Fish consumption and markers of colorectal cancer risk: a multicenter randomized controlled trial\textsuperscript{1–3}


ABSTRACT

Background: Diet is a major factor in the etiology of colorectal cancer, with high fish consumption possibly decreasing colorectal cancer risk, as was shown in several observational studies. To date, no intervention trials have examined the possible beneficial effects of fish intake on colorectal cancer risk.

Objective: The objective was to investigate the effects of a 6-mo intervention with oil-rich or lean fish on apoptosis and mitosis within the colonic crypt.

Design: In a multicenter, randomized, controlled intervention trial, patients with colorectal polyps, inactive ulcerative colitis, or no macroscopic signs of disease were recruited (n = 242) and randomly allocated to receive dietary advice plus either 300 g oil-rich fish (salmon) per week (n = 82), 300 g lean fish (cod) per week (n = 78), or only dietary advice (DA) (n = 82). Apoptosis and mitosis were measured in colonic biopsy samples collected before and after intervention (n = 213).

Results: The total number of apoptotic cells per crypt did not increase in the salmon or cod group: −0.10 (95% CI: −0.36, 0.16) and −0.06 (95% CI: −0.32, 0.20), respectively, compared with the DA group. The total number of mitotic cells per crypt decreased nonsignificantly in the salmon group (−0.87; 95% CI: −2.41, 0.68) and in the cod group (−1.04; 95% CI: −2.62, 0.53) compared with the DA group. Furthermore, the distribution of mitosis within the crypt did not significantly change in either group.

Conclusion: An increase in the consumption of either oil-rich or lean fish to 2 portions weekly over 6 mo does not markedly change apoptotic and mitotic rates in the colonic mucosa. This trial was registered at www.clinicaltrials.gov as NCT00145015.

INTRODUCTION

Colorectal cancer is one of the cancers most strongly related to dietary habits (1, 2), with 65–75% of the incidence of colorectal cancer attributed to dietary factors (3). One of the dietary habits that may reduce colorectal cancer risk is the consumption of fish (1). Several observational studies have shown that fish consumption could be related to a decreased risk of colorectal cancer (1). Recently, a meta-analysis of 19 prospective cohort studies showed a 12% decrease in the relative risk (RR) of colorectal cancer (RR: 0.88; 95% CI: 0.78, 1.00) in a comparison of high fish consumption with low fish consumption (4). The largest contributing study to this meta-analysis was the European Prospective Investigation into Cancer (EPIC) study, with a hazard ratio of 0.69 (95% CI: 0.54, 0.88) and a contrast of 70 g fish/day between the lowest and the highest intake category (5). The beneficial effects of fish consumption are generally attributed to their very-long-chain n-3 (omega-3) polyunsaturated fatty acid (VLC-PUFA) content, as established in animal and in vitro studies (6). However, fish contains other nutrients that have been associated with a reduced colorectal cancer risk, such as vitamin D and selenium (1, 7). Observational studies are currently unable to assess whether the possible protective effect of fish on colorectal cancer risk is only associated with the consumption of oil-rich fish or with fish in general, because no discrimination in type of fish is normally made in these studies. Hence, there is a need for intervention studies involving different types of fish. Because the assessment of colorectal cancer incidence as an outcome is not feasible, intermediate endpoints of colorectal cancer risk have to be used, despite their recognized limitations.

An intermediate endpoint often used for colorectal cancer risk in intervention studies is cell proliferation (8). A decrease in cell proliferation or mitosis indicates a longer cell cycle time, which thereby increases the time for cells to repair any replication errors (9). For many years, this has been considered one of the few available early biomarkers of colorectal cancer risk. Patients with ulcerative colitis (UC) and those with colorectal polyps have increased mitotic rates (9, 10) and are at an increased risk of colorectal cancer.

\textsuperscript{1} From the Division of Human Nutrition, Wageningen University, Netherlands (GKP, AG, GS, and EK); Gastrointestinal Biology and Health, Institute of Food Research, Norwich, United Kingdom (GM-N, LJH, KP-P, JRd, and EKL); UMC St Radboud, Nijmegen, Netherlands (FMN); Gelderse Vallei Hospital, Ede, Netherlands (BMW); Slingeland Hospital, Doetinchem, Netherlands (PCvdM); St Antonius Hospital, Nieuwegein, Netherlands (RT); Canisius Wilhelmina Hospital, Nijmegen, Netherlands (AT); Rijnstate Hospital, Arnhem, Netherlands (PJW); Norfolk & Norwich University Hospital, NHS Trust, Norwich, United Kingdom (ARH); and James Paget University Hospital, Great Yarmouth, United Kingdom (MPW).

\textsuperscript{2} Supported by the Integrated Project SEAFOOD, a grant from the European Union (contract no. 506359), and the Food Standards Agency UK.

\textsuperscript{3} Address correspondence to EK Lund, Gastrointestinal Biology and Health, Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, United Kingdom. E-mail: liz.lund@bbsrc.ac.uk.

Received February 12, 2009. Accepted for publication May 29, 2009. First published online June 24, 2009; doi: 10.3945/ajcn.2009.27630.
developing colorectal cancer. Gastrointestinal epithelial cell homeostasis is maintained by the balance between cell growth and apoptosis; therefore, more recently, apoptosis has been considered to be a useful marker of colorectal cancer risk (11). Higher levels of apoptosis indicate the faster removal of damaged cells, which can possibly prevent clonal expansion, and higher apoptotic levels have been linked to reduced colorectal cancer risk in both animal models (12) and humans (13). Patients with UC (14, 15) and those with polyps (16) have decreased apoptotic rates. Mitosis and apoptosis have been examined in several studies that investigated dietary supplementation with fish oil and have shown inconsistent results. Some studies showed a reduction in cell proliferation (17–20) and an increase in apoptosis (18, 21) in response to fish-oil supplementation, whereas other studies failed to show an effect (22–26). Also, many studies only confirmed an effect of fish oil in adenomatous tissue and not in normal tissue (17, 19, 25) or only showed an effect of fish oil within and not between treatment groups (17, 25).

To our knowledge, no intervention trials have examined the possible beneficial effects of fish consumption on colorectal cancer risk. Therefore, the aim of the current study was to investigate the effects of an intervention using either oil-rich or lean fish on apoptosis and mitosis in colonic crypts as markers of colorectal cancer risk in a high-risk population of patients, with either colorectal polyps or inactive UC, and in healthy control patients. We hypothesized that increased fish consumption would lead to increased apoptotic levels and decreased mitotic levels and that oil-rich fish would result in more pronounced effects than would lean fish.

SUBJECTS AND METHODS

Subjects and sample size

The trial [FISHGASTRO (FISH Consumption and GASTRO-intestinal Health) study] was carried out by 2 research centers: Wageningen University, Wageningen, Netherlands, and the Institute of Food Research, Norwich, United Kingdom. Potential participants were recruited from outpatient colonoscopy clinic lists in 8 clinical centers (6 in the Netherlands and 2 in the United Kingdom). The primary outcome of this trial was the change in apoptosis. On the basis of findings in a previous study, a sample size of 90 subjects per intervention group was calculated to provide power of ≥80% to detect a change of 0.2 apoptotic cells per crypt with an SD of 0.46 with the use of a 2-sided statistical significance level of \( P < 0.05 \) (23). To account for a dropout rate of 10%, we needed 100 subjects per intervention group (\( n = 300 \)).

Between November 2004 and July 2007, we recruited male and female volunteers aged 18–80 y for the study from outpatient clinic attendees visiting the hospital for a colonoscopy, which was part of their regular medical care. Three groups of subjects were recruited: 1) those with (previous) colorectal polyps that were histologically confirmed, 2) those with a diagnosis of UC (inactive), and 3) those without any macroscopic signs of disease in the colon, whose reasons for attending included irritable bowel syndrome, hemorrhoids, unexplained anemia, bowel complaints, or changes in defecation patterns. Approximately 10% of the invited patients were willing to participate in the trial. The main reasons for not participating were an unwillingness to increase their fish consumption or to undergo an extra sigmoidoscopy at the end of the trial, which was additional to their regular medical care. Subjects were excluded if they were allergic to fish, taking fish-oil supplements, taking nonsteroidal antiinflammatory drugs or acetylsalicylic acid, organ transplant recipients receiving immunosuppression therapy, patients with type 1 diabetes, or patients with an elevated infection risk. The Dutch study protocol was approved by the Medical Ethical Committee of Nijmegen University Medical Centre St Radboud (reference 2004/111), and the English study protocol was approved by King’s Lynn Local Research Ethics Committee (reference 04/Q0105/8). All subjects gave written informed consent after the study was explained to them, both in writing and verbally.

Design and treatment

The FISHGASTRO study is a multicenter parallel randomized controlled intervention trial (RCT). After an initial colonoscopy procedure was performed, eligible subjects were randomly allocated by an independent person to 1 of 3 dietary intervention groups: 1) oil-rich fish group (two 150-g portions farmed salmon/wk for 6 mo), 2) lean fish group (two 150-g portions of Icelandic cod/wk for 6 mo), and 3) dietary advice (DA) group. All 3 intervention groups received general dietary advice to achieve a healthy diet (27, 28). Treatment codes were generated by country and patient group in blocks of 6 by using a computer-generated randomization schedule. The fish was provided to the participants at their homes, and they were asked to consume it in addition to any regular fish consumption. Salmon and cod provided 1.4 and 0.99 g n-3 VLC-PUFAs/d, respectively, measured as described previously (29). We chose a study duration of 6 mo because this would be long enough to incorporate n-3 PUFAs in the colonic epithelium (23).

Volunteer compliance was checked by using food diaries, regular phone calls every 2–4 wk, and, for the salmon group, by pre- and postintervention measurements of serum n-3 VLC-PUFA concentrations.

Data collection

Colonial biopsy samples were collected before intervention during a routine colonoscopy procedure and after intervention during a sigmoidoscopy procedure. The preparation of the colonoscopy procedure consisted of macrogol (Kleanprep; Norgine BV, Amsterdam, Netherlands) in Netherlands or Picolax (Ferring Pharmaceuticals Limited, Berkshire, United Kingdom) in the United Kingdom; preparation for the sigmoidoscopy procedure consisted of an enema in both the Netherlands and the United Kingdom. Distal colon biopsy samples were obtained from mucosa of normal appearance at ~20–30 cm from the anal verge during the colonoscopy or sigmoidoscopy.

Fasting blood samples were taken on the day of the colonoscopy or sigmoidoscopy procedure, and serum was stored at −80°C before analysis. Serum cholesterol fatty acids were measured as previously described (29). Furthermore, we measured serum 25-hydroxyvitamin D (enzyme immunoassay; Immunodiagnostics Systems Ltd, Tyne and Wear, United Kingdom) and serum...
selenium concentrations using an Agilent 7500ce inductively coupled plasma mass spectrometry (Agilent UK Ltd, Stockport, United Kingdom) after ultraviolet-assisted wet digestion in a Metrohm 705 ultraviolet digester (Metrohm, Buckingham, United Kingdom). Dietary habits were assessed before and at the end of the intervention period with a self-administered food-frequency questionnaire (30, 31) and additionally with a 7-d food diary in the United Kingdom. Information on lifestyle, including physical activity (32) and smoking, weight, and height measures was obtained at baseline by questionnaire. The participants were asked to report any changes in well being and medication and supplement use during the intervention period.

Analysis of crypt cell apoptosis and proliferation in colonic biopsy samples

After being collected, the colonic biopsy samples were immediately fixed in ethanol:acetic acid (3:1) and stored at 4°C before analysis. Apoptosis and mitosis were determined in intact microdissected crypts according to morphologic criteria (33–35). Biopsy samples were dissected under low-power microscopy to yield thin strips of crypts, which were gently squashed beneath a cover-slip. Ten to twenty randomly selected intact crypts were viewed under a light microscope (×400). The length of each crypt was determined by comparison with a calibrated linear eyepiece graticule (Nikon United Kingdom, Kingston, United Kingdom), and the positions of mitotic cells were recorded along the length of the crypt. Data were expressed as the total number of apoptotic or mitotic cells per crypt. The microscope was blinded to both treatment and patient groups, and all analyses were performed in the same research center (Institute of Food Research).

Statistical analyses

Data analysis was carried out according to a predefined analysis plan. Subject compliance of the salmon group, based on serum n-3 VLC-PUFA changes, was tested by using a paired Student’s t test within the salmon group. After the intervention, changes in outcome variables were evaluated by using an analysis of covariance (ANCOVA), with adjustment for baseline values. We compared the changes in outcome measures in the salmon and cod groups with the changes in the DA group; therefore, changes are presented as the mean change compared with the DA group (95% CI). The distribution of mitosis within the crypt was also analyzed. Crypt lengths were normalized in tenths, and an ANCOVA was used to compare mitotic rates in the bottom 40% and top 40% of the crypts, as previously described by Anti et al (17). For all analyses, we explored whether the results were different per patient group and per country.

We performed analyses using the SAS statistical software program (version 9.1; SAS Institute Inc, Cary, NC) and considered a P value <0.05 as statistically significant. The researchers who performed the statistical analyses were blinded to the treatment and patient groups during the analyses.

RESULTS

Subjects and compliance

Of the 242 participants randomly assigned, 216 completed the 6-mo intervention, as is shown in Figure 1. Reasons for discontinuation are depicted in Figure 1 and consisted mainly of not wanting an extra sigmoidoscopy (n = 5), not willing to eat fish (n = 3), pregnancy (n = 2), or occurrence of prostate cancer (n = 2). Four serious adverse events were reported during the study period, but none were related to the study. Baseline

---

**FIGURE 1.** Flow diagram of the FISHGASTRO (FISH Consumption and GASTROintestinal Health) trial [based on the CONSORT (Consolidated Standards of Reporting Trials) statement (36)]. The subjects were randomly assigned to 1 of 3 groups: patients with a diagnosis of ulcerative colitis (UC), patients with a history of colorectal polyps, and patients with a “healthy colon” for whom no macroscopic signs of disease were observed during colonoscopy.
markers of colorectal cancer risk

Fish consumption had no effect on the number of apoptotic cells per crypt after the 6-mo intervention compared with the DA group, as is indicated in Table 4. Similarly, the changes in the number of mitotic cells per crypt were not significantly different between the fish groups and the DA group, although a non-statistically significant decrease of –0.9 mitotic cells per crypt (95% CI: –2.4, 0.7) was observed in the salmon group and of –1.0 mitotic cells per crypt (95% CI: –2.6, 0.5) in the cod group compared with the DA group (Table 4).

Comparison of the results per country at baseline showed a statistically significant differences between countries in the number of apoptotic cells per crypt (Netherlands: 0.5 ± 0.8; United Kingdom: 1.1 ± 0.9; P < 0.0001) and in the number of mitotic cells per crypt (Netherlands: 6.1 ± 3.6; United Kingdom: 9.1 ± 5.0; P < 0.0001). However, compared with the DA group, the changes in the number of apoptotic cells per crypt in the intervention groups did not differ between countries: –0.2 (95% CI: –0.5, 0.2) for salmon and –0.2 (95% CI: –0.5, 0.1) for cod in the Netherlands and 0.1 (95% CI: –0.4, 0.6) for salmon and 0.3 (95% CI: –0.2, 0.8) for cod in United Kingdom. The nonsignificant decrease in mitotic cells in the salmon and cod groups was more pronounced in the Netherlands participants than in the United Kingdom participants, although the decrease in mitotic cells in the Netherlands was not statistically significant; compared with the DA group, changes in the number of mitotic cells per crypt were –0.1 (95% CI: –1.3, 1.2) for salmon and –0.7 (95% CI: –2.0, 0.6) for cod in the Netherlands and 0.0 (95% CI: –3.3, 3.2) for salmon and 1.5 (95% CI: –1.7, 4.7) for cod in the United Kingdom.

Comparison of the different patient groups at baseline showed that the number of apoptotic cells per crypt was significantly higher in UC patients (0.9 ± 1.2; P = 0.04) and nonsignificantly higher in polypos patients (0.7 ± 0.9; P = 0.11) as compared with control subjects (0.6 ± 0.6). The number of mitotic cells per crypt was significantly higher in UC patients (8.1 ± 4.2; P = 0.02) but not in patients with polyps (6.6 ± 4.3; P = 0.33) compared with healthy control subjects (6.3 ± 4.0). The conclusions from our study did not change when we analyzed changes in apoptosis and mitosis stratified for patient group.

### Table 1

Baseline characteristics of the FISHGASTRO (FISH Consumption and GASTROintestinal Health) study population (n = 213)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Salmon group (n = 74)</th>
<th>Cod group (n = 70)</th>
<th>DA group (n = 69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>55.1 ± 11.5</td>
<td>57.4 ± 10.3</td>
<td>55.3 ± 9.5</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>51</td>
<td>41</td>
<td>54</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.5 ± 4.4</td>
<td>26.8 ± 4.3</td>
<td>26.7 ± 3.5</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>26.0</td>
<td>11.4</td>
<td>15.9</td>
</tr>
<tr>
<td>Family history of CRC (%)</td>
<td>1.3</td>
<td>4.3</td>
<td>4.4</td>
</tr>
<tr>
<td>Indication for baseline colonoscopy (%) screening</td>
<td>41</td>
<td>44</td>
<td>46</td>
</tr>
<tr>
<td>Research center, NL (%)</td>
<td>76</td>
<td>74</td>
<td>78</td>
</tr>
<tr>
<td>Patient group (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyps</td>
<td>38</td>
<td>46</td>
<td>42</td>
</tr>
<tr>
<td>UC</td>
<td>19</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Healthy control subjects</td>
<td>43</td>
<td>34</td>
<td>39</td>
</tr>
</tbody>
</table>

1 CRC, colorectal cancer; NL, Netherlands; UC, ulcerative colitis; DA, dietary advice. There were no statistically significant differences between the 3 intervention groups.

2 Mean ± SD (all such values).

### Table 2

Fish intake at baseline and at the end of the intervention

<table>
<thead>
<tr>
<th></th>
<th>Salmon group</th>
<th>Cod group</th>
<th>DA group</th>
</tr>
</thead>
<tbody>
<tr>
<td>frequency/wk</td>
<td>1.6 ± 1.3</td>
<td>1.6 ± 1.1</td>
<td>1.5 ± 1.1</td>
</tr>
<tr>
<td>Base line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>2.8 ± 1.3</td>
<td>2.9 ± 1.3</td>
<td>1.6 ± 1.3</td>
</tr>
<tr>
<td>End</td>
<td>1.4 ± 1.1</td>
<td>1.3 ± 1.4</td>
<td>0.1 ± 1.0</td>
</tr>
<tr>
<td>Difference in change compared with DA</td>
<td>1.3 (0.9, 1.7)</td>
<td>1.2 (0.8, 1.7)</td>
<td>—</td>
</tr>
</tbody>
</table>

1 n = 71 (salmon group), 65 (cod group), and 69 (dietary advice [DA] group) at baseline and 59 (salmon group), 61 (cod group), and 60 (DA group) after the intervention.

2 Mean ± SD (all such values).

3 Significantly different from baseline, P < 0.0001 (paired t test).

4 Mean; 95% CI in parentheses (all such values).

5 Significantly different from DA group, P < 0.05 (ANCOVA).
TABLE 3
Serum very-long-chain n–3 polyunsaturated fatty acid (eicosapentaenoic acid + docosahexaenoic acid) concentrations in the participants who completed the intervention

<table>
<thead>
<tr>
<th></th>
<th>Salmon group (n = 71)</th>
<th>Cod group (n = 69)</th>
<th>DA group (n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of total fatty acids in cholesteryl esters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.85 ± 1.41</td>
<td>2.71 ± 1.07</td>
<td>2.64 ± 1.20</td>
</tr>
<tr>
<td>End</td>
<td>3.59 ± 1.41</td>
<td>2.68 ± 1.04</td>
<td>2.49 ± 1.06</td>
</tr>
<tr>
<td>Change</td>
<td>0.74 ± 1.35</td>
<td>−0.04 ± 0.94</td>
<td>−0.14 ± 0.80</td>
</tr>
<tr>
<td>Difference in change compared with DA</td>
<td>0.88 (0.52, 1.24)</td>
<td>0.11 (−0.27, 0.47)</td>
<td>—</td>
</tr>
</tbody>
</table>

1. Missing values because of technical reasons: n = 3 (salmon group), 1 (cod group), and 1 [dietary advice (DA) group].
2. Mean ± SD (all such values).
3. Significantly different from baseline, P < 0.0001 (paired t test).
4. Mean; 95% CI in parentheses (all such values).
5. Significantly different from DA group, P < 0.05 (ANCOVA).

DISCUSSION

The results of this trial do not support the hypothesis that additional fish consumption over a 6-mo period changes the number of colonic apoptotic and mitotic cells or the distribution of mitotic cells within the crypt. Furthermore, no marked differences in these markers were observed between oil-rich and lean fish. To the best of our knowledge, this is the first RCT to have studied the effects of fish consumption on markers of colorectal cancer risk. It is generally considered preferable by many health professionals to encourage dietary changes rather than the use of supplements. Therefore, the consumption of fish in this study better reflects the effects of the current advice to consume 2 portions of fish per week as compared with previous fish-oil studies intervening with n–3 PUFA doses corresponding to 1–4 portions of salmon per day (17, 19). Another advantage of our study was that we compared oil-rich fish with lean fish. This allowed us to explore whether or not the possible effects of fish were mainly associated with n–3 PUFA.

There may be several reasons for not finding significant effects of fish on apoptotic and mitotic cell numbers per crypt. First, the baseline fish consumption of 1.5 portions/wk was already high in these subjects, which was also reflected in their higher serum n–3 VLC-PUFA concentrations compared with subjects in other fish-oil supplementation studies (37, 38). Perhaps a more pronounced effect of increasing fish consumption would be expected in a population of nonfish consumers. Second, it appeared that subjects did not consume the salmon or cod in addition to their habitual fish consumption, as requested, but partly substituted the fish they would normally consume with the study fish. Therefore, the intended increase of 2 additional portions of fish per week actually only resulted in an increase of 1.4 and 1.3 extra portions per week of salmon and cod, respectively. This resulted in an additional intake of 0.99 and 0.05 g n–3 VLC-PUFAs/d for salmon and cod, respectively. Thus, the contrasts in our study between the intervention groups may not have been large enough to observe a beneficial effect of additional fish consumption. On the other hand, an increase of 1.4 portions/wk may be the maximally achievable dietary modification in a population of fish eaters. The study duration of 6 mo was chosen as being of sufficient length to allow incorporation of n–3 VLC-PUFAs in the colonic epithelium (23). Such changes were expected to increase eicosapentaenoic acid and docosahexaenoic acid concentrations in mitochondrial phospholipids of colonocytes at the expense of n–6 PUFAs, also known as omega-6 PUFAs, thereby enhancing the deletion of colonic cells through apoptosis and reducing the level of DNA adducts through cell proliferation (39). Of all 7 studies that evaluated the effects of fish-oil supplementation on cell proliferation, 4 studies lasting 1–6 mo observed an effect (17–20). As far as we know, 3 studies assessed the effects of fish-oil supplementation on apoptosis: 2 studies observed an effect after 3 (18) and 24 (21) mo. Thus, for both markers, the 6-mo intervention appears to be long enough; however, for apoptosis, only one short-term study was not able to do so (22).

To measure apoptosis and mitosis, a morphologic method was used that has 2 advantages over other methods. Ki67 (8) and TUNEL (11) require well-orientated histologic sections to allow identification of full-length longitudinal crypt sections (40), whereas apoptosis, mitosis, and the distribution of mitosis within the crypt can be measured simultaneously in the whole crypt with the morphologic method. In a whole crypt mount, it is easier to detect the relatively rare apoptotic cells. However, the whole-crypt-mount approach does not identify all apoptotic cells on the luminal surface, whereas TUNEL and M30 stains may preferentially detect this normal programmed cell death (11). It is a moot point as to whether crypt-associated apoptosis or luminal

TABLE 4
Markers of colorectal cancer: number of apoptotic cells and mitotic cells per crypt in the FISHGASTRO study

<table>
<thead>
<tr>
<th></th>
<th>Salmon group (n = 74)</th>
<th>Cod group (n = 70)</th>
<th>DA group (n = 69)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Apoptotic cells (no./crypt)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.7 ± 1.02</td>
<td>0.7 ± 0.9</td>
<td>0.6 ± 0.7</td>
</tr>
<tr>
<td>End</td>
<td>0.6 ± 0.65</td>
<td>0.5 ± 0.7</td>
<td>0.5 ± 0.6</td>
</tr>
<tr>
<td>Change</td>
<td>−0.2 ± 0.9</td>
<td>−0.2 ± 0.8</td>
<td>−0.1 ± 0.6</td>
</tr>
<tr>
<td>Difference in change compared with DA</td>
<td>−0.1 (−0.4, 0.2)</td>
<td>−0.1 (−0.3, 0.2)</td>
<td>—</td>
</tr>
<tr>
<td><strong>Mitotic cells (no./crypt)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.0 ± 3.9</td>
<td>7.1 ± 4.5</td>
<td>6.2 ± 4.2</td>
</tr>
<tr>
<td>End</td>
<td>5.1 ± 3.3</td>
<td>5.0 ± 3.1</td>
<td>5.1 ± 3.3</td>
</tr>
<tr>
<td>Change</td>
<td>−1.9 ± 5.0</td>
<td>−2.1 ± 4.6</td>
<td>−1.1 ± 4.5</td>
</tr>
<tr>
<td>Difference in change compared with DA</td>
<td>−0.9 (−2.4, 0.7)</td>
<td>−1.0 (−2.6, 0.5)</td>
<td>—</td>
</tr>
</tbody>
</table>

1. DA, dietary advice.
2. Mean ± SD (all such values).
3. Mean; 95% CI in parentheses (all such values).
apoptosis is of most clinical significance (41). Different methods have been used to measure apoptosis and mitosis in various studies, which makes it difficult to compare our results with those of others. However, compared with a previous study using the same method to measure mitosis, our subjects had comparable levels of cell proliferation (42), yet the variation in mitotic levels was much higher despite the larger number of subjects in this study, and this could have affected the power of our study. One possible explanation as to why the variation was higher than in a previous study might be that the patient group used in this study was less homogenous than in this previous study, in which all patients were undergoing resection for colorectal cancer (23). Ideally, we would have studied the incidence of colorectal cancer as the outcome measure; however, because this is not feasible in an RCT, we studied apoptosis and mitosis as intermediate markers of colorectal cancer risk. In addition, other potential early markers of colorectal cancer risk may also be relevant, e.g., DNA damage leading to key mutations, DNA methylation, mitochondrial dysfunction, or presence of aberrant crypt foci. However, in this study, we chose to focus on apoptosis and mitosis as the most well-established markers for relatively short-term RCTs.

Because UC and polyp patients are at an increased risk of developing cancer, it could be hypothesized that the most pronounced effects would occur in these subjects. However, the number of subjects we were able to recruit in the UC group was lower \((n = 41)\) than was originally planned \((n = 90)\). Recruitment of patients with inactive UC was difficult because the number of eligible patients from those visiting the hospital for a colonoscopy was relatively low; thus, not reaching 90 subjects as planned would have decreased the power of our study. The changes in apoptotic numbers did not differ between patient groups for either salmon or cod. However, the most pronounced decrease in the number of mitotic cells was found in the UC patients, although it was not statistically significant. Thus, these data suggest that fish consumption might decrease the number of mitotic cells in UC patients, but this should be investigated in a larger UC population. It should also be noted that, of the patients referred to as having a “healthy colon,” ~60% had bowel complaints as an indication for their colonoscopy, whereas the reasons for the initial endoscopy included familiar occurrence of colorectal cancer, hemorrhoids, or anemia in the other 40%. Although their colonoscopy did not show any colonic abnormalities, these “healthy colon” patients do not all represent truly healthy individuals. Indeed, it was previously shown that a similar group of symptomatic patients with apparently normal mucosa had more mitotic cells per crypt than did those assessed as part of a routine screening program (43).

Another factor that could have affected our results was the use of different bowel preparations before intervention in the 2 countries and also after intervention compared with before intervention. First, preintervention Kleanprep was used in the Netherlands and Picolax was used in the United Kingdom as preparation for colonoscopy; Picolax was recently reported to result in higher levels of cell proliferation than Kleanprep (44). Indeed, we found significantly higher levels in the United Kingdom than in the Netherlands for both apoptosis and mitosis at baseline, which suggests that this could well have influenced our results. However, changes in apoptosis and mitosis were not significantly different between countries. Second, post-intervention colonic biopsy samples were collected by means of sigmoidoscopy, which was additional to the patient’s regular medical care, because it was considered unduly invasive to require subjects to undergo an additional colonoscopy. The switch from colonoscopy at baseline to sigmoidoscopy after the intervention might explain the reduction in mitosis seen in all 3 intervention groups. However, this might only explain the decrease in the United Kingdom, because a decrease in cell proliferation was found after the switch from Picolax to no bowel preparation but not after the switch from Kleanprep to no bowel preparation (44). In designing future studies using colonoscopy or sigmoidoscopy procedures, especially in multicenter studies, attention should be paid to standardizing bowel preparations if feasible.

To further elucidate the association of fish consumption with colorectal cancer, future intervention studies should also try to include nonfish eaters or individuals with a relatively low fish consumption, although this would practically be a major challenge. Alternatively, observational studies could be performed using more detailed questionnaires on fish intake and specifically inquiring as to the different types of fish and the preparation of fish, because this could affect the possible beneficial effects of fish.

### Table 5

<table>
<thead>
<tr>
<th></th>
<th>Salmon group ((n = 74))</th>
<th>Cod group ((n = 70))</th>
<th>DA group ((n = 69))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upper 40% of crypt</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>3.5 ± 5.9(^1)</td>
<td>2.8 ± 4.3</td>
<td>2.5 ± 4.4</td>
</tr>
<tr>
<td>After</td>
<td>2.7 ± 5.1</td>
<td>2.8 ± 5.3</td>
<td>2.2 ± 4.3</td>
</tr>
<tr>
<td>Change</td>
<td>−0.8 ± 5.9</td>
<td>−0.1 ± 5.6</td>
<td>−0.3 ± 3.8</td>
</tr>
<tr>
<td>Difference in change compared with DA</td>
<td>−0.5 (−2.2, 1.2)(^1)</td>
<td>0.2 (−1.6, 1.9)</td>
<td>—</td>
</tr>
<tr>
<td><strong>Lower 40% of crypt</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>81.7 ± 15.0</td>
<td>80.5 ± 13.4</td>
<td>86.0 ± 16.5</td>
</tr>
<tr>
<td>After</td>
<td>82.9 ± 12.2</td>
<td>84.4 ± 13.3</td>
<td>84.5 ± 10.8</td>
</tr>
<tr>
<td>Change</td>
<td>1.1 ± 15.5</td>
<td>4.0 ± 17.3</td>
<td>−1.5 ± 18.1</td>
</tr>
<tr>
<td>Difference in change compared with DA</td>
<td>2.7 (−2.9, 8.3)</td>
<td>5.5 (−0.2, 11.2)</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^1\) DA, dietary advice.
\(^2\) Mean ± SD (all such values).
\(^3\) Mean; 95% CI in parentheses (all such values).
In conclusion, this RCT of fish and markers of colorectal cancer showed that additional fish consumption of approximately 1.4 portions of either oil-rich or lean fish per week over 6 mo does not markedly change apoptotic and mitotic rates of the colonic mucosa in a population of fish eaters.

We are very grateful to all the people who kindly participated in this study. We also thank the endoscopy and gastroenterology staff of the following Dutch hospitals, where the participants were recruited: University Medical Centre Nijmegen Sint Radboud (Nijmegen), Hospital Gelderse Vallei (Ede), Slingeland Hospital (Doetinchem), Sint Antonius Hospital (Nieuwegein), Canisius Wilhelmina Hospital (Nijmegen), and Hospital Rijinstate (Arnhem). We also thank the clinical laboratories from all of these hospitals. From the United Kingdom, we thank the endoscopy and gastroenterology staff of the Norfolk & Norwich University Hospital NHS Trust and James Paget University Hospital. From the Division of Human Nutrition, Wageningen University, Netherlands, we thank all those who contributed to conducting this intervention study, including Yvonne ter Telge, Janneke van Wijngaarden, Celine Brattenga, Margriet Smits, Annemieke Kok, Liza van Steekenbergen, Monique Joriis, Susann Bellmann, Marlike Visser, Else-Mariette van Heijningen, Cathelijne Mieloo, Jantina Stol, Maaike Walters, Anke Emmann, Anne Maria Hilbers, and Betty van der Struijs for conducting the fatty acid analysis. From the National Institute of Public Health and the Environment, Bilthoven, Netherlands, we thank Maria Ocke for her help with the Dutch EPIC-food frequency questionnaire. From the Institute of Food Research, Norwich, United Kingdom, we thank all those who assisted in many ways with this study: Joanne Doleman, Jane Scarll, Noreen Neal, Dave Heart, Angela Twaiete, and the members of Human Nutrition Unit. We thank Marine Harvest, Norway, for donating the salmon and Pescanova, Spain, for donating the cod.

The authors’ responsibilities were as follows—GKP and GM-N planned and coordinated the study and collected and managed the data; GKP performed the statistical analyses, interpreted the results, and drafted the manuscript; AG, LHJ, FMN, BJMW, GS, EKL, and EK designed and initiated the trial; FMN, BJMW, PCvdM, RT, AT, PJW, ARH, and MPW: clinicians involved as part of the hospital research team; LJH, FMN, BJMW, GS, EKL, and EK: designed and initiated the trial; AG, GKP, PCvdM, and RT: contributed to the study’s execution; AG, GKP, PCvdM, and RT: supervised and ran the trial; AG, GKP, PCvdM, and RT: led the project; AG, GKP, PCvdM, and RT: have provided the necessary resources for the study; AG, GKP, PCvdM, and RT: contributed to the study’s interpretation; AG, GKP, PCvdM, and RT: drafted the manuscript; AG, GKP, PCvdM, and RT: critically reviewed the manuscript, and approved the final draft. None of the authors had any financial or personal conflicts of interest to disclose.

REFERENCES


31. Welch AA, Lund E, Amiano P, et al. Variability of fish consumption by guest on September 6, 2017 ajcn.nutrition.org Downloaded from


44. Croucher LJ, Bury JP, Williams EA, Riley SA, Corfe BM. Commonly used bowel preparations have significant and different effects upon cell proliferation in the colon: a pilot study. BMC Gastroenterol 2008;8:54.