.3) | 38.6 (36.0–41.7)              |
| Family history of diabetes (%)                                                        | 38.4                   | 41.0                          | 27.1             | 30.4                          |
| Body mass index (kg/m <sup>2</sup> )                                                  | 28.2 $\pm$ 5.6         | 28.9 $\pm$ 5.6                | 27.5 $\pm$ 5.2   | 27.9 $\pm$ 5.0                |
| Waist-hip ratio                                                                       | 0.86 $\pm$ 0.09        | 0.87 $\pm$ 0.08               | 0.86 $\pm$ 0.09  | 0.87 $\pm$ 0.08               |
| Fruits (portions/day)                                                                 | 1.13 (0.54–2.03)       | 1.76 (0.96–2.72) <sup>a</sup> | 1.16 (0.60–2.03) | 1.60 (0.93–1.44) <sup>a</sup> |
| Vegetables (portions/day)                                                             | 2.29 (1.47–3.32)       | 3.21 (2.37–4.60) <sup>a</sup> | 2.46 (1.60–3.52) | 3.32 (2.61–4.58) <sup>a</sup> |
| Energy intake (kcal/day)                                                              | 1805 $\pm$ 780         | 2152 $\pm$ 864 <sup>a</sup>   | 1772 $\pm$ 760   | 2036 $\pm$ 721 <sup>a</sup>   |
| Protein intake (g/day)                                                                | 73.1 $\pm$ 32.0        | 91.5 $\pm$ 37.6 <sup>a</sup>  | 73.8 $\pm$ 32.0  | 89.2 $\pm$ 32.3 <sup>a</sup>  |
| Fat intake (g/day)                                                                    | 72.2 $\pm$ 37.4        | 83.6 $\pm$ 41.5 <sup>a</sup>  | 69.1 $\pm$ 36.7  | 75.9 $\pm$ 32.3               |
| Insulin sensitivity ( $\times 10^{-4}$ min <sup>-1</sup> [ $\mu$ U/l] <sup>-1</sup> ) | 1.62 (0.91–2.90)       | 1.47 (0.76–3.01)              | 1.89 (1.15–3.29) | 1.98 (0.98–3.43)              |
| Fasting insulin (pmol/l)                                                              | 13 (9–18)              | 13 (9–20)                     | 11 (8–15)        | 12 (8–17)                     |
| Intact proinsulin (pmol/l)/C-peptide                                                  | 8.7 (6.4–12.9)         | 9.6 (6.8–14.1) <sup>a</sup>   | 8.0 (5.9–10.9)   | 9.4 (6.7–13.8) <sup>a</sup>   |
| Split proinsulin (pmol/l)/C-peptide                                                   | 11.1 (7.6–16.5)        | 11.9 (8.1–17.4)               | 9.6 (6.8–14.1)   | 11.4 (7.6–17.5) <sup>a</sup>  |
| Acute insulin response ( $\mu$ U/ml)                                                  | 52.5 (30.0–86.0)       | 47.5 (24.0–80.5) <sup>a</sup> | 44.0 (25.0–73.0) | 29.0 (20.0–58.0) <sup>a</sup> |
| Fasting glucose (mmol/l)                                                              | 5.4 (5.0–5.8)          | 5.5 (5.1–6.0) <sup>a</sup>    | 5.4 (5.0–5.8)    | 5.5 (5.0–6.2) <sup>a</sup>    |
| 2-h post-load glucose (mmol/l)                                                        | 6.8 (5.5–8.2)          | 6.8 (5.3–8.3)                 | 6.8 (5.4–8.1)    | 6.8 (5.3–8.4)                 |

Data in means  $\pm$  standard deviations or percentages or medians (interquartile ranges).

<sup>a</sup>  $p < 0.05$ .

<sup>b</sup> Current drinker refers to individuals who reported to drink any amount of alcohol currently when the questionnaire was administered.

**Table 2** Estimated regression coefficients (95% confidence intervals) on the association between fish consumption and measures of insulin sensitivity and beta-cell function.

|                                                     | <i>b</i> -coefficient (95% CI) | <i>p</i> -value |
|-----------------------------------------------------|--------------------------------|-----------------|
| Log insulin sensitivity                             |                                |                 |
| Unadjusted                                          | −0.031 (−0.11, 0.047)          | 0.436           |
| Model 1                                             | −0.030 (−0.11, 0.047)          | 0.444           |
| Model 2                                             | −0.039 (−0.12, 0.043)          | 0.350           |
| Model 3                                             | −0.031 (−0.12, 0.043)          | 0.363           |
| Model 4                                             | −0.015 (−0.083, 0.053)         | 0.664           |
| Log fasting insulin                                 |                                |                 |
| Unadjusted                                          | 0.071 (−0.019, 0.16)           | 0.124           |
| Model 1                                             | 0.070 (−0.020, 0.16)           | 0.128           |
| Model 2                                             | 0.053 (−0.041, 0.15)           | 0.270           |
| Model 3                                             | 0.037 (−0.044, 0.12)           | 0.373           |
| Model 4                                             | 0.016 (−0.066, 0.10)           | 0.708           |
| Log S <sub>1</sub> -adjusted acute insulin response |                                |                 |
| Unadjusted                                          | −0.13 (−0.24, −0.021)          | 0.020           |
| Model 1                                             | −0.13 (−0.24, −0.026)          | 0.015           |
| Model 2                                             | −0.10 (−0.22, −0.013)          | 0.083           |
| Model 3                                             | −0.10 (−0.22, 0.011)           | 0.077           |
| Model 4                                             | −0.13 (−0.25, −0.016)          | 0.025           |
| Log intact proinsulin/C-peptide ratio               |                                |                 |
| Unadjusted                                          | 0.10 (0.021, 0.19)             | 0.014           |
| Model 1                                             | 0.094 (0.010, 0.18)            | 0.028           |
| Model 2                                             | 0.12 (0.032, 0.21)             | 0.008           |
| Model 3                                             | 0.11 (0.024, 0.20)             | 0.012           |
| Model 4                                             | 0.073 (−0.014, 0.16)           | 0.100           |
| Log split proinsulin/C-peptide ratio                |                                |                 |
| Unadjusted                                          | 0.10 (0.0035, 0.20)            | 0.042           |
| Model 1                                             | 0.088 (−0.0079, 0.18)          | 0.072           |
| Model 2                                             | 0.087 (−0.015, 0.19)           | 0.094           |
| Model 3                                             | 0.073 (−0.024, 0.17)           | 0.141           |
| Model 4                                             | 0.031 (−0.065, 0.13)           | 0.522           |
| Fasting blood glucose                               |                                |                 |
| Unadjusted                                          | 2.68 (1.06, 4.29)              | 0.001           |
| Model 1                                             | 2.48 (0.89, 4.07)              | 0.002           |
| Model 2                                             | 2.78 (1.10, 4.47)              | 0.001           |
| Model 3                                             | 2.56 (0.98, 4.15)              | 0.002           |
| Model 4                                             | 2.27 (0.68, 3.86)              | 0.005           |
| 2-h post-load blood glucose                         |                                |                 |
| Unadjusted                                          | −0.38 (−5.26, 4.50)            | 0.880           |
| Model 1                                             | −0.061 (−4.86, 4.74)           | 0.980           |
| Model 2                                             | 0.78 (−4.31, 5.88)             | 0.763           |
| Model 3                                             | 0.088 (−4.66, 4.84)            | 0.971           |
| Model 4                                             | 0.085 (−4.71, 4.88)            | 0.972           |

Model 1 – adjusted for age and sex; Model 2 – Model 1 + education, income, smoking, alcohol consumption, caloric expenditure, family history of diabetes, dietary factors (kcal, protein, fat, fruits, vegetables); Model 3 – Model 2 + BMI, WHR; Model 4 – Model 3 + ethnicity.

( $\beta = -0.31 [-0.58, -0.043]$ ) and higher fasting blood glucose ( $\beta = 7.10 [3.31, 10.9]$ ) in Caucasians. Similarly, oily fish consumption was associated with lower S<sub>1</sub>-adjusted AIR ( $\beta = -0.28 [-0.54, -0.010]$ ), higher intact ( $\beta = 0.28 [0.080, 0.47]$ ) and split ( $\beta = 0.25 [0.012, 0.48]$ ) proinsulin/C-peptide ratios and higher fasting blood

glucose ( $\beta = 5.51 [1.74, 9.28]$ ) only among Caucasians. We found no consistent patterns of association between fried fish consumption and measures of insulin sensitivity and beta-cell function (data not shown).

## Discussion

In the multi-ethnic IRAS cohort, fish consumption was not associated with measures of insulin sensitivity, including fasting insulin and S<sub>1</sub>. However, Caucasians who consumed 2 or more portions of fish per week were likely to have lower S<sub>1</sub>-adjusted AIR, higher intact and split proinsulin/C-peptide ratios, and higher fasting blood glucose than those who consumed fewer than 2 portions per week. These associations remained after adjusting for potential confounders, including demographic, socio-economic, clinical, lifestyle and dietary factors. We did not observe any significant association between fish consumption and measures of beta-cell function among Africans–Americans and Hispanics.

Very few epidemiological studies have investigated the association between fish consumption and insulin sensitivity and beta-cell function. A cross-sectional analysis of the ATTICA study reported that fish consumption was positively associated with insulin resistance or insulin secretion; however, these associations were subjected to confounding by age, sex and BMI in a Greek cohort without diabetes. This study used less precise surrogate measures of insulin sensitivity and beta-cell function including fasting insulin, HOMA-IR and HOMA-B [5]. Our study extends the literature on fish consumption and diabetes by describing the association of fish consumption with insulin sensitivity and beta-cell function using direct and multiple measures of these important metabolic traits underlying type 2 diabetes in a well-characterized cohort of Caucasians, African–Americans and Hispanics.

Similar to the result of the ATTICA study [5], we did not observe a significant association between total, lean or oily fish consumption and measures of insulin sensitivity. In most animal studies, n-3 LC-PUFAs have been shown to improve insulin sensitivity through mechanisms such as activating peroxisome proliferator-activated receptor- $\alpha$ , decreasing tumor necrosis factor- $\alpha$  or stimulating insulin signaling cascade [15,16]. Clinical trials examining the effects of n-3 LC-PUFAs on insulin resistance have been conducted but collectively did not show an effect [9]. Previous observational studies on plasma n-3 LC-PUFAs, a biomarker for fish consumption, and insulin sensitivity have reported inconsistent results [17,18].

In the IRAS, higher fish consumption was significantly associated with beta-cell dysfunction, as indicated by decreased S<sub>1</sub>-adjusted AIR and elevated proinsulin/C-peptide ratios. We observed similar significant associations when modeling lean or oily fish consumption as the exposure. Evidence regarding fish consumption and beta cell function is scarce. The ATTICA epidemiological study did not observe an association between fish consumption and insulin secretion after accounting for potential confounders [5]. Clinical studies in human on n-3 LC-PUFAs showed either no significant effect on insulin secretion [19,20] or a reduced beta-cell response [8].

**Table 3** Estimated regression coefficients (95% confidence intervals) on the association between fish consumption and measures of insulin sensitivity and beta-cell function, stratified by ethnicity.

|                                 | Fish consumption ( $\geq 2$ portions/week vs. $< 2$ portions/week) |                       |                       | $P_{\text{ethnic difference}}$ |
|---------------------------------|--------------------------------------------------------------------|-----------------------|-----------------------|--------------------------------|
|                                 | Caucasians                                                         | African-Americans     | Hispanics             |                                |
| Log insulin sensitivity         | -0.022 (-0.13, 0.088)                                              | -0.072 (-0.20, 0.057) | 0.049 (-0.073, 0.17)  | 0.399                          |
| Log fasting insulin             | 0.087 (-0.046, 0.22)                                               | 0.077 (-0.081, 0.24)  | -0.088 (-0.23, 0.57)  | 0.711                          |
| Log $S_I$ -adjusted AIR         | -0.24 (-0.42, -0.056) <sup>a</sup>                                 | 0.034 (-0.21, 0.28)   | -0.12 (-0.30, 0.071)  | 0.584                          |
| Log intact proinsulin/C-peptide | 0.22 (0.085, 0.36) <sup>a</sup>                                    | -0.010 (-0.20, 0.18)  | -0.053 (-0.20, 0.093) | 0.019                          |
| Log split proinsulin/C-peptide  | 0.22 (0.057, 0.38) <sup>a</sup>                                    | -0.088 (-0.28, 0.099) | -0.11 (-0.27, 0.051)  | 0.006                          |
| Fasting blood glucose           | 4.50 (1.95, 7.06) <sup>a</sup>                                     | 2.61 (-0.86, 6.08)    | -0.46 (-2.98, 2.05)   | 0.024                          |
| 2-h post-load blood glucose     | 4.22 (-3.60, 12.0)                                                 | -2.48 (-11.2, 6.26)   | -3.11 (-11.9, 5.6)    | 0.383                          |

Multivariable-adjusted regression models adjusted for age, sex, education, income, smoking, alcohol consumption, caloric expenditure, family history of diabetes, dietary factors (kcal, protein, fat, fruits, vegetables), BMI and WHR.

<sup>a</sup>  $p < 0.05$  comparing  $\geq 2$  portions/week vs.  $< 2$  portion/week.

Potential mechanisms for the association between fish consumption and beta-cell dysfunction are unclear, and this observation may reflect a chance finding. Oral supplementation of n-3 LC-PUFAs has been shown to increase fasting blood glucose modestly in individuals with diabetes [21,22], possibly through mechanisms involving reduced response of pancreatic beta-cells to glucose-stimulated insulin secretion [23]. Alternatively, environmental contaminants, including methyl-mercury, dioxins and persistent organic pollutants (POPs) may play a role. Fish is a source of human exposure of these contaminants. Emerging evidence suggested that POPs predict the risk of incident diabetes [24,25]. Mechanistically, the oxidative stress induced by methyl-mercury has been shown to cause pancreatic beta-cell death and dysfunction in a mouse model [26]. Furthermore, POPs may impair beta-cell function as pancreatic islet cells are highly susceptible to oxidative damage [27]. Our findings that fish consumption is associated with beta-cell dysfunction and elevated fasting blood glucose provide a possible explanation for the positive associations between fish consumption and incident type 2 diabetes reported by previous studies [28–30].

We observed significant associations of fish consumption with beta-cell dysfunction and elevated fasting blood glucose in Caucasians only, and these associations were present regardless of the type of fish consumed. Of note is that all of the prospective studies that reported a positive association between fish consumption and incident type 2 diabetes were conducted in predominantly Caucasian populations [28–30]. Dietary habits and food choices vary across ethnic groups. Fish consumption may be associated with some ethnic-specific dietary patterns or lifestyle behaviors that modify the risk of beta-cell dysfunction. Ethnicity may also define the source and the type of fish consumed, based on locally-available fish species or traditional food culture. We attempted to account for a wide range of potential confounders including demographic, socioeconomic, clinical, lifestyle and dietary factors. However, we could not rule out the possibility of residual confounding. Alternatively, differential accuracy in the assessment of dietary intake across ethnic groups may play a role.

The strengths of our study are the well-characterized multi-ethnic cohort, and the detailed and direct

measurement of insulin sensitivity and beta-cell function. Our study also has potential limitations. First, the cross-sectional design did not allow us to explore the temporal relationship between fish consumption and measures of insulin sensitivity and secretion. Second, fish consumption was measured by self-report and participants had to estimate the frequency and portions of fish consumed which could introduce random measurement errors and bias the observed associations toward null. Although systematic differences in reporting dietary intake based on weight status or social desirability could bias the results in either direction, we did not observe an association between fish consumption and BMI, suggesting that reporting bias is unlikely to have occurred. Third, we have no information on environmental contaminants. Finally, our findings may apply to individuals with the demographic characteristics of these study populations and may not be generalized to populations with other ethnic backgrounds or with high fish consumption.

In summary, we found no evidence of an independent association between fish consumption and measures of insulin sensitivity in the multi-ethnic cohort of the IRAS. However, higher fish consumption may be associated with beta-cell dysfunction. Randomized controlled trials are needed to explore the causal relationships between fish consumption and these important metabolic traits.

## Conflict of interest

The authors declare that there is no conflict of interest associated with this manuscript.

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