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Fangzhou Chi Date

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Fish Intake and Risk for Incident, Sporadic Colorectal Adenomatous Polyps

By

Fangzhou Chi

MPH

Epidemiology

_____ [Chair's signature]

Roberd M. Bostick, M. D. M. P. H.

Committee Chair

Fish Intake and Risk for Incident, Sporadic Colorectal Adenomatous Polyps

By

Fangzhou Chi

Bachelor's Degree in Chinese Medicine

Shanghai University of Chinese Medicine

2011

Thesis Committee Chair: Roberd M. Bostick, M. D., M. P. H.

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in Epidemiology

2014

Abstract

Fish Intake and Risk for Incident, Sporadic Colorectal Adenomatous Polyps

By, Fangzhou Chi

Background: Previous studies have investigated an association between fish intake and risk for colorectal cancer, but a possible relationship between fish consumption and the precursor of colorectal cancer, adenomatous polyps, is unknown.

Purpose: To investigate an association between fish intake and risk of incident, sporadic colorectal adenomatous polyps, alone and according to other risk factors.

Methods: This case-control study was part of the Minnesota Cancer Prevention Research Unit (CPRU) program. Cases (n = 564) were patients aged 30 to 74 years with pathology-confirmed adenomatous polyps of the colon or rectum. Two control groups were recruited: colonoscopy-negative controls (n = 1,202) were colonoscopy/sigmoidoscopy-confirmed polyp-free participants, and community (CM) controls (n = 535) were subjects randomly selected from the state driver's license registry and frequency-matched to the cases on age, sex, and zip code. Data on dietary intake were collected using a modified, 153-item Willett food frequency questionnaire prior to colonoscopy.

Results: The multivariate-adjusted odds ratios and 95% confidence intervals (CI) for the second through the fourth quartiles of fish intake were, respectively, 1.29 (95% CI 0.91, 4.82), 0.88 (95% CI 0.62, 1.23), and 1.28 (95% CI 0.87, 4.88) in comparison with the colonoscopy-negative controls, and were similar to those in relation to the community controls. The association did not differ substantially according to other risk factors.

Conclusions: These results do not support the hypothesis that greater fish intake may reduce risk for incident, sporadic colorectal adenoma, although a possible modest U-shaped association cannot be ruled out.

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Table of Contents

CHAPTER I: BACKGROUND AND LITERATURE REVIEW

Introduction

In the United States, colorectal cancer is the third most common cancer and the third most frequent cause of cancer deaths in both men and women (1). Despite the high morbidity and mortality colorectal cancer is preventable. Evidence for this includes that several established or suspected risk factors for colorectal cancer are potentially modifiable. Diet is one of these important risk factors. Also, most colorectal cancers are preceded by precursor lesions, called adenomatous polyps, which can be identified by colonoscopy and removed.

Colorectal cancer

Descriptive epidemiology

Colorectal cancer is a cancer that forms in the tissues of colon or rectum. Approximately 95 percent of colorectal cancers are adenocarcinomas (2). It is the third most common type of cancer worldwide, with 1. 2 million cases reported in 2008. It is somewhat more common in men than in women. Incidence rates vary more than 20-fold around the world. It is much more common in high-income countries (1). Rates also vary by race and ethnic status with highest rates among Caucasians of northern European origin (3). Dietary and other environmental differences attribute to most of the international variation in rates. Migration studies also show that the risk for the disease is sensitive to environment changes (3). The fact that colon cancer is more frequent in some families and that some rare genetic syndromes are associated

with excess risk of colon cancer suggests that this is a disease subject to both genetic variation and environmental exposures.

Analytic epidemiology

The focus of most analytic studies considering diet and colorectal cancer has been on meat, fat, fruits, vegetables, fiber, calcium and vitamin D intakes, and alcohol consumption.

Meat

Overall red and processed meats are positively associated with risk of colorectal cancer. In a large prospective cohort study of 88,751 nurses 34 to 59 years old without a history of cancer, red meat intake was associated with a 2.5-fold increase in the risk of colon cancer (4). Another cohort study on 47,949 male health professionals 40 to 75 year of age also reported a statistically significantly increased risk of colon cancer in men who consumed more red meat (5). Two other cohort studies observed elevated risk with higher consumption of processed meat (6,7). Comparable findings have been seen in most case-control studies (8, 9). However, one large mortality follow-up study by American Cancer Society found no association between risk of colorectal cancer death and meat consumption (10). Another population-based case-control study in Sweden did not observe an association between colorectal cancer risk and meat preparation methods (11). Nimptsch *et al.*, investigated an association between meat and fish intake and risk of colorectal adenoma among 19,771 women in the Nurses Health Study II and found that red meat intake during adolescence was not associated with colorectal adenoma risk. Replacement of

one serving per day of red meat with one serving per day of fish was associated with an approximately 40 percent lower risk of adenomas (12).

Fruits and vegetables

Six cohort studies (13-18) and almost all case-control studies (13, 14) that investigated an association between vegetable and fruit intake and colorectal cancer risk reported modestly lower risk with higher consumption of vegetables or fruit. However, a prospective study among 88, 776 women and 47, 325 men found no association of intakes of fruit or vegetables with the risk of colorectal cancer (19). This difference may suggest that vegetable and fruit intake may be related to dietary patterns rather than simply to intakes of fruit and vegetables.

Dietary fiber

Reported associations of dietary fiber with risk of colorectal cancer have been inconsistent. A role of fiber was first proposed by Dennis Burkitt based on observations in Africa (20). A combined analysis based on 13 case-control studies found lower risk for colorectal cancer with higher intakes of dietary fiber (21). However, two cohort studies found no association (22,23). This discrepancy may be due to he heterogeneous nature of fiber and different ways of measuring of fiber intake.

Alcohol consumption

Four population-based cohort studies and about half of the case-control studies found higher risk with higher alcohol consumption (24). A dose-response association was also observed in a pooled cohort analysis (25).

This association was likely to be related to total ethanol intake, with no differences by type of alcoholic drink (24).

Calcium and vitamin D

One double-blinded, randomized clinical trial comparing 1,200 mg of calcium per day with placebo (26), one large case-control study comparing 1,993 cases and 2,410 population-based controls (27), and an intervention study investigating calcium supplements and colorectal epithelial cell proliferation rate found a reduced risk (28). Data from these studies further suggested that there was an interaction between vitamin D and calcium. Two studies found that higher calcium and vitamin D intakes were associated with lower risk of adenoma (29, 30). On the other hand, the WHI Calcium plus Vitamin D Supplementation Trial found no effect of calcium and vitamin D on colorectal cancer incidence; however, this trial had substantial levels of treatment drop in and drop out, making interpretation of its results problematic. (31).

Molecular basis of colorectal cancer

Colorectal cancer progress follows the adenoma-carcinoma sequence, which begins from abnormalities in the normal mucosa and developing into adenomas and cancer. Two heritable syndromes, Familial adenomatous polyposis (FAP), and Hereditary nonpolyposis colorectal cancer (HNPCC), provide a basis for research in the genetic pathways of colorectal cancer. Previous studies reported that it was the mutations in tumor-suppressor genes, oncogenes, and DNA repair genes, as well as other epigenetic changes such as methylation of DNA, that causes the normal cells to become malignant.

Impaired APC pathway

APC gene mutations are common in colorectal neoplasms. Somatic mutations in the *APC* gene occur in sporadic colorectal cancers. In FAP, both a germ line mutation and a somatic mutation is seen. *APC* gene mutation can cause an impaired APC pathway, which leads to alterations in cell cycle control including: increased cell proliferation, decreased cell differentiation, apoptosis, and cell adhesion. These alterations can result in the development of early adenoma from normal epithelium (32).

Impaired mismatch repair (MMR) pathway

Mutation in MMR genes, e. g., *MSH2*, *MLH1*, and hypermethylation of MMR genes can cause microsatellite insatiability (MSI), which further causes increased proliferation and decreased differentiation and apoptosis in the intestinal epithelium cells (32). This pathway can affect each step of colorectal cancer carcinogenesis.

Oncogenic mutation in *K-ras* gene

Mutations in the *K-ras* gene stimulate cell proliferation. They are rarely seen in adenoma smaller than 1 cm, which suggests that *K-ras* mutations occur later (after formation of early adenoma) in the pathogenesis of colorectal cancer (33).

Mutation in *TP53* gene

The *TP53* gene belongs to the tumor suppressor gene family. Normal function of p53 involves promoting the transcription of genes that regulate cell-cycle progression, apoptosis, and inhibiting angiogenesis. Mutations in *TP53* occur mainly in late adenomas (32).

Possible mechanisms for anti-cancer effects of fish (biological plausibility)

Omega-3 polyunsaturated fatty acids (PUFAs)

The most important component in fish that may explain its possible anti-cancer effect is omega-3 PUFA, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are found in highest quantities in oily fish such as sardines and mackerels. EPA and DHA can be defined as 'essential nutrients' because synthesizing omega-3 PUFAs is very low in the human body. Several molecular and cellular activities were composed to explain the mechanisms for the anti-neoplastic activity of omega-3 PUFAs. These include:

1. Anti-inflammatory activities

The anti-inflammatory activities of omega-3 PUFAs include two pathways. One is inhibition of cyclooxygenase-2 (COX-2) activity. The result is reduced synthesis of prostaglandins (PGs), which play an important role in the early stages of colorectal carcinogenesis (34, 35); another pathway is through the production of newly discovered anti-inflammatory mediators. EPA and DHA can produce the COX-2 dependent resolvin, especially in the presence of aspirin. Resolvin was found to reduce inflammation in animal models by inhibiting the production and transportation of inflammatory chemicals (36-38).

2. Production of reactive oxygen species (ROS) and increasing oxidative stress

Omega-3 PUFAs can generate ROS such as the superoxide radical and can increase intracellular ROS levels, which may induce cancer cell apoptosis (39).

3. Alteration of cell surface receptor function

There is evidence that omega-3 PUFAs can alter the membrane localization of the Ras protein by changing the structure and fluidity of the cell membrane. The membrane localization of Ras protein is critical in the regulation of cell growth, proliferation, and apoptosis. Omega-3 PUFAs can decrease the colonic localization of the Ras protein and reduce tumor formation in animal models (40, 41).

Calcium and vitamin D

One study suggested that the anti-cancer effect of fish may also due to the calcium and vitamin D contained in fish (42). Calcium and vitamin D have been investigated in numerous observational studies and clinical trials and found to be consistently associated with risk for colorectal cancer (43-47). However, the amount of calcium and vitamin D consumed through normal dietary fish intake may not be enough to exert an anti-cancer effect.

Association between fish intake and colorectal cancer

The results of studies that investigated an association between fish intake and colorectal cancer have been inconsistent. Five case-control studies (42, 48-51) and seven cohort studies (6, 12, 52-56) found inverse association of fish

intake with colorectal cancer. The strongest inverse association was observed in large multi-center case-control study undertaken in Italy by Franceschi *et al.*, who found an approximately 30% and 60% lower risk for colorectal cancer and colon cancer, respectively.

However, the results from one case-control study in Burgundy, France (57) and three large population-based cohort studies (58-60) reported no association between fish intake and colorectal cancer risk. One multi-center, randomized controlled intervention trial by Pot *et al.* (61) that tested the effects of a 6-month intervention with oil-rich fish or lean fish on apoptosis and mitosis (early biomarkers of colorectal cancer risk) within colonic crypts in the normal colon mucosa found that the increase in fish consumption did not markedly change apoptotic and mitotic rates in the colonic mucosa. In addition, in one cohort study, the Shanghai Womens' Health Study, cholesterol-rich fish, such as eel, shrimp, and shellfish were positively associated with colorectal cancer risk (62).

Several factors may contribute to the discrepancies in the association between fish consumption and colorectal cancer risk found in different studies. First, the amount, frequency, and type of fish consumed were different across studies. Some studies used servings of fish intake per week while some studies use the weight of fish consumed as a measure of fish intake amount. Some studies did not consider the difference between fish types whereas other studies divided fish into different types, for example, lean and oil-rich, fresh water and sea water fish and so on. Second, most studies did not mention the fish cooking

methods. Anti-cancer components in fish may undergo chemical changes due to cooking and lose their protective effect against cancer. On the other hand, some cooking methods, such as smoking, salting, and cooking at high temperature, can produce carcinogenic compounds, which may explain the elevated risk for colorectal cancer in some studies. Third, study endpoints are not consistent. Colorectal cancer, colon cancer, and rectal cancer were all defined as cases in examining associations with fish intake. Fourth, heterogeneity across the study populations may have also played a role in the discrepancies of study findings. Different populations tend to have different dietary habits, life styles, and different susceptibilities to cancer due to genetic variances.

Summary

Observational findings about the association between fish intake and colorectal cancer are inconsistent. No previous studies have investigated an association of fish intake with the precursor lesion of colorectal cancer, adenomatous polyps. The potential anti-cancer effect of fish may be mainly due to the n-3 polyunsaturated fatty acids contained in fish. Potential mechanisms involve anti-inflammatory activities, reducing ROS levels, and altering cell surface receptor function. Future studies should investigate an association between fish intake and adenomas, the exact mechanism underlying anti-cancer effect of fish or n-3 PUFAs, and the appropriate amount of fish consumption or effective dose of EPA or DHA intake.

CHAPTER II: MANUSCRIPT

Title: Fish Intake and colorectal adenomatous polyps

Author: Fangzhou Chi, Roberd M. Bostick

ABSTRACT

Background: Previous studies have investigated an association between fish intake and risk for colorectal cancer, but a possible relationship between fish consumption and the precursor of colorectal cancer, adenomatous polyps, is unknown.

Purpose: To investigate an association between fish intake and risk of incident, sporadic colorectal adenomatous polyps, alone and according to other risk factors.

Methods: This case-control study was part of the Minnesota Cancer Prevention Research Unit (CPRU) program. Cases (n = 564) were patients aged 30 to 74 years with pathology-confirmed adenomatous polyps of the colon or rectum. Two control groups were recruited: colonoscopy-negative controls (n = 1,202) were colonoscopy/sigmoidoscopy-confirmed polyp-free participants, and community (CM) controls (n = 535) were subjects randomly selected from the state driver's license registry and frequency-matched to the cases on age, sex, and zip code. Data on dietary intake were collected using a modified, 153-item Willett food frequency questionnaire prior to colonoscopy.

Results: The multivariate-adjusted odds ratios and 95% confidence intervals (CI) for the second through the fourth quartiles of fish intake were, respectively, 1.29 (95% CI 0.91, 4.82), 0.88 (95% CI 0.62, 1. 23), and 1.28 (95% CI 0.87, 4.88) in comparison with the colonoscopy-negative controls, and were similar to

those in relation to the community controls. The association did not differ substantially according to other risk factors.

Conclusions: These results do not support the hypothesis that greater fish intake may reduce risk for incident, sporadic colorectal adenoma, although a possible modest U-shaped association cannot be ruled out.

INTRODUCTION

In the United States, colorectal cancer is the third most common cancer and the third most frequent cause of cancer deaths in both men and women (1). The morbidity and mortality from the disease is high, but the disease appears to be preventable, as suggested by, two features. First, colorectal cancer has several established risk factors that are modifiable. Second, the existence of a pre-neoplastic lesion, the adenomatous polyp, and the use of colonoscopy have enabled further study on molecular basis of the pathogenesis of colorectal cancer.

Diet is one of the most important and modifiable risk factors for colorectal cancer. Previous studies suggested that higher consumption of red meat and higher fat intake was associated with higher risk for colorectal cancer (4, 5, 12-14) whereas higher consumption of vegetables, fruits (13, 14, 63), higher intake of calcium and vitamin D were associated with lower risk for colorectal cancer (13, 14, 27). Numerous studies have been conducted on an association between omega-3 polyunsaturated fatty acids (PUFAs), which were found in highest quantity in oily fishes such as salmon, mackerel, and sardines and risk for colorectal cancers (40). There is emerging evidence that two main omega-3 PUFAS, eicosapentaenoic acid (EPA) and docosahexaenoic acid

(DHA), have anti-cancer activities, especially against colorectal cancer (64-66). EPA and DHA could reduce risk for cancer via anti-inflammatory pathways, which are similar to the mechanism of the anti-cancer effect of aspirin. Some studies have investigated fish oil or omega-3 PUFAs and aspirin combined (67, 68). Reported associations between fish intake and colorectal cancer have been inconsistent. One large sample case-control study by Yang (48) found that frequent raw/cooked fish intake was associated with approximately 32% lower risk for colorectal cancer in men aged 40-79 years. A randomized clinical trial by Pot *et al.* (61) found no effect on apoptotic and mitotic rates in the colonic mucosa after six months of fish intake. Another case-control study by Poole *et al.* (69) found fish intake to be inversely associated with risk for hyperplastic polyps.

Adenomatous polyps are established precursors of colorectal cancer. Studies show that adenomatous polyps share common risk factors with colorectal cancer (70-73). To our knowledge, no studies have examined an association between fish intake, alone or in combination with regular use aspirin, and risk of adenomatous polyps. Accordingly, we evaluated an association of fish consumption alone and in combination with aspirin, with risk for adenomatous polyps in a large case-control study.

MATERIALS AND METHODS

The University of Minnesota Cancer Prevention Research Unit (CPRU) was a National Cancer Institute-funded program that combined several units of the University of Minnesota and a large multi-sited private gastroenterology

clinic, Digestive Healthcare (DH), which performed approximately 60 percent of all the colonoscopies in the Minneapolis metropolitan area. This case-control study of adenomatous polyps was conducted as part of the CPRU and was aimed at investigating factors associated with the formation of adenomatous polyps. The present study was approved by the Institutional Review Board of Emory University, Atlanta, Georgia.

Study subjects

Cases and Digestive Healthcare (DH) controls were recruited by DH staff through the usual scheduling of outpatient elective, colonoscopies, and screening flexible sigmoidoscopies, between April 1991 and April 1994. Patients were screened for eligibility and recruited before colonoscopy or sigmoidoscopy. Eligibility criteria for all participants included (a) residents of Minneapolis-St Paul metropolitan area; (b) 30 – 74 years of age; (c) English speaking and willing to participate; (d) no known genetic syndromes associated with colonic neoplasia; (e) no previous history of adenomatous polyps; (f) no individual history of cancer (except non-melanoma skin cancer); and (g) no history of ulcerative colitis, familial polyposis, Crohn's Disease, or Gardner Syndrome. Introductory materials describing the study, four study questionnaires, and a consent form were mailed to eligible subjects. Upon consent the questionnaires were completed before the colonoscopy or simoidoscopy. At colonoscopy or sigmoidoscopy, completed consent forms and questionnaires were collected and blood was drawn. Cases (n = 574) were those patients with at least one adenoma adenomatous polyp at the colonoscopy. All identified polyps were removed and examined by the study pathologist using

diagnostic criteria established for the National Polyp Study (74). DH controls (n = 1,202) were polyp free at colonoscopy (n = 574) or sigmoidoscopy (n = 628). The study participation rate was 68 percent.

A community-control group (CM controls) was also recruited. The CM controls (n = 550) were randomly selected from the 1991 Minnesota State Drivers License Registry. One control was selected for each case, frequency-matched to the age (5-year intervals), sex, and zip. A history of no previous adenomas in the CM controls was self-reported. Prospective CM controls were contacted via phone call. Upon consent, eligible participants were mailed a packet identical to that sent to DH clinic subjects. Completed forms were returned by prepaid mail. No blood samples were collected from the CM controls. The participation rate for the population controls was 65 percent.

Data collection

Information was collected using questionnaires on personal medical history, family history of colon cancer in first-degree relatives, anthropometrics (height, weight, and waist circumference and hip circumferences), tobacco use, physical activity during the previous 12 months, reproductive history (female subjects only), and sociodemographic and anthropometric characteristics. Dietary intake over the previous 12 months, including alcohol intake, were obtained using a modified, 153-item, semiquantitative Willett Food Frequency Questionnaire, which had been studied for validity and repeatability within the Nurses' Health Study cohort (75), the Iowa Women's Health Study cohort (76), and the Health Professionals Follow-up Study cohort (77). Information on fish

intake was obtained from four questions concerning the frequency with which participants ate various types of fish, including white fish, fatty fish, and canned tuna. The responses were combined to form a total fish intake variable. Regular aspirin use was defined as at least one pill per week for at least 1 year.

We excluded from analysis participants with more than 10 percent of the items on the food frequency questionnaire left blank and with implausible energy intakes (> 6000 or < 600 kcal/day). On the basis of these criteria, 48 participants (10 cases, 23 DH controls, 15 CM controls) were excluded from the analyses.

Statistical analysis

The normality of the distributions of the continuous variables was assessed, and descriptive comparisons of the cases with each of the two controls groups were tabulated and analyzed using the Student t-test for continuous variables (transformed to normalize them if indicated) and the chi-square test for categorical variables. Associations between fish intake and adenomatous polyps were assessed using unconditional logistic regression; the odds ratios with 95% confidence intervals were used to describe the strengths of association. A P-value for trend across the categories was calculated by including a quartile indicator variable as a continuous variable as a covariate in the models. Fish intake was classified into quartiles according to the sex-specific distribution in the community controls. Analyses were conducted separately for comparisons of the cases versus the DH controls and for the cases versus the CM controls. Odds ratios (ORs) were adjusted for potential confounders. First potential confounders were fitted to a full multivariate model with all potential

confounders. Then we used a backwards elimination method to include variables which altered the crude OR by at least 10%. Covariates to be included in the final model are age, sex, alcohol consumption, smoking (pack-years), hormone replacement therapy use (1 for men, 2 for women without hormone replacement therapy use, and 3 for women with hormone replacement therapy use), total energy intake, total fat intake, body mass index, family history of colon cancer in first degree relatives, and total oxidative balance score. Odds ratios were also stratified on aspirin use to compare the effect of fish intake combined with aspirin use on incident adenomatous polyps. All tests of statistical significance were two sided. All analyses were carried out using SAS software, version 9. 3 (SAS Institute, Cary, North Carolina).

RESULTS

Population characteristics

Selected characteristics of the study subjects are summarized in Table 1. Study participants were, on average, 53-58 years of age, and had a mean body mass index of approximately 27 kg/m^2. More than 97% of participants were white.

Cases and controls were similar with respect to height (1.7 m), waist-to-hip ratio (0. 9), and years of education (14 years). There was a greater proportion of men (61.7%) among the cases than among either of the control groups. Cases smoked more cigarettes (3.8 pack-years) and had higher alcohol consumption (10.1 gm / day). Female cases were less likely to have taken

hormone replacement therapy (HRT). The DH controls were more likely to have a family history of colorectal cancer in a first-degree relative.

Cases, on average, had higher total energy intakes, higher total fat, higher red and processed meat intakes, and lower fruit and vegetable intakes. Cases, on average, also had lower calcium and vitamin D intakes and lower total oxidative balance scores compared to both the DH and CM controls. Mean fish intakes (just under 2 servings/week) were similar across the three groups.

Fish intake and adenoma risk

The associations of fish intake with adenomas are presented in Table2. There was no definitive pattern in the fish-adenoma association across the quartiles, and none of the point estimates or p-values for trend was statistically significant. If anything, there was a suggestion of a U-shaped association and higher risk for those in the upper quartiles of intake in the analyses involving each of the control groups (25% and 37% higher in the analyses involving the colonoscopy and community controls, respectively).

Analyses of the fish intake-adenoma association according to potential effect modifying risk factors and according to adenoma characteristics are presented in Table3. The sample size for these analyses was small, and the estimates were quite unstable. There were no clear patterns of differences across the strata.

DISCUSSION

The results from this study provide no support for an association of fish intake with risk for incident, sporadic colorectal adenoma, although there was some suggestion of a possible U-shaped association. To our knowledge, this is the first epidemiological study to investigate an association of fish intake with colorectal adenoma.

The results of previous studies that investigated an association between fish intake and colorectal cancer are inconsistent. Five case-control studies (42, 48-51) and seven cohort studies (6, 12, 53-56) found an inverse association of fish intake with colorectal cancer. The finding was in a large-scale multicenter case-control study undertaken in Italy by Franceschi *et al.*, who found an approximately 30 percent and 60 percent lower risk for colorectal cancer and colon cancer, respectively. However, one case-control study in Burgundy, France (57) and three large population-based cohort studies (58-60) found no association between fish intake and colorectal cancer risk. One multicenter, randomized controlled intervention trial by Pot *et al.* (61) tested the effects of a 6-month intervention with oil-rich fish or lean fish on apoptosis and mitosis (early biomarkers of colorectal cancer risk) within the colonic crypts of the normal rectal mucosa and found no substantial change in the apoptotic and mitotic rates. In addition, in one cohort study, the Shanghai Womens' Health Study, cholesterol-rich fish, such as eel, shrimp, and shellfish were positively associated with colorectal cancer risk (62).

Most previous studies suggested that the effective anti-cancer component in fish may be omega-3 polyunsaturated fatty acids (n-3 PUFAs),

especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are found in highest quantities in oily fish such as sardines and mackerels. Several potential mechanisms may explain the possible anti-cancer activity of n-3 PUFAs. These include:

1. Anti-inflammatory activities

The anti-inflammatory activities of omega-3 PUFAs involve two pathways. One is the inhibition of cyclooxygenase-2 (COX-2) activity; the result is reduced synthesis of prostaglandins (PGs), which play an important role in the early stages of colorectal carcinogenesis (34, 35). The second is through the production of newly discovered anti-inflammatory mediators. EPA and DHA can produce COX-2-dependent resolvin, especially in the presence of aspirin. Resolvin was found to reduce inflammation in animal models by inhibiting the production and transportation of inflammatory chemicals (36-38).

2. Production of reactive oxygen species (ROS) and increasing oxidative stress

Omega-3 PUFAs can generate ROS such as the superoxide radical and can increase intracellular ROS levels, which may induce cancer cell apoptosis (39).

3. Alteration of cell surface receptor function

There is evidence that omega-3 PUFAs can alter the membrane localization of the Ras protein by changing the structure and fluidity of membranes. The membrane localization of the Ras protein is critical in the regulation of cell growth, proliferation, and apoptosis. Omega-3 PUFAs can

decreasing colonic localization of the Ras protein and reduce tumor formation in animal models (40, 41).

The results of this study could be construed as being consistent with a U-shaped association between fish consumption and risk of adenomas. If this pattern is true, it possibly would explain some of the contradictory study results from previous observational studies. Two previous studies also found a U-shaped effect pattern of n-3 PUFAs on atrial fibrillation and cognitive performance. The first study, an observational study conducted by de Groot *et al.* (78), investigated an association between fish consumption and cognitive performance in 700 Dutch high school students aged 12-18 years. Overall, higher fish consumption was associated with better vocabulary scores and better academic achievement (measures of cognitive performance). However, among those who consumed more than 450 mg of EPA/DHA daily, fish consumption was associated with lower vocabulary scores and lower academic achievement. The second study by Rix *et al.*, (79) investigated an association between consumption of fish and n-3 PUFA and the development of atrial fibrillation (AF) in the Diet, Cancer and Health cohort of 57,053 participants aged 50-64 years in Denmark. The associations of both fish and n-3 PUFA intakes with AF were U-shaped. A possible explanation is that persons with the highest intakes may be exposed to excessively higher amounts of toxic substances in fish, such as mercury, polychlorinated dibenzo-p-dioxins, organochlorine residues, and other chemicals, some of which have been shown to be mutagens or carcinogens (35, 80-82). Another explanation is that oily fish are also rich in cholesterol. Previous studies found a direct association between dietary cholesterol intake

and cancer risk (83, 84). However, an exact mechanism underlying this U-shaped association is unclear.

Several studies found an inverse association between regular aspirin use and risk of colorectal cancer (85-87). Aspirin can cause irreversible inactivation of COX isoenzymes through acetylation, thereby inhibiting the generation of prostaglandin G2 (PGG2). This mechanism is similar to that of n-3 PUFA, which means that they may have an additive or synergistic effect in preventing colorectal cancer. There is an ongoing randomized controlled trial of EPA and/or aspirin for colorectal adenoma prevention (the seAFOod Polyp Prevention Trial) by Hull *et al.* aiming at gaining insights into the mechanisms of action of EPA and aspirin, alone and in combination (88).

The present study has several strengths. The use of two control groups is the primary advantage. One control group was drawn from the same colonoscopy/sigmoidoscopy population as the cases. All endoscopy-negative controls went through a complete colonoscopy/sigmoidoscopy and were determined to be polyp free, thereby decreasing the possibility of outcome misclassification bias. However, the use of colonoscopy/sigmoidoscopy controls introduces the possibility of selection bias. Because participants in this control group may have a clinical indication for receiving a colonoscopy or sigmoidoscopy, they may have been more similar to the cases than were the community controls, resulting in attenuated associations and less generalizability to the general population. So, a second, community-based control group was also used; however, because their control status was not confirmed by

colonoscopy, there may also have been some outcome misclassification in relation to the findings involving this control group, again attenuating the associations. Another strength of this study is that the food frequency questionnaires, of which the validity had been previously tested, were collected before colonoscopy/sigmoidoscopy, therefore reducing the possibility of recall bias.

Other limitations of this study not noted above include those inherent in the case-control design, such as the inability to assess temporality, and the use of a food frequency questionnaire with limited questions on fish intakes, to assess long-term diet. Also, there was a limited range of fish intakes in this population. Finally, the sample size in this study was limited for conducting stratified analyses.

In conclusion, the results from this study provide no support for an association of fish intake with risk for incident, sporadic colorectal adenoma, although there was some suggestion of a possible U-shaped association. Further research should aim at identifying a possible beneficial dose of fish intake in the prevention of adenomas in the general population.

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Tables of Results

Table 1. Selected characteristics by group, Minnesota Cancer Prevention Research case-control study, 1991-1994

Characteristic	Cases		Colonoscopy Controls		Community Controls		P values	
	Mean (SD[*])	%	Mean (SD)	%	Mean (SD)	%	DH [h]	CM [i]
No.	564		1202		535			
Age, years	58.1 (9.6)		52.8 (11.1)		57.7 (10.4)		< 0.0001	0.46
Men, %		61.7		37.6		55.1	< 0.0001	0.03
BMI [a], kg/m^2	27.4 (4.7)		26.6 (4.9)		26.8 (4.5)		0.001	0.05
Height, m	1.7 (0.1)		1.7 (0.1)		1.7 (0.1)		< 0.0001	0.11
Waist-to-hip ratio	0.9 (0.1)		0.9 (0.1)		0.9 (0.1)		< 0.0001	0.003
Education, years	14.0 (3.3)		14.5 (3.2)		14.1 (2.9)		0.001	0.007
Cigarette smoking [b]	3.8 (9.1)		2.4 (7.5)		2.7 (7.4)		0.001	0.02
Alcohol intake, gm/d	10.1 (16.6)		6.4 (12.9)		8.1 (15.5)		< 0.0001	0.05
Family history [c], %		20.0		25.9		9.4	0.02	< 0.0001
Physical activity, MET-hrs/week	37.4 (39.4)		34.8 (32.8)		38.2 (39.2)		0.15	0.72
Dietary intakes								
Total energy, kcal/day	2,091 (776)		2,002 (718)		2,054 (719)		0.02	0.42
Total fat, gm/day	73.1 (34.4)		66.8 (30.3)		70.2 (31.3)		0.0001	0.15
Red meat, servings/week	4.7 (3.6)		4.3 (3.4)		4.4 (3.2)		0.03	0.07

Table 1 Continued

Processed meat, servings/week	2.6 (3.9)		1.8 (2.6)		2.5 (3.8)	< 0.0001	0.59
Total fruit, servings /week	16.8 (12.4)		18.7 (13.1)		18.5 (12.9)	0.005	0.03
Total vegetable, servings/week	25.5 (15.7)		27.0 (18.0)		26.0 (14.8)	0.10	0.57
Total calcium, mg/day	959.4 (531.1)		990.1 (518.3)		987.7 (552.4)	0.25	0.39
Total vitamin D, IU/day	325.8 (257.4)		349.6 (259.6)		354.7 (264.4)	0.07	0.07
Fish, servings/week	1.9 (1.5)		1.9 (1.7)		1.8 (1.6)	0.80	0.88
Aspirin use [d]		20.2		26.3	18.7	0.006	0.52
NSAID use [e]		8.9		18.5	11.2	< 0.0001	0.19
HRT [f]		38.8		50.4	44.2	< 0.0001	0.04
OBS [g]	-1.2 (5.4)		0.6 (5.6)		0.1 (5.8)	< 0.0001	0.0002

* SD: standard deviation.

a. BMI: body mass index.

b. Cigarette smoking: pack-years smoking, including non-smokers.

c. Family history: family history of colon cancer in first-degree relative.

d. Use aspirin at least once per week.

e. Take NSAID at least once per week.

f. Ever on hormone replacement therapy, in women.

g. OBS: total oxidative balance score.

h. P value for comparing cases and DH controls.

i. P value for comparing cases and CM controls.

Table 2. Crude and multivariate-adjusted associations of fish intake with incident, sporadic adenomatous polyps; Minnesota Cancer Prevention Research Unit case-control study, 1991-1994

Fish intake quartile	Median intake (Servings/week)	Cases vs. colonoscopy controls				Cases vs. community controls			
		crude		adjusted [a]		crude		adjusted [a]	
		OR*	95% CI*	OR	95% CI	OR	95% CI	OR	95% CI
1	0.5	1.00		1.00		1.00		1.00	
2	1.5	1.07	0.81, 1.42	1.29	0.91, 1.82	1.12	0.80, 1.57	1.15	0.78, 1.71
3	2.0	0.83	0.64, 1.09	0.88	0.62, 1.23	0.92	0.67, 1.27	1.03	0.70, 1.51
4	4.0	1.14	0.86, 1.49	1.28	0.87, 1.88	1.10	0.80, 1.52	1.37	0.89, 2.11
p trend		*0.24*		*0.14*		*0.71*		*0.49*	

* OR, odds ratio; CI, confidence interval.

a. Adjusted for age, sex, alcohol consumption, smoking, hormone replacement therapy use, total energy intake, fat intake, body mass index, total oxidative balance score, family history of colorectal cancer in a first-degree relative.

Table 3. Multivariate-adjusted associations of fish intake with incident, sporadic adenomatous polyps, stratified on aspirin use; Minnesota Cancer Prevention Research Unit case-control study, 1991-1994

Regular aspirin use[a]	Fish intake quartile	Cases vs. DH controls		Cases vs. CM controls	
		OR*[b]	95% CI*[b]	OR	95% CI
Yes	1	1.00		1.00	
	2	1.22	0.60, 2.48	0.97	0.40, 2.34
	3	0.69	0.32, 1.45	0.99	0.40, 2.47
	4	0.93	0.41, 2.12	1.28	0.48, 3.41
No	1	1.00		1.00	
	2	1.36	0.90, 2.05	1.19	0.76, 1.87
	3	0.92	0.62, 1.36	1.03	0.67, 1.59
	4	1.40	0.90, 2.19	1.36	0.84, 2.21

*OR, odds ratio; CI, confidence interval.

a. Take aspirin once a week or more.

b. Adjusted for age, sex, alcohol consumption, smoking, hormone replacement therapy use, total energy intake, fat intake, body mass index, total oxidative balance score, family history of colorectal cancer in a first-degree relative.

CHAPTER III: SUMMARY AND FUTURE DIRECTIONS

The results from this study provide no support for an association of fish intake with risk for incident, sporadic colorectal adenoma, although there was some suggestion of a possible U-shaped association.

We investigated an association of fish intake with risk of adenoma stratified on regular aspirin use. We also stratified on other potential effect modifiers including sex, median age (56.0 years), median body mass index, family history of colon cancer in first-degree relatives, regular NSAID use, and size of largest adenoma (< 1.0 cm and ≥ 1.0 cm). Results are provided in the Appendices. Future larger studies are needed to investigate whether there are effect modifiers of the association between fish intake and adenoma as well as whether the associations may differ according to adenoma characteristics. These characteristics would include the adenoma location (distal/proximal), histology, and levels of dysplasia (mild, moderate, and severe). By stratification on these characteristics, researchers could assess whether fish acts in certain locations of colon or rectum, in which stage does fish intake may work, and whether fish intake is associated with adenoma dysplasia. Further studies on related mechanisms could provide guidance whether fish or n-3 omega PUFAs may prevent colorectal cancer, and if so, what “doses” would be optimal.

In addition, further studies could examine whether a fish-colorectal neoplasm association differs according to the various types of fish. N-3 PUFAs, highly abundant in oil-rich fish, such as salmon and mackerel, may increase lipid

peroxidation and oxidative stress causing DNA damage. Thus, it would be helpful to compare the effects of oil-rich fish and lean fish based on this potential genotoxic effect of oil-rich fish.

Furthermore, researchers should also pay attention to whether fish preparation methods can influence fish's possible anti-cancer effect. Heterocyclic amines (HCAs) can be produced when fish are cooked using high-temperature methods, such as deep-frying or grilling. Previous studies in animal models and some population studies found that HCAs are mutagenic and can increase risk of cancer. Therefore the results of such studies could provide evidence for future dietary suggestions on fish.

APPENDICES

Table. Multivariate-adjusted associations of fish intake with incident, sporadic adenomatous polyps, stratified on median age; Minnesota Cancer Prevention Research Unit case-control study, 1991-1994

Age	Fish intake quartile	Cases vs. colonoscopy controls		Cases vs. community controls	
		OR*[b]	95% CI*[b]	OR	95% CI
< 56 [a]	1	1.00		1.00	
	2	1.50	0.65, 3.44	1.31	0.78, 2.22
	3	0.92	0.42, 2.02	1.05	0.61, 1.81
	4	2.40	0.98, 5.88	1.69	0.94, 3.06
>= 56	1	1.00		1.00	
	2	1.31	0.78, 2.22	1.24	0.62, 2.50
	3	1.05	0.61, 1.81	1.10	0.55, 2.19
	4	1.69	0.94, 3.06	1.69	0.78, 3.66

*OR, odds ratio; CI, confidence interval.

a. Median age.

b. Adjusted for sex, alcohol consumption, smoking, hormone replacement therapy use, total energy intake, fat intake, body mass index, total oxidative balance score, family history of colorectal cancer in a first-degree relative.

Table. Multivariate-adjusted associations of fish intake with incident, sporadic adenomatous polyps, stratified on sex; Minnesota Cancer Prevention Research Unit case-control study, 1991-1994

Sex	Fish intake quartile	Cases vs. colonoscopy controls		Cases vs. community controls	
		OR*[a]	95% CI*[a]	OR	95% CI
Male	1	1.00		1.00	
	2	1.06	0.66, 1.72	1.02	0.61, 1.71
	3	0.63	0.39, 1.01	0.80	0.48, 1.34
	4	1.08	0.64, 1.83	1.39	0.80, 2.43
Female	1	1.00		1.00	
	2	1.64	0.98, 2.73	1.43	0.77, 2.66
	3	1.30	0.79, 2.12	1.45	0.79, 2.65
	4	1.75	0.97, 3.15	1.29	0.64, 2.58

*OR, odds ratio; CI, confidence interval.

a. Adjusted for age, alcohol consumption, smoking, hormone replacement therapy use, total energy intake, fat intake, body mass index, total oxidative balance score, family history of colorectal cancer in a first-degree relative.

Table. Multivariate-adjusted associations of fish intake with incident, sporadic adenomatous polyps, stratified on family history; Minnesota Cancer Prevention Research Unit case-control study, 1991-1994

Family history	Fish intake quartile	Cases vs. colonoscopy controls		Cases vs. community controls	
		OR*[a]	95% CI*[a]	OR	95% CI
Yes	1	1.00		1.00	
	2	1.33	0.59, 2.98	0.71	0.23, 2.21
	3	1.38	0.63, 3.03	1.93	0.56, 6.62
	4	2.55	1.11, 5.82	2.75	0.68, 11.06
No	1	1.00		1.00	
	2	1.20	0.81, 1.77	1.24	0.81, 1.90
	3	0.78	0.53, 1.13	0.98	0.65, 1.49
	4	0.99	0.64, 1.54	1.20	0.76, 1.91

*OR, odds ratio; CI, confidence interval.

a. Adjusted for age, sex, alcohol consumption, smoking, hormone replacement therapy use, total energy intake, fat intake, body mass index, total oxidative balance score.

Table. Multivariate-adjusted associations of fish intake with incident, sporadic adenomatous polyps, stratified on median BMI*; Minnesota Cancer Prevention Research Unit case-control study, 1991-1994

BMI Kg/m ^{2}	Fish intake quartile	Cases vs. colonoscopy controls		Cases vs. community controls	
		OR*[b]	95% CI*[b]	OR	95% CI
< 26.4 [a]	1	1.00		1.00	
	2	1.33	0.80, 2.19	1.16	0.72, 1.93
	3	1.01	0.62, 1.63	1.01	0.48, 1.27
	4	1.24	0.71, 2.17	0.96	0.75, 2.22
>= 26.4	1	1.00		1.00	
	2	1.18	0.72, 1.93	1.21	0.71, 2.05
	3	0.78	0.48, 1.27	1.03	0.60, 1.78
	4	1.29	0.75, 2.22	1.80	1.01, 3.22

*OR, odds ratio; CI, confidence interval; BMI: body mass index.

a. Median BMI in community controls.

b. Adjusted for age, sex, alcohol consumption, smoking, hormone replacement therapy use, total energy intake, fat intake, total oxidative balance score, family history of colorectal cancer in a first-degree relative.

Table. Multivariate-adjusted associations of fish intake with incident, sporadic adenomatous polyps, stratified on regular NSAID use; Minnesota Cancer Prevention Research Unit case-control study, 1991-1994

Regular NSAID use	Fish intake quartile	Cases vs. colonoscopy controls		Cases vs. community controls	
		OR*[a]	95% CI*[a]	OR	95% CI
Yes	1	1.00		1.00	
	2	0.92	0.26, 3.20	0.90	0.18, 4.56
	3	1.16	0.42, 3.18	4.74	1.08, 20.80
	4	2.34	0.70, 8.04	2.77	0.63, 12.11
No	1	1.00		1.00	
	2	1.35	0.93, 1.95	1.17	0.77, 1.77
	3	0.89	0.61, 1.28	0.90	0.60, 1.35
	4	1.21	0.80, 1.82	1.28	0.81, 2.02

*OR, odds ratio; CI, confidence interval.

a. Adjusted for age, sex, alcohol consumption, smoking, hormone replacement therapy use, total energy intake, fat intake, body mass index, total oxidative balance score, family history of colorectal cancer in a first-degree relative.

Table. Multivariate-adjusted associations of fish intake with incident, sporadic adenomatous polyps, stratified on size of largest adenoma; Minnesota Cancer Prevention Research Unit case-control study, 1991-1994

Size of largest adenoma	Fish intake quartile	Cases vs. colonoscopy controls		Cases vs. community controls	
		OR*[a]	95% CI*[a]	OR	95% CI
< 1 cm	1	1.00		1.00	
	2	1.38	0.93, 2.05	1.26	0.81, 1.96
	3	1.01	0.69, 1.48	1.20	0.79, 1.84
	4	1.24	0.80, 1.92	1.39	0.87, 2.24
>= 1 cm	1	1.00		1.00	
	2	1.08	0.64, 1.80	1.24	0.56, 1.71
	3	0.59	0.34, 1.03	0.98	0.43, 1.41
	4	1.32	0.74, 2.34	1.20	0.85, 2.86

*OR, odds ratio; CI, confidence interval.

a. Adjusted for age, sex, alcohol consumption, smoking, hormone replacement therapy use, total energy intake, fat intake, body mass index, total oxidative balance score, family history of colorectal cancer in a first-degree relative.