Long term gluten consumption in adults without celiac disease and risk of coronary heart disease: prospective cohort study

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ABSTRACT

OBJECTIVE
To examine the association of long term intake of gluten with the development of incident coronary heart disease.

DESIGN
Prospective cohort study.

SETTING AND PARTICIPANTS
64 714 women in the Nurses’ Health Study and 45 303 men in the Health Professionals Follow-up Study without a history of coronary heart disease who completed a 131 item semiquantitative food frequency questionnaire in 1986 that was updated every four years through 2010.

EXPOSURE
Consumption of gluten, estimated from food frequency questionnaires.

MAIN OUTCOME MEASURE
Development of coronary heart disease (fatal or non-fatal myocardial infarction).

RESULTS
During 26 years of follow-up encompassing 2 273 931 person years, 2 431 women and 4 098 men developed coronary heart disease. Compared with participants in the lowest fifth of gluten intake, who had a coronary heart disease incidence rate of 352 per 100 000 person years, those in the highest fifth had a rate of 277 events per 100 000 person years, leading to an unadjusted rate difference of 75 (95% confidence interval 51 to 98) fewer cases of coronary heart disease per 100 000 person years. After adjustment for known risk factors, participants in the highest fifth of estimated gluten intake had a multivariable hazard ratio for coronary heart disease of 0.95 (95% confidence interval 0.88 to 1.02; P for trend=0.29).

Conclusions
A reduction in dietary gluten may result in the reduced consumption of whole grains, which are associated with lower cardiovascular risk.
RESEARCH

were available for analysis. Return of the mailed questionnaires on health and lifestyle habits, anthropometrics, environmental exposures, and medical conditions. In 1986, diet in both cohorts was assessed with a validated 136 item semiquantitative food frequency questionnaire. Among the 73,666 women in NHS and 49,934 men in HPFS who completed a food frequency questionnaire in 1986, we excluded participants if they reported implausible daily energy intake (<600 or >3500 kcal/d for women and <800 or >4200 kcal/d for men) or missing gluten data (NHS 48; HPFS 39); a diagnosis of myocardial infarction, angina, or stroke or coronary artery bypass graft surgery (NHS 4015; HPFS 2647); or cancer (NHS 4689; HPFS 1785). Participants were specifically asked about a history of celiac disease in 2014; we excluded from this analysis anyone who reported a previous diagnosis of celiac disease (NHS 200; HPFS 160). After these exclusions, 64,714 women and 45,303 men were available for analysis. Return of the mailed questionnaire was considered to imply informed consent.

Methods

Study population

The Nurses’ Health Study (NHS) is a prospective cohort of 121,700 female nurses from 11 US states who were enrolled in 1976. The Health Professionals Follow-up Study (HPFS) is a prospective cohort of 51,529 male health professionals from all 50 states who were enrolled in 1986. Participants in NHS and HPFS have been followed via biennial self administered questionnaires on health and lifestyle habits, anthropometrics, environmental exposures, and medical conditions. In 1986, diet in both cohorts was assessed with a validated 136 item semiquantitative food frequency questionnaire. Among the 73,666 women in NHS and 49,934 men in HPFS who completed a food frequency questionnaire in 1986, we excluded participants if they reported implausible daily energy intake (<600 or >3500 kcal/d for women and <800 or >4200 kcal/d for men) or missing gluten data (NHS 48; HPFS 39); a diagnosis of myocardial infarction, angina, or stroke or coronary artery bypass graft surgery (NHS 4015; HPFS 2647); or cancer (NHS 4689; HPFS 1785). Participants were specifically asked about a history of celiac disease in 2014; we excluded from this analysis anyone who reported a previous diagnosis of celiac disease (NHS 200; HPFS 160). After these exclusions, 64,714 women and 45,303 men were available for analysis. Return of the mailed questionnaire was considered to imply informed consent.

Measurement of exposure and outcome

In both cohorts, diet was assessed in 1986, 1990, 1994, 1998, 2002, 2006, and 2010. For each food item, participants were asked about the frequency with which they consumed a commonly used portion size for each food over the previous year; available responses ranged from never or less than once a month to six or more times a day. We calculated nutrients by using the Harvard T. H. Chan School of Public Health nutrient database, which was updated every two to four years during the period of food frequency questionnaire distribution. We used year specific nutrient tables for ingredient level foods. Previous validation studies have shown that the derivation of nutrient values correlates highly with nutrient intake as measured by one week food diaries in women and men.

For each of these two cohorts, we derived the quantity of gluten consumed. We calculated the quantity of gluten on the basis of the protein content of wheat, rye, and barley based on recipe ingredient lists from product labels provided by manufacturers or cookbooks in the case of home prepared items. Previous studies have used conversion factors of 75% or 80% when calculating the proportion of protein content that comprises gluten; we used the more conservative estimate of 75%). Although gluten’s proportion of total protein may be more variable for rye and barley than for wheat, we used the same conversion factor for all three grains, consistent with previous studies.

Although trace amounts of gluten can be present in oats and in condiments (for example, soy sauce), we did not calculate gluten on the basis of these items as the quantity of gluten is much lower than that in cereals and grains and the contribution to total gluten intake would be negligible.

In 1986 the five largest contributors to gluten in both cohorts were dark bread, pasta, cold cereal, white bread, and pizza (supplementary table A). Previous validation studies within these cohorts found that the Pearson correlation coefficients between the number of servings of these items reported on food frequency questionnaires and that reported on seven day dietary records ranged from 0.35 (pasta) to 0.79 (cold cereal) for women and from 0.37 (dark bread) to 0.86 (cold cereal) for men. A separate validation study of this food frequency questionnaire found that this method of measuring vegetable (that is, plant based) protein intake, of which gluten is the major contributor, correlated highly with that measured in seven day dietary records (Spearman correlation coefficient 0.66).

We divided cohort participants into fifths of estimated gluten consumption, according to energy adjusted grams of gluten per day. We obtained energy adjusted values by regression using the residual method, as described previously. To quantify long term dietary habits, we used cumulative averages through the questionnaires preceding the diagnosis of coronary heart disease, death, or the end of follow-up. For example, we calculated cumulative average estimated gluten intake in 1994 by averaging the daily consumption of gluten reported in 1986, 1990, and 1994. We treated cumulative average estimated gluten intake as a time varying covariate. For participants with missing dietary data, we used the most recent previous dietary response on record. Because the development of a significant illness may cause a major change in dietary habits, and so as to reduce the possibility of reverse causality, we suspended updating dietary response data for participants who developed diabetes, cardiovascular disease (including stroke, angioplasty, or coronary artery bypass graft surgery), or cancer. For such patients, the cumulative average dietary gluten value before the development of this diagnosis was carried forward until the end of follow-up.

The primary outcome of incident coronary heart disease consisted of a composite outcome of non-fatal myocardial infarction or fatal myocardial infarction. For all participants who recorded such a diagnosis, we requested and reviewed medical records. We classified myocardial infarctions meeting World Health...
Organization criteria, which require typical symptoms plus either diagnostic electrocardiographic findings or elevated cardiac enzyme concentrations, as definite, and we considered myocardial infarctions requiring hospital admission and corroborated by phone interview or letter only as probable. Deaths were identified from state vital records and the National Death Index or reported by participants’ next of kin. We classified coronary heart disease deaths by examining autopsy reports, hospital records, or death certificates. Fatal coronary heart disease was confirmed via medical records or autopsy reports or if coronary heart disease was listed as the cause of death on the death certificate and there was previous evidence of coronary heart disease in the medical records. We designated as probable those cases in which coronary heart disease was the underlying cause on the death certificate but no previous knowledge of coronary heart disease was indicated and medical records concerning the death were unavailable. We considered definite and probable myocardial infarction together as our primary outcome, as we have previously found that results were similar when probable cases were excluded.33

Statistical analyses
Patients were followed from 1986 until the development of coronary heart disease, death, or the end of follow-up in 2012 (June 2012 for NHS; January 2012 for HPFS). We tested for the association between cumulative average gluten intake and the development of coronary heart disease, comparing each fifth of gluten intake with the lowest fifth. We used Cox proportional hazards models conditioning on age in months and follow-up cycle to calculate age adjusted and multivariable adjusted hazard ratios and 95% confidence intervals. We first generated these estimates in each cohort and tested for heterogeneity of the associations by meta-analysis of aggregate data using the Q statistic. Because we did not observe any significant heterogeneity for the association of gluten with coronary heart disease in the two cohorts (P for heterogeneity>0.10), we then did a pooled analysis combining the participants of NHS and HPFS and estimated the hazard ratios by using Cox modeling stratified by study cohort. We tested the assumption of proportional hazards by testing the interaction term between gluten intake and the period of follow-up and found no violations of this assumption (P>0.05).

We tested the hypothesis that increasing amounts of energy adjusted dietary gluten is associated with an increased risk of coronary heart disease. Our main model included non-dietary and dietary covariates, constructed a priori. Non-dietary covariates consisted of age, race (white, non-white), body mass index (by fifth), height (in inches), history of diabetes, regular (at least twice weekly) use of aspirin and non-steroidal anti-inflammatory drugs, current use of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), current use of a multivitamin, smoking history (pack years), parental history of myocardial infarction, history of hypertension, history of hypercholesterolemia, use of physical activity as measured in metabolic equivalents (METs) per week, and (in NHS) menopausal status and menopausal hormone use. Dietary covariates were energy adjusted and consisted of daily consumption of alcohol (grams), trans fats (grams), red meats (servings), processed meats (servings), polyunsaturated fats (grams), fruits (servings), and vegetables (servings). We did several secondary analyses, constructed a priori. Firstly, because gluten is a component of both refined grains and whole grains, which are each purported to be associated with coronary heart disease, we used multivariable models examining the association between estimated gluten intake and coronary heart disease with additional adjustment for refined grain consumption and whole grain consumption. Secondly, we stratified analyses by age (<65 v≥65 years), body mass index (<25 v≥25), physical activity (<18 v≥18 MET-hours/week), and smoking status (current v never v past smoking). Thirdly, we separately considered the outcomes of fatal and non-fatal myocardial infarction. Fourthly, we considered the possibility that an association of estimated gluten intake with coronary heart disease may be evident only when extreme levels of intake are considered; we therefore examined participants according to tenths (instead of fifths) of gluten intake. Fifthly, because identification and treatment of risk factors for coronary heart disease may have changed over time, we repeated the primary analysis, restricting the time period first to 1986-97 and then to 1998-2012. Sixthly, instead of suspending dietary updates on the diagnosis of cardiovascular disease, diabetes, or cancer (as we did for the primary analysis), we repeated the primary analysis, updating dietary responses regardless of the development of these conditions. Finally, in addition to these a priori analyses, we did post-hoc analyses, including each of the following additional dietary variables in our full model: the Alternate Healthy Eating Index score, percentage protein, percentage total fat, and intake of dairy, saturated fatty acids, monounsaturated fatty acids, sodium, and dietary fiber. We used SAS version 9.4 for all analyses and considered two sided P values of <0.05 to be statistically significant.

Patient involvement
No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for recruitment, design, or implementation of the study. No patients were asked to advise on interpretation or writing up of results. Although this specific analysis concerning gluten and coronary heart disease was not conceived in direct collaboration with the research participants, they have been actively engaged in the broad research direction of the cohorts. For example, participants are mailed an annual newsletter that communicates results and highlights notable findings. In response, participants return feedback, including suggestions for future studies. Findings are also disseminated on study websites (www.nursehealthstudy.org and https://www.hsph.harvard.edu/hfps/index.html)
Results
Among 64,714 women and 45,303 men eligible for analysis, the mean daily estimated intake of gluten at baseline was 7.5 (SD 1.4) g among women and 10.0 (2.0) g among men in the highest fifth and 2.6 (0.6) g among women and 3.3 (0.8) g among men in the lowest fifth. In 2010 the mean daily estimated gluten intake was 7.9 (2.4) g among women and 9.2 (2.8) g among men in the highest fifth and 3.1 (1.2) g among women and 3.7 (1.3) g among men in the lowest fifth. Table 1 shows baseline demographic characteristics according to fifth of gluten intake, and table 2 shows dietary characteristics. Gluten intake correlated inversely with alcohol intake, smoking, total fat intake, and unprocessed red meat intake. Glu-</p>
Table 2 | Age adjusted baseline dietary characteristics of study participants by fifths of energy adjusted gluten intake. Values are means (SD) unless stated otherwise and are standardized to age distribution of study population

| Characteristics | Nurses’ Health Study (1986) | Health Professionals Follow-up Study (1986) |
|----------------|-----------------------------|-----------------------------------------------|
|                | 1 (lowest) | 2 | 3 | 4 | 5 (highest) | 1 (lowest) | 2 | 3 | 4 | 5 (highest) |
| Median (range) gluten intake, g/d | 2.8 (0.3-4.4) | 4.7 (4.3-5.1) | 7.1 (6.2-26.7) | 3.5 (0.4-3.6) | 6.0 (5.1-6.6) | 9.4 (8.1-38.4) |
| Total energy, kcal | 1738 (554) | 1815 (525) | 1683 (492) | 1966 (654) | 2045 (619) | 1904 (583) |
| Energy adjusted gluten intake, g/d | 2.6 (0.6) | 4.7 (0.2) | 7.5 (1.4) | 3.3 (0.8) | 6.0 (0.3) | 10.0 (2.0) |
| Median (IQR) whole grains, g/d | 5.9 (2.6-11.2) | 10.3 (5.2-17.1) | 20.7 (10.6-32.9) | 9.4 (4.1-17.9) | 16.1 (8.6-25.9) | 32.7 (19.0-48.3) |
| Refined grains, g/d | 31.7 (12.5) | 47.0 (10.8) | 61.4 (18.3) | 39.0 (18.8) | 57.6 (15.6) | 77.1 (24.6) |
| Cereal fiber, g/d | 2.4 (1.4) | 4.1 (1.9) | 7.2 (4.4) | 3.2 (2.0) | 5.4 (2.5) | 9.7 (5.6) |
| Median (IQR) bran, g/d | 0.8 (0.3-1.9) | 1.7 (0.7-3.5) | 3.9 (1.6-7.8) | 1.2 (0.4-2.9) | 2.6 (1.1-5.7) | 6.3 (2.9-11.6) |
| Polyunsaturated fat, g/d | 10.8 (3.4) | 11.0 (2.7) | 10.9 (2.7) | 13.3 (4.1) | 13.3 (3.3) | 12.9 (3.3) |
| Trans fat, g/d | 2.2 (0.8) | 2.5 (0.9) | 2.6 (1.0) | 2.6 (1.0) | 2.9 (1.1) | 2.8 (1.2) |
| Sodium, mg/d | 2759 (1144) | 2849 (999) | 2836 (967) | 3229 (1325) | 3276 (1086) | 3261 (1059) |
| Glycemic load | 90.4 (22.9) | 97.7 (16.2) | 108.7 (16.2) | 112.2 (29.7) | 122.6 (22.5) | 140.2 (22.4) |
| Glycemic index | 50.5 (4.6) | 52.1 (3.2) | 53.7 (3.0) | 51.7 (4.5) | 53.1 (3.2) | 54.2 (3.0) |
| Median (IQR) processed meat, servings/week | 1.4 (0.5-2.5) | 1.5 (0.9-3.0) | 1.4 (0.5-2.5) | 1.5 (0.9-4.0) | 1.9 (0.9-3.9) | 1.0 (0.5-2.5) |
| Carbohydrate, % of energy (median) | 45.6 | 47.7 | 51.2 | 45.2 | 46.0 | 51.0 |
| Protein, % of energy (median) | 19.1 | 18.4 | 17.9 | 19.0 | 18.3 | 18.0 |
| Total fat, % of energy (median) | 33.7 | 37.2 | 31.2 | 34.0 | 32.7 | 29.4 |
| Unprocessed red meat, servings/week | 4.4 (3.2) | 4.2 (2.6) | 3.3 (2.2) | 5.0 (3.8) | 4.5 (3.1) | 3.1 (2.6) |
| Vegetables, servings/d | 4.3 (2.6) | 4.0 (2.1) | 3.5 (1.9) | 3.6 (2.6) | 3.4 (1.9) | 3.2 (1.9) |
| Fruits, servings/d | 1.9 (1.5) | 1.8 (1.2) | 1.5 (1.0) | 1.7 (1.6) | 1.6 (1.2) | 1.6 (1.2) |
| Alternate Healthy Eating Index score* | 51.9 (11.9) | 51.2 (10.8) | 52.4 (11.0) | 52.0 (11.9) | 52.1 (11.2) | 55.0 (11.5) |
| Carbohydrate, % of energy | 46.5 | 47.1 | 51.2 | 45.2 | 46.0 | 51.0 |
| Protein, % of energy | 19.1 | 18.4 | 17.9 | 19.0 | 18.3 | 18.0 |
| Total fat, % of energy | 33.7 | 37.2 | 31.2 | 34.0 | 32.7 | 29.4 |
| Unprocessed red meat, servings/week | 4.4 (3.2) | 4.2 (2.6) | 3.3 (2.2) | 5.0 (3.8) | 4.5 (3.1) | 3.1 (2.6) |
| Vegetables, servings/d | 4.3 (2.6) | 4.0 (2.1) | 3.5 (1.9) | 3.6 (2.6) | 3.4 (1.9) | 3.2 (1.9) |
| Fruits, servings/d | 1.9 (1.5) | 1.8 (1.2) | 1.5 (1.0) | 1.7 (1.6) | 1.6 (1.2) | 1.6 (1.2) |
| Alternate Healthy Eating Index score* | 51.9 (11.9) | 51.2 (10.8) | 52.4 (11.0) | 52.0 (11.9) | 52.1 (11.2) | 55.0 (11.5) |

*Score of diet quality that incorporates vegetables, fruits, whole grains, sugar sweetened drinks and juices, nuts and legumes, red and/or processed meat, and alcohol, score ranges from 0 (lowest quality) to 110 (highest quality).

Table 3 | Gluten and risk of coronary heart disease (fatal and non-fatal myocardial infarctions)

| Fifth of energy adjusted gluten intake | 1 (lowest) | 2 | 3 | 4 | 5 (highest) | P for trend |
|----------------------------------------|------------|---|---|---|------------|-----------|
| Mean, median (range) gluten intake, g/d | 2.6; 2.8 (0-3.4) | 3.8; 3.8 (3.4-4.3) | 4.7; 4.7 (4.3-5.1) | 5.6; 5.6 (5.1-6.2) | 7.5; 7.1 (6.2-26.7) | -- |
| No of events | 492 | 470 | 494 | 471 | 504 | -- |
| Person years | 246 539 | 280 655 | 290 265 | 296 789 | 293 279 | -- |
| Incidence per 100 000 person years | 200 | 167 | 170 | 159 | 172 | -- |
| Age adjusted HR (95% CI)* | 1.0 (reference) | 0.88 (0.77 to 1.00) | 0.90 (0.80 to 1.02) | 0.84 (0.74 to 0.96) | 0.89 (0.79 to 1.01) | 0.08 |
| Multivariable adjusted HR (95% CI)* | 1.0 (reference) | 0.97 (0.86 to 1.10) | 1.02 (0.90 to 1.16) | 0.97 (0.85 to 1.10) | 1.00 (0.88 to 1.14) | 0.98 |
| Full model HR (95% CI)* | 1.0 (reference) | 0.96 (0.85 to 1.10) | 1.02 (0.90 to 1.16) | 0.96 (0.84 to 1.09) | 1.01 (0.89 to 1.15) | 0.92 |

*Additionally adjusted for race, body mass index, height, history of diabetes, regular non-steroidal anti-inflammatory drug use, current use of multivitamin, alcohol intake (g/d), smoking (pack years), aspirin use, statin use, parental history of myocardial infarction, history of hypertension, history of hypercholesterolemia, physical activity (MET, h/wk), menopausal status (Nurses’ Health Study only), and menopausal hormone use (Nurses’ Health Study only).

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**Additional information:**

- GL values are means (SD) unless stated otherwise and are standardized to age distribution of study population.
- Table 2: Age adjusted baseline dietary characteristics of study participants by fifths of energy adjusted gluten intake. Values are means (SD) unless stated otherwise and are standardized to age distribution of study population.
- Table 3: Gluten and risk of coronary heart disease (fatal and non-fatal myocardial infarctions).

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remaining variance of gluten intake correlating with whole grain intake), we found an inverse relation between estimated gluten intake and coronary heart disease; participants in the highest fifth of gluten intake had a lower coronary heart disease risk (hazard ratio 0.85, 0.77 to 0.93). When we instead adjusted for whole grains (leaving the variance of gluten intake correlating with refined grain intake), we found no association between gluten intake and incident coronary heart disease; participants in the highest fifth of gluten intake had a risk of coronary heart disease that was not different from those in the lowest group (hazard ratio 1.00, 0.92 to 1.09).

Table 5 shows results according to subgroups defined by age, body mass index, physical activity, and smoking status. The association between estimated gluten intake and coronary heart disease remained null across all of these strata with the exception of smoking status. Among current smokers, the highest fifth of gluten intake was associated with increased risk of coronary heart disease (hazard ratio 1.34, 1.09 to 1.66; P for trend = 0.02). However, when we additionally adjusted for refined grains (leaving the variance of gluten intake correlating with whole grain intake), the association between gluten intake and coronary heart disease was no longer significant (hazard ratio for highest fifth 1.25, 0.95 to 1.64; P for trend = 0.21).

We found no significant association between estimated gluten intake and either fatal myocardial infarction or non-fatal myocardial infarction when considered as separate outcomes (see supplementary table B). We did not observe any significant associations between tenth categories of gluten intake with risk of coronary heart disease (see supplementary table C). Nor did we find a significant association between gluten intake and coronary heart disease when separately considering the time strata 1986-97 and 1998-2012 or when updating dietary responses regardless of the development of the comorbid conditions of cardiovascular disease, diabetes, or cancer (see supplementary tables D and E).

Discussion

In two prospective cohorts with updated dietary information over 20 years of follow-up, we found no significant association between estimated gluten intake and the risk of subsequent overall coronary heart disease, non-fatal myocardial infarction, and fatal myocardial infarction. The lack of association was consistent in both men and women, as well among other subgroups defined by cardiovascular risk factors.

Comparison with other studies

Dietary gluten has been the subject of increased attention and concern in recent years. Much of the data on gluten and coronary heart disease are limited to people with celiac disease, in whom gluten elicits an inflammatory response characterized by small intestinal villous atrophy and the development of antibodies to tissue transglutaminase, a ubiquitous enzyme that is present on vascular endothelial cells.34 35 Patients with celiac disease may have an increased risk of myocardial
Table 5 | Hazard ratios for coronary heart disease events (fatal and non-fatal myocardial infarctions) by fifths of energy adjusted gluten intake, stratified by age, body mass index, physical activity, and smoking

| Fifth of energy adjusted gluten intake | Nurses' Health Study | Health Professionals Follow-up Study | P for trend | P for interaction |
|---------------------------------------|----------------------|--------------------------------------|------------|------------------|
| Mean; median (range) gluten intake, g/d |                      |                                      |            |                  |
| 1 (lowest)                            | 2.6; 2.8 (0-3.4)     | 3.3; 3.5 (0-4.3)                     |            |                  |
| 2                                    | 3.8; 3.8 (3.4-4.3)   | 4.9; (4.9 3-5.5)                     |            |                  |
| 3                                    | 4.7; 4.7 (4.3-5.1)   | 6.0; 6.0 (5.5-6.6)                   |            |                  |
| 4                                    | 5.6; 5.6 (5.1-6.2)   | 7.3; 7.3 (6.6-8.1)                   |            |                  |
| 5 (highest)                           | 7.5; 7.5 (6.2-26.7)  | 10.0; 9.4 (8.1-38.4)                 |            |                  |

| Age                                    |                      |                                      |            |                  |
| <65 years                              |                      |                                      |            |                  |
| No of events                           | 469; 423             | 231 320                              |            |                  |
| Person years                           | 269 283              | 282 702                              |            |                  |
| Incidence per 100 000 person years     | 460; 404             | 288 484                              |            |                  |
| Multivariable adjusted HR (95% CI)*    | 1.0 (reference)      | 0.85 (0.75 to 0.97)                  | 0.11       |                  |
| ≥65 years                              |                      |                                      |            |                  |
| No of events                           | 953; 815             | 173 129                              |            |                  |
| Person years                           | 883; 821             | 187 115                              |            |                  |
| Incidence per 100 000 person years     | 140; 155             | 188 976                              |            |                  |
| Multivariable adjusted HR (95% CI)*    | 1.0 (reference)      | 0.90 (0.82 to 0.99)                  | 0.16       |                  |

| Body mass index                        |                      |                                      |            |                  |
| <25                                    |                      |                                      |            |                  |
| No of events                           | 516; 463             | 188 630                              |            |                  |
| Person years                           | 540; 521             | 225 115                              |            |                  |
| Incidence per 100 000 person years     | 521; 570             | 241 864                              |            |                  |
| Multivariable adjusted HR (95% CI)*    | 1.0 (reference)      | 0.90 (0.79 to 1.02)                  | 0.36       |                  |
| ≥25                                    |                      |                                      |            |                  |
| No of events                           | 899; 768             | 214 254                              |            |                  |
| Person years                           | 798; 702             | 243 559                              |            |                  |
| Incidence per 100 000 person years     | 702; 727             | 274 408                              |            |                  |
| Multivariable adjusted HR (95% CI)*    | 1.0 (reference)      | 0.94 (0.85 to 1.03)                  | 0.77       |                  |

| Physical activity                      |                      |                                      |            |                  |
| <18 METS/week                          |                      |                                      |            |                  |
| No of events                           | 858; 743             | 235 824                              |            |                  |
| Person years                           | 730; 724             | 268 065                              |            |                  |
| Incidence per 100 000 person years     | 724; 739             | 272 513                              |            |                  |
| Multivariable adjusted HR (95% CI)*    | 1.0 (reference)      | 0.91 (0.82 to 1.00)                  | 0.77       |                  |
| ≥18 METS/week                          |                      |                                      |            |                  |
| No of events                           | 563; 493             | 167 954                              |            |                  |
| Person years                           | 610; 503             | 201 348                              |            |                  |
| Incidence per 100 000 person years     | 503; 558             | 204 611                              |            |                  |
| Multivariable adjusted HR (95% CI)*    | 1.0 (reference)      | 0.94 (0.85 to 0.96)                  | 0.07       |                  |

| Smoking history                        |                      |                                      |            |                  |
| Never smokers                          |                      |                                      |            |                  |
| No of events                           | 385; 392             | 150 734                              |            |                  |
| Person years                           | 413; 411             | 202 494                              |            |                  |
| Incidence per 100 000 person years     | 411; 407             | 213 124                              |            |                  |
| Multivariable adjusted HR (95% CI)*    | 1.0 (reference)      | 0.92 (0.80 to 1.07)                  | <0.001     |                  |
| Past smokers                           |                      |                                      |            |                  |
| No of events                           | 662; 561             | 175 686                              |            |                  |
| Person years                           | 635; 554             | 201 222                              |            |                  |
| Incidence per 100 000 person years     | 554; 594             | 202 857                              |            |                  |
| Multivariable adjusted HR (95% CI)*    | 1.0 (reference)      | 0.92 (0.80 to 1.06)                  |            |                  |
| Current smokers                        |                      |                                      |            |                  |
| No of events                           | 208; 176             | 53 776                               |            |                  |
| Person years                           | 179; 140             | 44 216                               |            |                  |
| Incidence per 100 000 person years     | 140; 166             | 39 800                               |            |                  |
| Multivariable adjusted HR (95% CI)*    | 1.0 (reference)      | 0.94 (0.78 to 1.08)                  |            |                  |

HR = hazard ratio.

*Adjusted for age, race, body mass index, height, history of diabetes, regular non-steroidal anti-inflammatory drug use, current use of multivitamin, alcohol intake (g/d), smoking (pack years), aspirin use, statin use, parental history of myocardial infarction, history of hypertension, history of hypercholesterolemia, physical activity (MET, h/wk), menopausal status and menopausal hormone use (Nurses' Health Study only), trans fat, red meat, processed meat, polyunsaturated fats, fruits, and vegetables.
infarction and death from cardiovascular disease that is reduced after diagnosis of celiac disease, possibly owing to the beneficial effect of a gluten-free diet, although this association is controversial. Patients with celiac disease who develop a myocardial infarction are less likely to have classic cardiac risk factors, such as smoking and dyslipidemia, leading to the hypothesis that the pro-inflammatory effect of gluten exerts an independent cardiac risk.

The popularity of a low gluten or gluten-free diet in the general population has markedly increased in recent years. Despite the limited evidence that gluten plays a role in cardiovascular health, this increasing adoption of a gluten-free diet by people without celiac disease has occurred in conjunction with speculation that gluten may have a deleterious role in health outcomes even in the absence of gluten sensitivity. The rationale for this concern includes the observation that foods containing gluten often have a high glycemic index, which has been linked to cardiovascular risk. Studies in mice have shown pro-inflammatory effects of gluten administration and a protective association of gluten restriction with the development of diabetes.

In one cross-sectional study in young adults, gluten intake was correlated with higher plasma concentrations of α2-macroglobulin, an acute phase reactant that is associated with inflammation. Gluten has been found to cause gastrointestinal symptoms in patients without celiac disease, although the mechanism for this remains uncertain.

We found that estimated gluten intake correlated moderately with whole grain and refined grain intake, as expected given the prominence of wheat among dietary grains; gluten also correlated with glycemic index. We noted a significant inverse relation between estimated gluten intake and coronary heart disease when we adjusted for refined grain intake. Although the absolute risk difference was modest (75 coronary heart disease events per 100,000 person years when we compared the highest fifth of gluten intake with the lowest fifth in the pooled analysis), this lower risk likely reflects the fact that adjustment for refined grains leaves the remainder of the variance of gluten intake correlated with whole grain intake. Whole grain intake has been found to be inversely associated with coronary heart disease risk and cardiovascular mortality.

These findings underscore the potential that people who severely restrict gluten intake may also significantly limit their intake of whole grains, which may actually be associated with adverse cardiovascular outcomes.

Strengths and limitations of study

Strengths of our study include its large sample size, long term follow-up, prospective and repeated assessments of diet with validated questionnaires, and validated outcome measurement. Our study also has several limitations. Unmeasured or residual negative confounding is a possibility, although our main model included multiple dietary and non-dietary covariates. We did not specifically ask about the intake of gluten-free substitute foods, and participants were not asked about whether they specifically adhered to a gluten-free diet. Although we excluded participants who reported a diagnosis of celiac disease, we could not identify which people without celiac disease nonetheless maintained a very low gluten or gluten-free diet. Nevertheless, the observation period (1986-2012) largely preceded the widespread interest in gluten as a health concern that has arisen more recently in the US. We likewise were unable to determine whether gluten was present in trace amounts in certain foods, such as soy sauce or oats that were not harvested on separate fields; therefore, potential exists for misclassification at the low end of gluten intake. Although trace amounts of gluten (such as 50 mg daily) can induce symptoms and inflammation in patients with celiac disease, measurement of such gluten exposure would have a small effect on gluten quantity even in the lowest fifth of baseline daily gluten intake in our cohorts (2.6 g daily in women and 3.3 g in men). Therefore, although we were unable to determine the association of a strict gluten-free diet with coronary heart disease, we did not observe any association of very low estimated gluten intake with coronary heart disease, as might be realistically expected among people who maintain a gluten-free diet in usual practice.

Our measurement of gluten intake was based on the assumption that gluten comprised 75% of the protein content of wheat, rye, and barley, following the convention of a single conversion factor for all three grains. Although this may overestimate the amount of gluten intake for rye and barley, it is unlikely to bias our results given the overall low intake of rye and barley in these cohorts. Although gluten has not been specifically quantified in validation studies of food frequency questionnaires, this instrument has shown good validity with regard to reasonable correlation with seven day dietary recall of foods containing gluten (supplementary table A) and intake of vegetable protein, to which gluten is a significant contributor. In addition, participants with undiagnosed celiac disease were not uniformly identified in these cohorts. However, according to population based estimates, such people would account for less than 1% of the cohort. Moreover, inclusion of these participants would be expected to bias the results toward an association of gluten with coronary heart disease, which was not observed.

In this analysis, we did not examine change in body mass index in relation to gluten ingestion. However, body mass index is unlikely to mediate an association between gluten and coronary heart disease, as the risk estimate did not change from positive toward a null association when we added body mass index to the model. Finally, in secondary subgroup analyses, we observed a higher risk of coronary heart disease among participants in the highest fifth of gluten intake among current smokers. However, these associations were no longer significant once we adjusted the models for refined grain consumption. Given the relatively small number of cases of coronary heart disease among current smokers in the highest fifth of gluten intake and the
lack of a clear mechanistic basis for this heterogeneity, these results should be viewed in the context of multiple testing. When we applied the Bonferroni correction to smoking categories (which contained three strata), our finding regarding gluten intake and coronary heart disease among current smokers was no longer statistically significant.

Conclusion and public health implications

In these two large, prospective cohorts, the consumption of foods containing gluten was not significantly associated with risk of coronary heart disease. Although people with and without celiac disease may avoid gluten owing to a symptomatic response to this dietary protein, these findings do not support the promotion of a gluten restricted diet with a goal of reducing coronary heart disease risk. In addition, the avoidance of dietary gluten may result in a low intake of whole grains, which are associated with cardiovascular benefits. The promotion of gluten-free diets for the purpose of coronary heart disease prevention among asymptomatic people without celiac disease should not be recommended.

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Ethical approval: The Institutional Review Boards of Brigham and Women’s Hospital and the Harvard T. H. Chan School of Public Health approved this study. Return of the mailed questionnaire was considered to imply informed consent. Protocol number: 1999-P-011114/754.

Transparency declaration: The lead author (the manuscript’s guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Data sharing: Data, the statistical code, questionnaires, and technical processes are available from the corresponding author at achan@mgh.harvard.edu.

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Supplementary tables