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## **Fish and salad consumption are inversely associated with levels of oxidatively damaged DNA in a Danish adult cohort**

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Running head: Dietary items and oxidatively damaged DNA

### Highlights

- Oxidatively damaged DNA was measured with the comet assay in blood cells.
- Intake of fruit, vegetable, whole grains, fish, salad and potatoes were assessed.
- Levels of Fpg-sensitive sites were inversely associated with intake of fish.
- Levels of Fpg-sensitive sites were inversely associated with intake of salad.
- There was no association between dietary items and DNA repair activity.

### Abstract

This study investigated associations between levels of oxidatively damaged DNA, measured by the formamidopyrimidine DNA glycosylase (Fpg)-modified comet assay and intake of fish, salad, fruits, vegetables, wholegrain items, and potatoes in a cross-sectional study of 382 men and 591 women between 18 and 93 years. Intake of dietary items was obtained from questionnaires, and stratified into less than once per week, weekly or daily consumption. Intake of fish as main course was inversely associated with levels of Fpg-sensitive sites in peripheral blood mononuclear cells (PBMCs) in especially women ( $P < 0.001$  multivariate linear regression). Intake of fish was also inversely associated with lower levels of Fpg-sensitive sites in men ( $P < 0.05$ , univariate analysis), although it was not statistically significant in analysis adjusted for lifestyle and other dietary factors. Intake of salad was inversely associated with levels of Fpg-sensitive sites in men ( $P < 0.001$ , multivariate linear regression). Statistically significant associations were also observed for intake of vegetables and potatoes in men, although these were weak and not robust in all statistical models. The sum of the six individual dietary items was inversely associated with levels of Fpg-sensitive sites in the strata of men ( $P < 0.001$ , multivariate linear regression). Finally, levels of DNA repair incision activity were not associated with individual food categories or the total dietary food score. In summary, consumption of health-promoting foods is associated with lower levels of Fpg-sensitive sites in human PBMCs and strongest effects in the present population were ingestions of fish and salad.

Keywords: biomonitoring, comet assay, DNA repair, nutrition, oxidatively damaged DNA.

## 1. Introduction

The comet assay has been used to investigate beneficial effects on DNA damage in various supplementation trials with predominantly antioxidants and phytochemicals [1]. The most

convincing results have been obtained with the enzyme-modified comet assay for the measurement of oxidatively damaged DNA, whereas DNA strand breaks appear to be the least sensitive comet assay endpoint in antioxidant supplementation trials [2]. The oxidatively generated nucleobase lesion 8-oxo,7,8-dihydroguanine (8-oxoGua) can be measured with the comet assay by incubating the gel-embedded DNA with excision repair enzymes from bacteria (formamidopyrimidine DNA glycosylase (Fpg)) or humans (human 8-oxoguanine DNA glycosylase (hOGG1)). The Fpg-modified comet assay seems to detect higher levels of oxidatively damaged DNA in human leukocytes than the hOGG1 version of the assay [3]. The most recent dietary intervention studies, published in the 2010s, on levels of Fpg- or hOGG1-sensitive sites have focussed on blueberries [4-6], kiwi fruits [7] and beverages such as green tea [8, 9] and coffee [10-13]. Certain older intervention studies did not detect effects on levels of oxidatively damaged DNA after dietary intervention with fruits and vegetables in well-nourished subjects [14, 15]. Antioxidant and phytochemical supplementation might only be effective as DNA-protective substances in subjects who suffer from oxidative stress due to low habitual intake or high turn-over of antioxidants, for instance due to smoking or disease [16].

Recent epidemiologic evidence indicates that adherence to five different lifestyle factors have a profound impact on risk of all-cause mortality [17]. These lifestyle factors include diet (low consumption of fats and processed meat; high consumption of fish, grains, fruits and vegetables), modest alcohol consumption, physical activity, short waist circumference and no smoking. A new trend has also emerged, which integrates traditional Nordic food items with expected health-promoting effects (fish, cabbages, rye bread, oatmeal, apples, pears and root vegetables) into a Nordic food index, which is inversely associated with risk of all-cause mortality in especially men, colorectal cancer in women and type-2 diabetes in both men and women [18-20]. The present study investigated the effect of dietary habits in a large cross-sectional study of healthy humans that were

recruited for a national health survey in Denmark [21]. Levels of oxidatively damaged DNA in peripheral blood mononuclear blood cells (PBMCs) have been reported previously, whereas associations between DNA damage and dietary habits were not investigated [22]. DNA repair incision activity of oxidatively damaged DNA has been assessed as a possible mechanism of action of associations between dietary habits and levels of Fpg-sensitive sites in PBMCs.

## 2. Methods

### *2.1. Study description and collection of blood samples*

The subjects were invited to participate in a health examination, and an internet-based questionnaire on socio-demography, health behaviour, self-reported health status, and living condition. There were no inclusion or exclusion criteria. The collection of blood samples has been described previously [23]. We collected blood from 1,019 volunteers in Copenhagen, Denmark. The number of subjects was based on the original aim of investigating the influence of the most prevalent polymorphism (Ser326Cys) on DNA repair incision activity, assessed by the comet assay. A power calculation showed that 1,000 samples were required to detect a 25% difference between the wild-type and variant genotype. There was no association between the Ser326Cys polymorphism and the DNA repair incision activity. In a subsequent study, we reported associations between DNA damage levels and age, sex, body mass index (BMI), blood pressure, smoking habit, alcohol consumption (number of drinks per week), cholesterol, triglycerides, C-reactive protein and glycosylated haemoglobin (HbA1c) [22]. The statistical analysis revealed a residual variation of 0.17 lesions/ $10^6$  bp for the level of Fpg-sensitive sites (mean level of Fpg-sensitive sites = 0.48 lesions/ $10^6$  bp, coefficient of variation = 35%). In comparison, the mean and standard deviation of the assay controls were 1.07 and 0.17 lesions/ $10^6$  bp, respectively (coefficient of variation = 16%). The higher level of residual variation in Fpg-sensitive sites in the blood samples as compared to assay controls indicated that there was unexplained variation in the dataset. The assessment was

based on the coefficient of variation rather than the standard deviations because the assay controls had higher level of Fpg-sensitive sites than blood samples and the variation in the comet assay is typically proportional to the level of DNA damage. We hypothesized that the differences in dietary habits were important contributors to the unexplained residual variation.

The present study is based on results from 382 men and 591 women as questionnaire results are missing from some subjects. In addition, questionnaires from two women had missing information of consumption of salad. The age range was 18-85 years (mean = 49.7 years, standard deviation = 16.2 years) and 18-93 years (mean = 48.6 years, standard deviation = 16.5 years) for the men and women, respectively. The body mass index was 25.0 kg/m<sup>2</sup> (standard deviation = 3.2 kg/m<sup>2</sup>, range: 17.4 – 39.4 kg/m<sup>2</sup>) and 23.7 kg/m<sup>2</sup> (standard deviation = 3.6 kg/m<sup>2</sup>, range: 15.8 – 41.2 kg/m<sup>2</sup>) for the men and women, respectively.

## *2.2. Questionnaire*

The dietary questions have been developed by The National Institute of Public Health and the Danish Veterinary and Food Administration, and the questions have been used in the National Representative Health and Morbidity Studies in Denmark with three 3 to five year intervals since 2000 [24]. The questions (in Danish) are available as supplementary file 1. The questions in the questionnaire assessed the consumption of 1) wholegrain bread and cereals, 2) fruit (the question in Danish was followed by apples, bananas and oranges as examples of fresh fruit), 3) vegetables (boiled, fried or baked), 4) salad (the question also highlighted the name of a special type of Danish salad that consists of shredded or tiny raw vegetables, e.g. carrots and different types of cabbage mixed with e.g. apples, raisins and oranges as main ingredients), 5) potatoes, and 6) fish (the question specified that it was “fish for supper”). The responses were segregated into six categories: 1) never or very seldom, 2) less than once per week, 3) once per week, 4) a couple of times per

week, 5) almost daily, and 6) several times per day. However, this segregation of results produces relatively small group sizes in especially sex-specific strata (table 1). Thus, we have pooled the results to three groups referred to as *less than once per week* (original group 1 and 2, score = 0), *weekly* (original group 3 and 4, score = 1), and *daily* (original group 5 and 6, score = 2) consumption of the dietary items.

We have calculated a *total dietary intake score*. The total dietary intake score has a range from 0 to 12. The highest score is equivalent to the 2 multiplied with 6 dietary item groups). There were no subjects in categories 0, and a subjects in category 1 (n = 3) and 2 (n = 6) have been incorporated in category 3. Likewise, the result from one subject in category 12 was incorporated into category 11 in order to avoid too small group sizes. In this score, ingestion of potatoes is considered to be healthy because the Danish Dietary Recommendations highlighted daily intake of potatoes as beneficial to health due to the content of certain vitamins and fibres [25]. In addition, the recommendations noted that diets with high carbohydrate content are healthy because of the associated lower intake of saturated fatty acids.

### *2.3. Measurement of Fpg-sensitive sites*

The comet assay procedures and original results on DNA damage levels have been published previously [22]. In brief, the cells were embedded in 0.75% low melting point agarose. We used 45 min incubation for the treatment with Fpg (gift from Professor Andrew Collins, University of Oslo, Norway) in a humidified box. The duration of electrophoresis was 20 min (0.83 V/cm from anode to cathode, 25 V, 300 mA on the power supply). The level of Fpg-sensitive sites is the difference in scores of parallel slides incubated with Fpg or buffer, respectively. They are reported as lesions/10<sup>6</sup> base pairs (bp) after transformation of the primary comet assay descriptor (i.e. visual classification) by use of X-ray calibration curves as described previously [26]. We prefer to report comet assay



results as lesions/ $10^6$  bp because it is more informative to report the number of lesions than the primary comet assay descriptor and the standardization should remove the inter-investigator variation [27]. The present report describes results on Fpg-sensitive sites, whereas hOGG1-sensitive sites and DNA strand breaks have not been reported because they only showed minor associations with lifestyle factors and there was no association with food categories in a preliminary assessment of the dataset.

#### *2.4. Measurement of DNA repair incision activity of oxidatively damaged DNA*

Results on DNA repair incision activity of oxidatively damaged DNA have been published by Jensen et al. 2012 [23] and Løhr et al. 2015 [28] on subsets of the samples. Jensen et al. 2012 [23] analysed the DNA repair incision activity in 147 samples; 49 samples from each of the Ser326Cys single nucleotide polymorphism groups of hOGG1 were analysed in comet assay batches as group-matched samples. Løhr et al. 2015 [28] assessed the effect of age on DNA repair incision activity, using age-matched samples of subjects with the same sex ( $n = 78$ ). In addition, the studies used different assay conditions and substrate DNA for the repair incision activity (Ro19-8022 + light and  $\text{KBrO}_3$ , respectively). Thus, the DNA repair incision activities are not directly comparable on a quantitative scale, although the assays detect repair activity toward 8-oxoGua. For the present study we have reported the results separately in the two datasets. In addition, we have pooled Z-scores from the two different datasets using the equation:  $Z\text{-value} = (X_i - \mu_i) / \sigma_i$  ( $X_i$ : value of the sample,  $\mu_i$ : mean of the population,  $\sigma_i$ : standard deviation of the population).

#### *2.5. Statistical analysis*

Associations between dietary items and levels of oxidatively damaged DNA have been assessed in multivariate linear regression models with increasing complexity: unadjusted univariate analysis (model 1) and adjustment for lifestyle factors (age, BMI, blood pressure, smoking habit, alcohol

consumption (number of drinks per week), cholesterol, triglycerides, C-reactive protein and HbA1c; model 2), other dietary items (fruits, fish, salad, whole grains, vegetables or potatoes; model 3), and both lifestyle factors and other dietary items (model 4). All models were applied on separate strata of men and women because our previous study showed differences in the associations between lifestyle factors and levels of oxidatively damaged DNA in men and women [22]. Analyses were considered statistically significant at a 5% level. Percent differences were calculated from linear regression coefficients, using the slope and intercept). Statistical analyses were carried out in Stata version 5.5 for Windows (Stata Corp, College Station, TX, USA).

### 3. Results

#### *3.1. Association between dietary items and levels of Fpg-sensitive sites in PBMCs*

The association between intake of fish and Fpg-sensitive sites is shown in figure 1. There were statistically significant associations in both women ( $P < 0.01$ ) and men ( $P < 0.05$ ) in unadjusted analyses. As can be seen in figure 1, the statistically significant inverse associations were mainly driven by the difference between subjects with less than once per week versus weekly consumption of fish in both women (-8.8%, 95% CI: -13.8%, -3.8%) and men (-7.2%, 95% CI: -14.5%, -0.5%). Ingestion of fish was inversely associated with lower level of Fpg-sensitive sites in all statistical models in women, whereas the statistically significant difference in unadjusted analysis was attenuated in adjusted analyses in the strata of men (table 2). The fully adjusted analysis (model 4) showed a stronger effect in women (-30%, 95% CI: -46%, -15%) as compared to men (-7.1%, 95% CI: -16%, 2.1%) between the groups of less than once per week versus weekly fish consumption. It should be noted that the inverse association in men was similar in unadjusted and fully adjusted analyses, whereas the confidence interval increased. Thus, the statistically non-significant association with fish consumption in men might be due to the statistical power rather than attenuation of the association.

The association between intake of salad and Fpg-sensitive sites is shown in figure 2. Ingestion of salad was inversely associated with lower levels of Fpg-sensitive sites in all statistical models in the strata of men (table 2). In the fully adjusted model, there was 19% lower level of Fpg-sensitive sites per unit increase in salad consumption (95% CI: -29%, -8.8%). There was also a weak and not entirely robust inversely association between intake of potatoes and lower levels of Fpg-sensitive sites (table 2). The inverse association between intake of vegetables and Fpg-sensitive sites was statistically significant in the strata of men in analyses that were adjusted for other dietary items (table 2). It should be noted that the association changed direction from inverse (unadjusted analysis, model 1) to positive association (fully adjusted analysis, model 4). The equivocal associations between the intake of vegetables and Fpg-sensitive sites are most likely due to covariance with other dietary items. Although, the associations between Fpg-sensitive sites and intake of wholegrain items were generally inverse and strongest in men, none were statistically significant.

Figure 3 shows the association between the total dietary food score and levels of Fpg-sensitive sites. Unadjusted analysis showed an inverse association in the strata of men (slope = -0.016,  $P < 0.001$ , model 1), which was robust in statistical analysis adjusted for lifestyle factors (slope = -0.015,  $P < 0.001$ , model 2). There was no association in the strata of women in unadjusted (slope = -0.004,  $P > 0.05$ , model 1) and adjusted analyses (slope = -0.005,  $P > 0.05$ , model 2). The group of men had slightly lower total dietary food score (median, 5<sup>th</sup> and 95<sup>th</sup> percentile of 8, 4 and 11, respectively) than women (median, 5<sup>th</sup> and 95<sup>th</sup> percentile 9, 5 and 11, respectively ( $\chi^2 = 16.5$ ,  $P < 0.05$ ).

### *3.2. DNA incision repair activity toward oxidatively damaged DNA*

The mean levels of DNA repair incision activity are outlined in table 3. The results of the two different studies are shown separately because of differences in assay conditions [22, 23]. In the

combined analysis, Z-scores from the two studies have been pooled. In general, there was no association between the dietary components and the level of DNA repair incisions.

#### 4. Discussion

The present study shows an inverse association between the intake of fish and oxidatively damaged DNA in PBMCs of healthy humans. The same trend was observed in both sexes, although statistically significant results were only maintained in the strata of women in fully adjusted statistical models. Especially, the adjustment for other dietary items affected the statistical outcome in the strata of men, whereas the effect size was not attenuated (7.2% and 7.1% lower levels of Fpg-sensitive sites in unadjusted and fully adjusted models). It suggests that there could be a protective effect of fish consumption in men, although the statistical power was insufficient to detect a statistically significant effect at 5% level. The term “fish” in our study refers to any species of fish. The most commonly consumed types of fish in Denmark are cod, saithe, herring, plaice and salmon. Fish contains a number of constituents that promote good health, including polyunsaturated fatty acids, selenium, iodine and vitamin D. Certain intervention studies have indicated that ingestion of polyunsaturated fatty acids did not alter the level of oxidatively damaged DNA [29]. Likewise, an intervention study showed no effect of vitamin D supplementation on the basal level of Fpg-sensitive sites in lymphocytes [30]. Two selenium supplementation trials showed unaltered levels of 8-oxo-7,8-dihydroguanine-2'-deoxyguanosine (8-oxodG) and Fpg-sensitive sites in leukocytes [31, 32]. To the best of our knowledge, there are no studies on iodine intake and levels of Fpg-sensitive sites in humans. The Danish Health Authority recommends ingestion of fish as main course at least twice a week. The only exceptions are women who are pregnant, plan pregnancy, or are lactating, and children between 3 and 14 years old. It is recommended to these vulnerable groups that they should avoid the ingestion of large predator fish (e.g. tuna or swordfish) due to the possibility of high mercury content. As the population in the present study was 18 years and above, the

observations should not be generalized to populations of children and adolescents. To the best of our knowledge, there are no studies that have assessed the association between habitual intake of seafood and levels of oxidatively damaged DNA in leukocytes, whereas some studies have shown a lack of association between fish consumption and urinary excretion of 8-oxodG [33, 34]. It should be noted that 8-oxodG excreted in urine is not derived from DNA, as the product of hOGG1-mediated repair is 8-oxoGua [35]. Nevertheless, 8-oxoGua and 8-oxodG are closely correlated and both have been shown to be associated with increased risk of cancer in prospective cohort studies [36-38].

The present study showed an inverse association between intake of salad and levels of Fpg-sensitive sites in men. In the Danish questionnaire, “salad” would most likely be interpreted as a green salad, although it might contain plant materials with other colours (e.g. tomatoes or nuts). The question mentioned a special type of Danish salad, which is based on grated carrots and apples, and therefore has an orange rather than green colour. In general, the consumption of salad displays positive covariance with intake of fruit, wholegrain items and vegetables, which also were negatively associated with levels of Fpg-sensitive sites in men in unadjusted analyses (table 2). The difference in the association of salad ingestion and Fpg-sensitive sites between men and women might be related to the general lower habitual intake of health promoting dietary items as demonstrated by the lower total dietary food score in the strata of men. Although it has not been assessed in the present study, it is possible that men also have a higher intake of “unhealthy” dietary items such as red or processed meat. Similarly, the protective effect of potatoes in the strata of men might be a direct effect of vitamins and fibres or an indirect effect because potatoes are ingested at the expense of unhealthy dietary items.

The statistical analyses showed inconsistent associations between the intake of vegetables and levels of Fpg-sensitive sites. The opposite associations in unadjusted and adjusted analyses suggest

a strong influence of the covariates (i.e. other dietary items) on the association between vegetable intake and Fpg-sensitive sites. In addition, the results contradict the notion that antioxidants and other phytochemicals in vegetables protect against oxidative damage to DNA. Results from a previous cross-sectional study showed a positive association between vegetable intake and hOGG1-sensitive sites in lymphocytes in 31 elderly from Croatia [39]. A study from Florence, Italy showed mainly positive associations between food items and Fpg-sensitive sites in PBMCs from 71 subjects, which reached statistical significance for intake of tomatoes and coffee, whereas there were no statistical significance for vegetables, fruits, potatoes, bread and fish [40]. Using results from a large biomonitoring study on occupational exposure to fibres, it was shown that levels of Fpg-sensitive sites in lymphocytes correlated inversely with intake of fruits, vegetables and cereals [41]. On the other hand, there was no difference in levels of Fpg-sensitive sites in lymphocytes between vegetarians and non-vegetarians [42].

It is important to stress that our observations stem from a cross-sectional study and it has some limitations. 1) The response rate was relatively low (less than 20% of the invited subjects participated in the survey) and generalization from the study population to a wider population should be done with caution. The source population (i.e. residents in Frederiksberg municipality) is a wealthy area in Copenhagen. Thus, it can be speculated that the exposure gradient is relatively small in the present study. Especially, there were few subjects with rare intake of fruits, vegetables, salad and wholegrain items. The Danish Health Authority recommends “increased” ingestion of these food categories. The ingestion of potatoes is competing with pasta and rice, although it traditionally has been a major constituent in hot suppers in Denmark. The ingestion of fish is relatively low in this study population, which is similar to the general Danish population. The study population has a relatively high socioeconomic status and the subjects are generally healthy. Thus, it can be speculated that stronger effects will be seen in poorly nourished subjects. 2) The relatively

crude questionnaire-based assessment of dietary intake might result in non-differential misclassification of the intake of the dietary items, which would increase the within-group variation in biomarker levels and attenuate the true associations. Especially, the questionnaire neither assesses the amount of ingested dietary items per serving nor distinguishes between cooking processes of the fish and vegetables such as boiling or frying. This may also miss the intake of specific dietary items (e.g. cabbage) or vegetables that were trendy before or after the cross-sectional study. 3) Residual confounding in the observed associations may be a problem. High intake of food products from the plant kingdom may be associated with a generally healthy lifestyle. A practical outcome of this limitation is that ingestion of salad should not be recommended if ingestion of this food product occurs at the expense of fruits or other types of vegetables (e.g. cabbage). 4) Reverse causality can be a limitation in cross-sectional studies, although it is not directly a problem in our study as the outcome is a biomarker. The study design has a strong influence on the statistical outcome of human intervention trials on oxidatively damaged DNA [43, 44]. The problem relates mainly to sequential studies that do not control for period effects, i.e. variation in biomarkers over time that may erroneously be interpreted as an effect of the intervention. Placebo-controlled trials with salad and fish are challenging as true placebo items do not exist. Nevertheless, intervention trials have the advantage of being fully quantitative by design as the amount of dietary item is controlled by the investigators rather than the subjects.

## **5. Conclusion**

This cross-sectional study shows that intake of fish and salad, as well as the total dietary food score, is associated and lower levels of Fpg-sensitive sites in PBMCs from healthy humans. The lack of association between dietary items and DNA repair incision activity of oxidatively damaged DNA indicates that the beneficial effects of fish and salad are related to an intake of healthy chemical components (or avoidance of unhealthy dietary items in case of salad and potatoes) rather than

secondary effects related to increased DNA repair activity. The study suggests that beneficial effects of these food items may be more relevant to investigate in short-term dietary supplementation trials than specific chemical components in foods.

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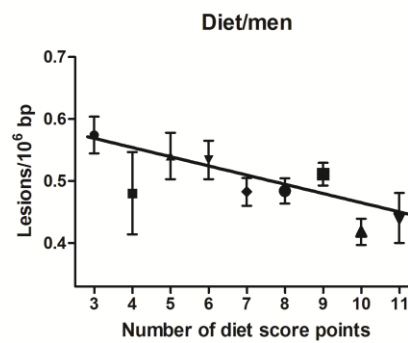
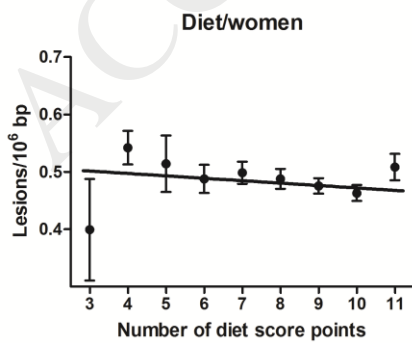
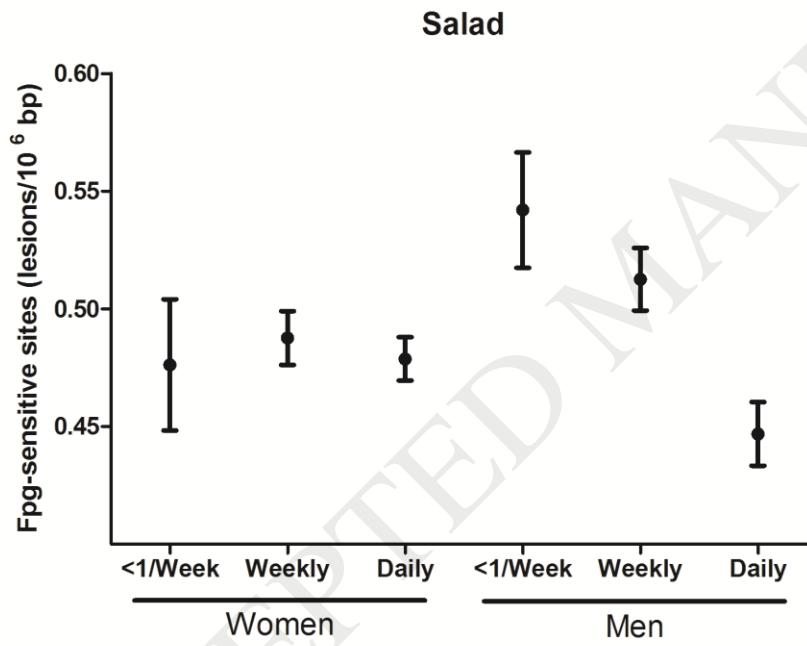
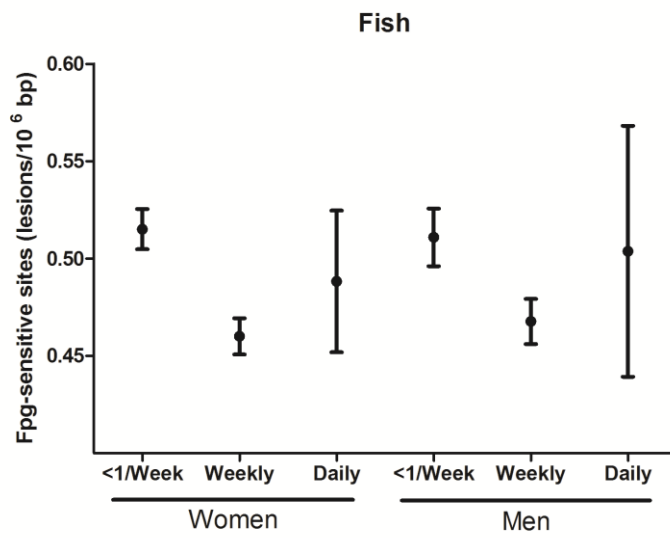
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### Legends to figures

**Figure 1.** Association between intake of fish as main course and levels of Fpg-sensitive sites in peripheral blood mononuclear cells (depicted as lesions/ $10^6$  bp). There are inverse associations in the strata of women ( $P < 0.001$ ) and men ( $P < 0.05$ , unadjusted linear regression analysis). The symbols are mean and SEM values. The number of subjects are 227, 349 and 15 (women) and 144, 232 and 6 (men) in the groups with less than once per week, weekly and daily consumption of fish, respectively.

**Figure 2.** Association between intake of salad and levels of Fpg-sensitive sites in peripheral blood mononuclear cells (depicted as lesions/ $10^6$  bp). There is an inverse association in the strata of men ( $P < 0.001$ , unadjusted linear regression analysis). The symbols are mean and SEM values. The number of subjects are 43, 209 and 337 (women) and 38, 164 and 180 (men) in the groups with less than once per week, weekly and daily consumption of salad, respectively.

**Figure 3.** Association between dietary food score and levels of Fpg-sensitive sites in in peripheral blood mononuclear cells (depicted as lesions/ $10^6$  bp). There is an inverse association in the strata of men ( $P < 0.001$ , unadjusted linear regression analysis). The symbols are mean and SEM values. The number of subjects are 6/12, 10/11, 22/24, 48/36, 67/50, 101/68, 158/80, 143/77 and 34/24 in groups score 3 to 11 (women/men).



**Table 1.** Distribution of subjects in categories of dietary items in women and men

<b>Category</b>	<b>Less than once per week</b>	<b>Weekly</b>	<b>Daily</b>
<i>Women</i>			
Whole grains	2% (2/10)	11% (14/51)	87% (179/335)
Fruits	3% (5/13)	13% (14/64)	84% (130/365)
Vegetables	4% (3/23)	36% (45/167)	60% (167/229)
Salad	7% (8/35)	35% (46/163)	57% (219/118)
Potatoes	31% (28/117)	51% (107/224)	18% (111/4)
Fish	38% (47/180)	59% (211/138)	3% (13/2)
<i>Men</i>			
Whole grains	4% (5/12)	13% (4/47)	82% (144/170)
Fruits	5% (4/14)	24% (22/68)	72% (116/158)
Vegetables	9% (5/29)	42% (40/122)	49% (139/47)
Salad	10% (11/27)	43% (52/112)	47% (130/50)
Potatoes	18% (5/64)	60% (70/158)	22% (81/4)
Fish	38% (32/112)	61% (153/79)	2% (5/1)

Numbers in brackets correspond to original questionnaire classes “never or very seldom” and “less than once per week” (i.e. “less than once per week”), “once per week” and “couple of times per week” (i.e. “weekly”), and “almost daily” and “several times per day” (i.e. “daily”). “Whole grains”

refers to wholegrain bread and cereals. The total number of subjects are 591 women and 382 men, except for the salad consumption in the strata of women because of missing information in two questionnaires (n = 589).

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**Table 2.** Slopes of linear regression analysis of Fpg-sensitive sites in peripheral blood mononuclear cells of humans

<b>Model</b>	<b>Fruits</b>	<b>Fish</b>	<b>Salad</b>	<b>Whole grains</b>	<b>Vegetables</b>	<b>Potatoes</b>
<i>Women</i>						
Model 1	0.008	<b>-0.044**</b>	-0.003	-0.003	-0.007	0.003
Model 2	0.013	<b>-0.050***</b>	-0.001	-0.006	-0.007	-0.006
Model 3	0.018	<b>-0.046**</b>	-0.002	-0.005	-0.001	0.005
Model 4	0.023	<b>-0.052***</b>	0.003	-0.009	-0.005	-0.005
<i>Men</i>						
Model 1	-0.011	<b>-0.038*</b>	<b>-0.054***</b>	-0.029	-0.025	<b>-0.029*</b>
Model 2	-0.009	<b>-0.037*</b>	<b>-0.048**</b>	-0.027	-0.020	<b>-0.038*</b>
Model 3	0.003	-0.024	<b>-0.082***</b>	-0.018	<b>0.051*</b>	-0.027
Model 4	0.004	-0.028	<b>-0.074***</b>	-0.020	<b>0.055*</b>	<b>-0.038*</b>

Model 1 (unadjusted linear regression), model 2 (adjusted for effect of life-style factor), model 3 (adjusted for other dietary items) and model 4 (adjusted for both lifestyle factors and other dietary items). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001. “Whole grains” refers to wholegrain bread and cereals.

**Table 3.** DNA repair activity of oxidatively damaged DNA in cell extracts from peripheral blood mononuclear cells of humans

<b>Study</b>	<b>Fruits</b>	<b>Fish</b>	<b>Salad</b>	<b>Whole grains</b>	<b>Vegetables</b>	<b>Potatoes</b>
<i>Study 1</i> (Ro19-8022 + light)						
Less than once per week	0.31±0.08	0.29±0.02	0.22±0.05	0.32±0.18	0.24±0.07	0.26±0.03
Weekly	0.28±0.03	0.24±0.01	0.26±0.02	0.22±0.03	0.26±0.02	0.24±0.01
Daily	0.25±0.01	0.22±0.03	0.26±0.02	0.26±0.01	0.25±0.01	0.30±0.03
<i>Study 2</i> (KBrO <sub>3</sub> )						
Less than once per week	0.41±0.15	0.39±0.04	0.40±0.06	None	0.38±0.09	0.49±0.04
Weekly	0.35±0.03	0.43±0.02	0.38±0.02	0.42±0.03	0.41±0.03	0.40±0.03
Daily	0.43±0.02	None	0.44±0.03	0.41±0.02	0.42±0.03	0.36±0.04
<i>Combined</i>						
Less than once per week	0.28±0.50 (8)	0.09±0.14 (73)	-0.16±0.25 (17)	0.53±1.41 (2)	-0.14±0.38 (11)	0.22±0.16 (47)

Weekly	-0.03±0.16 (44)	-0.03±0.08 (140)	-0.04±0.10 (81)	-0.14±0.15 (31)	0.05±0.12 (69)	-0.09±0.08 (130)
Daily	0.01±0.08 (165)	-0.30±0.26 (4)	0.07±0.10 (119)	0.03±0.08 (184)	0.01±0.09 (137)	0.08±0.17 (40)

The results are mean and SEM (number of subjects) in stratified groups. Results in study 1 [23] and 2 [22] are reported as incisions/10<sup>6</sup> bp. The results in the combined analysis of study 1 and 2 are Z-scores. “Whole grains” refers to wholegrain bread and cereals.



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