Published in final edited form as:

Am J Gastroenterol. 2019 January ; 114(1): 127–134. doi:10.1038/s41395-018-0267-5.

Dietary Gluten Intake and Risk of Microscopic Colitis Among US Women without Celiac Disease: A Prospective Cohort Study

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Critical revision of the manuscript for important intellectual content: all coauthors

Obtained funding: A.T.C., H.K.

Administrative, technical, or material support: A.T.C., H.K.

Study supervision: A.T.C., H.K.

Competing interests:

Hamed Khalili receives consulting fees from Abbvie, Takeda, and Samsung Bioepis. Hamed Khalili also receives funding from Takeda. Benjamin Lebwohl receives consulting fees from Takeda. Ashwin N. Ananthakrishnan serves on the scientific advisory board of Abbvie, Takeda, and Merck. Andrew T. Chan receives consulting fees from Pfizer Inc., and Bayer A.G. The remaining authors have no conflicts to disclose.

IRB Protocol Title:

Environmental Factors and Inflammatory Bowel Disease

IRB Protocol Number:

2001-P- 001128/25

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Abstract

Objective: Microscopic colitis is a common cause of chronic watery diarrhea among the elderly. Although the prevalence of celiac disease appears to be higher in patients with microscopic colitis, the relationship between dietary gluten intake and risk of microscopic colitis among individuals without celiac disease has not been explored.

Methods: We conducted a prospective study of 160,744 US women without celiac disease enrolled in the Nurses' Health Study (NHS) and the NHSII. Dietary gluten intake was estimated using validated food frequency questionnaires every 4 years. Microscopic colitis was confirmed through medical records review. We used Cox proportional hazard modeling to estimate the multivariable-adjusted hazard ratio (HR) and 95% confidence interval (CI).

Results: We documented 224 incident cases of microscopic colitis over more than 20 years of follow-up encompassing 3,715,945 person-years (crude incidence rate: 5.9/100,000 person-years) in NHS and NHSII. Dietary gluten intake was not associated with risk of microscopic colitis ($P_{\text{trend}} = 0.69$). Compared to individuals in the lowest quintile of energy-adjusted gluten intake, the adjusted HR of microscopic colitis was 1.17 (95% CI: 0.78–1.76) for the middle quintile and 1.08 (95% CI: 0.71–1.64) for the highest quintile. Additional adjustment for primary dietary sources of gluten including refined and whole grains did not materially alter the effect estimates (All $P_{\text{trend}} = 0.64$). The null association did not differ according to lymphocytic or collagenous subtypes ($P_{\text{heterogeneity}} = 0.60$) and was not modified by age, smoking status, or body mass index (All $P_{\text{interaction}} = 0.25$).

Conclusion: Dietary gluten intake during adulthood was not associated with risk of microscopic colitis among women without celiac disease.

INTRODUCTION

Microscopic colitis is a common cause of chronic watery diarrhea characterized by lymphocytic infiltration of colonic mucosa with or without expansion of collagen fiber in the lamina propria (1). The cause of microscopic colitis is largely unknown, but may be due to an inappropriate immune response to the gut luminal microenvironment in genetically-susceptible individuals (2). The disease has emerged as a common cause of non-bloody diarrhea in the past decade with recent data suggesting that up to 10% to 20% of patients with chronic diarrhea are diagnosed with microscopic colitis (3). Microscopic colitis and celiac disease share many clinical manifestations with a number of studies demonstrating a

higher prevalence (4–15%) of celiac disease among patients with lymphocytic subtype of microscopic colitis (4–6). Previous reports have suggested that some patients with concomitant celiac disease may have improvements in disease activity following a gluten-free diet, minimizing the need for medical therapy (6). Nevertheless, the role of gluten, the dietary antigen in celiac disease, in the pathogenesis of microscopic colitis remains largely unknown.

Recently, gluten has been implicated in the etiology of a wide range of conditions, encompassing metabolic syndrome, neurologic disorders, and cardiovascular diseases (7). The increasing concern about dietary gluten intake both in the medical community and lay public has resulted in the reduction or avoidance of gluten intake by segments of the general population without celiac disease, particularly among individuals with chronic gastrointestinal disorders (8). In microscopic colitis, where the best-established treatment is corticosteroids, patients may opt to adhere to a gluten-free diet for its perceived safety and benefits in relieving intestinal symptoms. On the other hand, gluten avoidance may, at least in part, be associated with less healthy eating pattern through reduction in consumption of whole grains. As such, systematic evaluation of the role of gluten in chronic gastrointestinal disorders may have a significant role in informing public health recommendations regarding intake of gluten (8). We therefore sought to examine the association between dietary gluten intake and risk of incident microscopic colitis in two large prospective cohorts of US women, the Nurses' Health Study (NHS) and NHSII. With over 20 years of validated and updated data on lifestyle and dietary information, these cohorts offer a unique opportunity to examine the relationship between dietary gluten intake and risk of microscopic colitis.

METHODS

Study Population

The prospective NHS began in 1976 when 121,530 female nurses, aged 30 to 55 years, residing in eleven US states provided detailed information on their lifestyle and medical history. Similarly, NHSII was established in 1989 when 116,533 female nurses, aged 25 to 42 years, provided information on lifestyle, reproductive factors, and medical diagnoses. In both cohorts, participants have been followed biennially by self-administered questionnaires on lifestyle factors, anthropometric measurements, medications, and medical conditions until 2014 in NHS and 2015 in NHSII. The overall follow-up rate is above 90% for NHS and above 85% for NHSII (9). The study was approved by the institutional review boards of both the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health.

We excluded participants who reported a diagnosis of celiac disease at any time during the study period. Among eligible participants who completed a semi-quantitative food frequency questionnaire (SFFQ) at baseline (1986 in NHS and 1991 in NHSII, $n = 167,026$), we additionally excluded participants with pre-existing inflammatory bowel disease, microscopic colitis, cancer, or missing body weight (Figure). As shown previously, the baseline characteristics of participants who completed SFFQ were similar to those of the entire cohort (10).

Ascertainment of Microscopic Colitis

In both cohorts, a diagnosis of microscopic colitis was self-reported initially and was subsequently confirmed by two gastroenterologists (K.E.B. and H.K.) (11). Since 1976 in NHS and 1989 in NHSII, participants reported diagnoses of inflammatory bowel diseases, including microscopic colitis, ulcerative colitis, and Crohn's disease during biennial questionnaires. When a diagnosis of microscopic colitis was reported, participants were sent supplementary questionnaires and requests to obtain relevant medical records. We collected data on diagnostic tests, endoscopy results, histopathology, and laboratory findings. Confirmation of cases was based on histopathology records review by two independent gastroenterologists blinded to exposure information. When possible, we also obtained information on microscopic colitis subtypes (lymphocytic colitis and collagenous colitis). We excluded participants who subsequently denied microscopic colitis on the supplementary questionnaires, participants who did not give permission to review their medical records, and participants whose diagnoses of microscopic colitis were not confirmed after review of medical records.

Assessment of Gluten Intake

In NHS, dietary intake was assessed by validated SFFQ every four years since 1986 (12). In NHSII, a similar SFFQ was administered every four years since 1991. The SFFQ evaluated the habitual frequency of consumption of various food items in standard portion sizes during the previous year. Available responses ranged from "never or less than once per month" to "6 or more times per day." We derived individual dietary components based on the Harvard Food Composition Database (13).

Measurements of dietary gluten intake had been reported in detail previously (8). Briefly, the quantity of dietary gluten intake was calculated based on the protein content of wheat, rye, and barley within each food item. Consistent with previous studies, we used the more conservative estimate of conversion factor of 75% when calculating the proportion of protein content that comprised gluten to avoid overestimating the intakes (14). We did not include trace sources of gluten (for example, gluten from oats and condiments) as the quantity is likely to have been trivial compared to that from grains and cereals (15). The SFFQ method of measuring plant-derived protein (mainly gluten) has previously been demonstrated to correlate reasonably with measurements by seven-day dietary records (Spearman correlation coefficient 0.66) (16). Additionally, in two recent validation studies nested in NHSII ($n = 732$) and the parallel Health Professionals Follow-Up Study ($n = 650$), the energy-adjusted Spearman rank correlation coefficient (95% CI) comparing gluten intake from the second SFFQ with average of both 7-day dietary records were 0.55 (0.48–0.61) and 0.58 (0.52 – 0.64), respectively, confirming the validity of SFFQ in measuring dietary gluten intake.

We obtained energy-adjusted gluten intake using the residual method, which allowed us to focus on the nutrient component independent of total energy intake and reduce effects of over and under reporting by participants (17). To reduce measurement errors and to represent long-term dietary patterns, we used the cumulative average of energy-adjusted gluten intake as our main exposure (18). As an example, we calculated the cumulative average gluten intake in 1999 as the average of daily gluten consumption reported in 1991,

1995, and 1999. We also performed a sensitivity analysis using simple-updated energy-adjusted gluten intake (using the most recent SFFQ) to focus on the influence of more recent, short term gluten intake on disease risk. For example, estimated gluten intake from the 1999 SFFQ was used for follow-up cycle from 1999–2001. Finally, to minimize the possibility that subclinical or undiagnosed microscopic colitis may influence intake of gluten, we also introduced a 4-year lag in our sensitivity analyses where energy-adjusted gluten intake was derived from a SFFQ at least 4 years prior to each follow up cycle.

Assessment of Covariates

In both cohorts, height was recorded at baseline when cohorts were initiated (1976 in NHS and 1989 in NHSII). Body weight was updated every 2 years via questionnaires. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Assessment of smoking status has been reported in detail previously (19, 20). Participants provided their past smoking habits, including the age at which they started or stopped smoking, during the baseline questionnaire. Current smoking status and the number of cigarettes smoked daily were updated in the biennial questionnaires. We categorized smoking status as never, past, and current. Participants who smoked less than 20 cigarettes in their lifetime were categorized as never-smokers.

Physical activity was self-reported using a validated questionnaire on average every four years (21). Participants were asked to provide the average time per week spent in recreational activities (for example, walking, jogging, or bicycling) during the past year. A metabolic equivalent of tasks (MET) score was assigned to each physical activity based on energy expenditure (22). We calculated the amount of physical activity by multiplying the average time spent in each activity by the MET score. We also assessed diet quality using the Alternative Healthy Eating Index (AHEI) score, which was constructed based on dietary factors that have been consistently associated with a lower risk of chronic diseases (23). Menopausal status and the use of menopausal hormones were also evaluated using biennial questionnaires. In a subset of NHS participants, the reproducibility of self-reported menopausal status was > 98% between questionnaire cycles (24). Starting from 1989 in NHSII and 1990 in NHS, we asked information on current regular use (at least twice weekly) of non-steroid anti-inflammatory drugs (NSAIDs). Finally, we also inquired regular use of proton pump inhibitors (PPIs) since 2000 in NHS and 2001 in NHSII. Regular use of anti-depressants (e.g. selective serotonin reuptake inhibitors, etc.) was assessed in the biennial questionnaires since 1996 in NHS and 1993 in NHSII. As these medications have previously been strongly associated with risk of microscopic colitis,^{29, 30} we performed secondary analysis limiting the study period to 2000–2014 in NHS and 2001–2015 in NHSII to account for the use of these medications.

Statistical Analysis

Person time was calculated from the date of return of baseline questionnaire (1986 in NHS and 1991 in NHSII) to the date of diagnosis of microscopic colitis, death from any cause, last returned questionnaire, or end of follow up (2014 in NHS and 2015 in NHSII), whichever came first. We modeled gluten intake as quintiles of cumulative average intake updated every 4 years. We estimated age-adjusted and multivariable-adjusted hazard ratios

(HR) and 95% confidence intervals (CI) using Cox proportional hazard modeling stratified by age (in months) and study period (2-year intervals). Models were adjusted for the following variables, chosen *a priori*: age, smoking status, menopausal hormone therapy, AHEI score (in quintiles), physical activities (quintiles), current regular use of NSAIDs (yes/no), and BMI (<25 kg/m², 25–29.9 kg/m² and ≥ 30 kg/m²). All covariates, including dietary gluten intake, were assessed as time-varying exposures. Test of linear trend was performed by analyzing dietary gluten intake as a continuous variable. We examined whether the association between gluten intake and risk of microscopic colitis differed according to disease subtype using the log likelihood ratio test to compare model fit between models assuming a common effect and models allowing separate associations across collagenous and lymphocytic colitis subtypes (25).

In exploratory analyses, we evaluated the relationship between dietary gluten intake and risk of microscopic colitis according to strata defined by age (< 60 vs. ≥ 60 years), BMI (< 25 vs. ≥ 25 kg/m²), and smoking (never vs. ever). We tested for effect modification by comparing models with cross-classified categories of gluten and each stratum with those with main effects only using log likelihood ratio tests. All statistical analyses were conducted with SAS version 9.4 (SAS Institute, Cary, NC). Significance was set as a *p* value < 0.05 in a two-tailed test.

RESULTS

After exclusion, a total of 160,743 participants were eligible for our study, which included 67,835 women in NHS and 92,908 women in NHSII. Table 1 demonstrates the baseline characteristics of participants according to quintiles of gluten intake. On average, participants in the highest quintile of gluten intake consumed more whole grains and refined grains and were more likely to be never-smokers. In contrast, there did not appear to be any differences in age, BMI, physical activity, and menopausal status according to quintiles of gluten intake.

Through 2014 in NHS and 2015 in NHSII, we documented 224 incident cases of microscopic colitis over 3,715,945 person-years of follow up. Of the 224 patients with microscopic colitis, we were able to confirm 107 cases of collagenous and 105 cases of lymphocytic colitis. The remaining 12 cases had histopathology reports of either a diagnosis of “microscopic colitis” only or did not provide sufficient information about collagen band thickness.

Dietary gluten intake was not associated with risk of microscopic colitis ($P_{\text{trend}} = 0.69$, Table 2). Compared to women in the lowest quintile of energy-adjusted gluten intake, the multivariable-adjusted HRs for microscopic colitis were 1.17 (95% CI: 0.78–1.76) for women in the middle quintile and 1.08 (95% CI: 0.71–1.64) for women in the highest quintile of gluten intake. The associations remained non-significant when we additionally adjusted for the primary sources of gluten. When additionally adjusted for refined grains, the multivariable-adjusted HRs were 1.21 (95% CI: 0.78–1.87) for the middle quintile and 1.16 (95% CI: 0.67–1.98) for the highest quintile of gluten intake ($P_{\text{trend}} = 0.64$). When additionally adjusted for whole grains intake, the multivariable-adjusted HRs were 1.15

(95% CI: 0.76–1.74 for women in the middle quintile and 1.03 (95% CI: 0.66–1.61) for women in the highest quintile of dietary gluten intake ($P_{\text{trend}} = 0.92$). We also repeated these analyses using deciles of cumulative intake of gluten and observed similar results. Compared to individuals in the lowest decile of cumulative-average intake of gluten (average gluten intake = 3.1 ± 0.7 g), the multivariable-adjusted HR for the highest decile (average gluten intake = 9.1 ± 1.5 g) was 1.05, 95% CI: 0.56–1.96. In our secondary analysis restricting our follow-up period to after 2000 in NHS and 2001 in NHSII to account for the use of SSRIs and PPIs, we found no association between dietary gluten intake and risk of microscopic colitis ($P_{\text{trend}} = 0.67$).

We also examined the influence of more recent short-term intake of gluten on risk of microscopic colitis and observed similar results ($P_{\text{trend}} = 0.43$). Compared to participants in the lowest quintile of simple-updated dietary gluten intake, the multivariable-adjusted HR of microscopic among those in the highest quintile was 0.92, 95% CI: 0.59–1.45. Finally, we considered the possibility that undiagnosed or subclinical symptoms of microscopic colitis may influence intake of gluten intake and therefore performed a 4-year lagged analysis to account for the possibility of reverse causation. Similar to our primary analyses, we did not observe an association between dietary gluten intake and risk of microscopic colitis ($P_{\text{trend}} = 0.59$). Compared to the participants in the lowest quintile of dietary gluten intake, the multivariable-adjusted HR of microscopic among those in the highest quintile was 1.28, 95% CI: 0.83–1.98.

We explored the relationship between dietary gluten intake and risk of microscopic colitis according to disease subtypes (Table 3). The association between gluten intake and risk of microscopic colitis did not differ according to disease subtypes ($P_{\text{heterogeneity}} = 0.60$). For women in the highest quintile of gluten intake, the multivariable-adjusted HR for collagenous colitis was 1.05 (95% CI: 0.55–2.00) compared to women in the lowest quintile of gluten intake. Similarly, for women in the highest quintile of gluten intake, the multivariable-adjusted HR for lymphocytic colitis was 1.14 (95% CI: 0.64–2.01) compared to women in the lowest quintile of gluten intake.

Finally, we examined the association between gluten intake and risk of microscopic colitis according to strata defined by age, BMI, and smoking (Table 4) and did not observe any evidence for effect modification (all $P_{\text{interaction}} = 0.25$).

DISCUSSION

In two large prospective cohort studies of US women, we found no association between dietary gluten intake and risk of microscopic colitis among patients without celiac disease. The results were consistent in multiple sensitivity analyses, across different subtypes of microscopic colitis and subgroups defined by age, BMI, and smoking status.

The exact mechanism by which microscopic colitis develops remains largely uncharacterized (1, 2). Nevertheless, the presence of marked female preponderance and findings from a recent genome wide association study demonstrating variants within *HLA* gene to be associated with risk of collagenous colitis, indicate an immune-mediated etiology

(26, 27). Studies of environmental risk factors for microscopic colitis have largely focused on medications, with NSAIDs and proton pump inhibitors previously been associated with increased risk (28, 29). Celiac disease, in addition to being more prevalent among patients with microscopic colitis, also has histologic characteristics that are remarkably similar to that of microscopic colitis, particularly the lymphocytic colitis subtype (5). Therefore, it is possible that gluten may represent a common luminal antigen for both disorders. This hypothesis is further supported by the previous observation that a gluten enema can induce lymphocytic colitis-like changes in patients with celiac disease (30). A number of case studies have shown that among patients with co-existing microscopic colitis and celiac disease, both diseases may respond to a gluten-free diet (1, 6, 31). Despite these lines of evidence, we did not observe an association between gluten intake and risk of microscopic colitis among individuals without celiac disease. Additionally, we evaluated this association in the context of the primary sources of gluten intake namely refined and whole grains and observed no significant changes in our effect estimates, suggesting that regardless of the source, gluten is not associated with risk of microscopic colitis. These results are particularly relevant at a time when a low gluten diet or gluten-free diet are gaining popularity in the general population especially in patients with chronic intestinal symptoms (32, 33).

To the best of our knowledge, no prior study has examined the role of gluten-free diet in treatment of microscopic colitis. Nevertheless, our data suggest that the pathogenesis of microscopic colitis, at least in patients without celiac disease, is likely not related to gluten consumption and therefore in contrast to celiac disease, it is unlikely that gluten withdrawal would lead to improvement in histologic inflammation or clinical symptoms. This hypothesis is supported by earlier work from Freeman who demonstrated that large consumption of dietary gluten for over one month in two patients with established lymphocytic colitis was not associated with any histologic changes in the proximal small intestine or changes in severity of inflammation in the colon (34). Interestingly, even in a large series of patients with established celiac disease ($n = 1,009$), the majority of cases of microscopic colitis occurred after the diagnosis of celiac disease while patients were already on gluten-free diet with no clear correlations in changes in duodenal and colonic biopsies following dietary changes, indicating that even in patients with celiac disease, adherence to a gluten-free diet may not alter the development or progression of microscopic colitis (6). Nevertheless, we acknowledge that similar to inflammatory bowel disease, microscopic colitis may also represent a heterogeneous group of disorders and therefore whether gluten intake can play a role in a subgroup of cases with specific genetic susceptibility cannot be entirely ruled out.

The strengths of our study include large, well-established cohorts with high response rates and over 20 years of follow up. The repeated assessments of lifestyle, diet, medications, and outcome data over time allowed us to account for changes in the distribution of confounders during the study period and reduced the possibility of recall bias. The detailed and validated information on diet also minimized measurement errors. Finally, with diagnosis confirmation by medical records review, we were able to distinguish whether the associations varied according to disease subtype.

Our study has several limitations that are worth noting. First, our study comprised mainly Caucasian women. While this may influence the generalizability of our findings, previous studies suggested that the risk of microscopic colitis is much higher among women with a female-to-male gender ratio up to 9:1 (35, 36). The all-female composition makes our cohorts particularly suitable to study risk factors for the disease. Second, our number of cases of microscopic colitis may have limited our power to detect more modest associations. We estimated that we would have an 80% power to detect a HR of at least 1.81 between the highest and lowest quintiles in a 2-tailed test. However, as previously reported in our cohort and consistent with prior studies (11, 28, 29), use of NSAIDs, PPIs, SSRIs, and smoking are strongly associated with risk of microscopic colitis, indicating that the contribution of gluten to the risk of microscopic colitis, if any, is minimal. Third, this study relied on self-reported questionnaires for assessing dietary gluten intake which may be subject to measurement errors. However, our validation studies have demonstrated strong correlation between gluten intake derived from SFFQ to that of diet records. Nevertheless, we were unable to directly identify participants who were on a gluten-free diet and cannot examine whether such diet is protective against the development of microscopic colitis. Fourth, data on diagnosis of microscopic colitis was initially reported by the participants which may have led to under-ascertainment of the outcome. However, as previously demonstrated the incidence rates of microscopic colitis in our cohorts are similar to those of other population-based cohorts and our participants are nurses with high health literacy increasing the likelihood of reporting the diagnosis (11). The slightly lower overall incidence rate compared to some of the prior studies is largely due to the younger age of our participants relative to the peak age of diagnosis. Finally, the observational nature of our cohorts prevents us from excluding residual confounding. However, we were able to adjust for a variety of established risk factors for risk of microscopic colitis, including smoking and NSAIDs use using prospectively collected data and adjustment for these factors did not materially alter our effect estimates (11, 36).

CONCLUSION

In summary, we found no association between dietary gluten intake during adulthood and risk of microscopic colitis in two large, prospective cohorts of US women without celiac disease. Together with prior studies, these data provide compelling evidence that microscopic colitis is largely not a gluten-induced disorder.

Acknowledgement:

We would like to thank the participants and staff of the NHS and NHS2 for their valuable contributions.

Funding:

K. Burke is supported by F32 DK115134. H. Khalili is supported by K23 DK099681. P. Lochhead is supported by a career development award from the Crohn's and Colitis Foundation. A. Chan is supported by K24 DK098311, a Crohn's and Colitis Foundation Senior Investigator Award, and a Stuart and Suzanne Steele MGH Research Scholars Award. The Nurses' Health Study and Nurses' Health Study II are supported by UM1 CA186107 and UM1 CA176726, respectively. The salary of K. Ivey is supported by a National Health and Medical Research Council early career fellowship.

REFERENCES

1. Pardi DS, Kelly CP. Microscopic colitis. *Gastroenterology* 2011;140:1155–65. [PubMed: 21303675]
2. Munch A, Langner C. Microscopic colitis: clinical and pathologic perspectives. *Clin Gastroenterol Hepatol* 2015;13:228–36. [PubMed: 24407107]
3. Munch A, Aust D, Bohr J, et al. Microscopic colitis: Current status, present and future challenges: statements of the European Microscopic Colitis Group. *J Crohns Colitis* 2012;6:932–45. [PubMed: 22704658]
4. Matteoni CA, Goldblum JR, Wang N, et al. Celiac disease is highly prevalent in lymphocytic colitis. *J Clin Gastroenterol* 2001;32:225–7. [PubMed: 11246349]
5. Wolber R, Owen D, Freeman H. Colonic lymphocytosis in patients with celiac sprue. *Hum Pathol* 1990;21:1092–6. [PubMed: 2227917]
6. Green PH, Yang J, Cheng J, et al. An association between microscopic colitis and celiac disease. *Clin Gastroenterol Hepatol* 2009;7:1210–6. [PubMed: 19631283]
7. Lebowitz B, Ludvigsson JF, Green PH. Celiac disease and non-celiac gluten sensitivity. *BMJ* 2015;351:h4347. [PubMed: 26438584]
8. Lebowitz B, Cao Y, Zong G, et al. Long term gluten consumption in adults without celiac disease and risk of coronary heart disease: prospective cohort study. *BMJ* 2017;357:j1892. [PubMed: 28465308]
9. History of The Nurses' Health Study (NHS). <http://www.nurseshealthstudy.org/about-nhs/history>. Accessed 12/05/2017.
10. Nimptsch K, Bernstein AM, Giovannucci E, et al. Dietary intakes of red meat, poultry, and fish during high school and risk of colorectal adenomas in women. *Am J Epidemiol* 2013;178:172–83. [PubMed: 23785116]
11. Burke KE, Ananthakrishnan AN, Lochhead P, et al. Smoking is Associated with an Increased Risk of Microscopic Colitis: Results From Two Large Prospective Cohort Studies of US Women. *J Crohns Colitis* 2018.
12. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985;122:51–65. [PubMed: 4014201]
13. Harvard TH Chan School of Public Health Nutrition Department. <https://regepi.bwh.harvard.edu/health/>. Accessed 12/05/2017. [cited; Available from:]
14. Andren Aronsson C, Lee HS, Koletzko S, et al. Effects of Gluten Intake on Risk of Celiac Disease: A Case-Control Study on a Swedish Birth Cohort. *Clin Gastroenterol Hepatol* 2016;14:403–409 e3. [PubMed: 26453955]
15. See JA, Kaukinen K, Makharia GK, et al. Practical insights into gluten-free diets. *Nat Rev Gastroenterol Hepatol* 2015;12:580–91. [PubMed: 26392070]
16. Yuan C, Spiegelman D, Rimm EB, et al. Validity of a Dietary Questionnaire Assessed by Comparison With Multiple Weighed Dietary Records or 24-Hour Recalls. *Am J Epidemiol* 2017;185:570–584. [PubMed: 28338828]
17. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol* 1986;124:17–27. [PubMed: 3521261]
18. Willett W *Nutritional Epidemiology*: Oxford University Press; 2012.
19. Nishihara R, Morikawa T, Kuchiba A, et al. A prospective study of duration of smoking cessation and colorectal cancer risk by epigenetics-related tumor classification. *Am J Epidemiol* 2013;178:84–100. [PubMed: 23788674]
20. Higuchi LM, Khalili H, Chan AT, et al. A prospective study of cigarette smoking and the risk of inflammatory bowel disease in women. *Am J Gastroenterol* 2012;107:1399–406. [PubMed: 22777340]
21. Wolf AM, Hunter DJ, Colditz GA, et al. Reproducibility and validity of a self-administered physical activity questionnaire. *Int J Epidemiol* 1994;23:991–9. [PubMed: 7860180]
22. Ainsworth BE, Haskell WL, Leon AS, et al. Compendium of physical activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc* 1993;25:71–80. [PubMed: 8292105]

23. Chiuvè SE, Fung TT, Rimm EB, et al. Alternative dietary indices both strongly predict risk of chronic disease. *J Nutr* 2012;142:1009–18. [PubMed: 22513989]
24. Colditz GA, Stampfer MJ, Willett WC, et al. Reproducibility and validity of self-reported menopausal status in a prospective cohort study. *Am J Epidemiol* 1987;126:319–25. [PubMed: 3605058]
25. Wang M, Spiegelman D, Kuchiba A, et al. Statistical methods for studying disease subtype heterogeneity. *Stat Med* 2016;35:782–800. [PubMed: 26619806]
26. Westerlind H, Mellander MR, Bresso F, et al. Dense genotyping of immune-related loci identifies HLA variants associated with increased risk of collagenous colitis. *Gut* 2017;66:421–428. [PubMed: 26525574]
27. Westerlind H, Bonfiglio F, Mellander MR, et al. HLA Associations Distinguish Collagenous From Lymphocytic Colitis. *Am J Gastroenterol* 2016;111:1211–3. [PubMed: 27481432]
28. Fernandez-Banares F, de Sousa MR, Salas A, et al. Impact of current smoking on the clinical course of microscopic colitis. *Inflamm Bowel Dis* 2013;19:1470–6. [PubMed: 23552765]
29. Masclee GM, Coloma PM, Kuipers EJ, et al. Increased risk of microscopic colitis with use of proton pump inhibitors and non-steroidal anti-inflammatory drugs. *Am J Gastroenterol* 2015;110:749–59. [PubMed: 25916221]
30. Dobbins WO, 3rd, Rubin CE Studies of the Rectal Mucosa in Celiac Sprue. *Gastroenterology* 1964;47:471–9. [PubMed: 14226217]
31. Fernandez-Banares F, Esteve M, Farre C, et al. Predisposing HLA-DQ2 and HLA-DQ8 haplotypes of coeliac disease and associated enteropathy in microscopic colitis. *Eur J Gastroenterol Hepatol* 2005;17:1333–8. [PubMed: 16292086]
32. Kim HS, Patel KG, Orosz E, et al. Time Trends in the Prevalence of Celiac Disease and Gluten-Free Diet in the US Population: Results From the National Health and Nutrition Examination Surveys 2009–2014. *JAMA Intern Med* 2016;176:1716–1717. [PubMed: 27598396]
33. Fasano A, Sapone A, Zavallos V, et al. Nonceliac gluten sensitivity. *Gastroenterology* 2015;148:1195–204. [PubMed: 25583468]
34. Freeman HJ. Failure of added dietary gluten to induce small intestinal histopathological changes in patients with watery diarrhea and lymphocytic colitis. *Can J Gastroenterol* 1996;10:436–9. [PubMed: 9113884]
35. Williams JJ, Kaplan GG, Makhija S, et al. Microscopic colitis-defining incidence rates and risk factors: a population-based study. *Clin Gastroenterol Hepatol* 2008;6:35–40. [PubMed: 18166476]
36. Pardi DS. Diagnosis and Management of Microscopic Colitis. *Am J Gastroenterol* 2017;112:78–85. [PubMed: 27897155]

STUDY HIGHLIGHTS**WHAT IS THE CURRENT KNOWLEDGE**

- Microscopic colitis is a common cause of chronic diarrhea among the elderly.
- Microscopic colitis and celiac disease shared clinical and pathologic features, and the role of gluten in microscopic colitis is largely unknown.

WHAT IS NEW HERE:

- Dietary gluten intake during adulthood is not associated with risk of microscopic colitis among women without celiac disease.

Figure.
Flow diagram of the study population

Table 1.
Age-adjusted Baseline Demographics of Study Participants by Quintiles of Energy-adjusted Gluten Intake

Characteristics ¹	Nurses' Health Study (1986)			Nurses' Health Study II (1991)		
	1 (lowest) n = 13,478	3 (middle) n = 13,639	5 (highest) n = 13,548	1 (lowest) n = 18,450	3 (middle) n = 18,501	5 (highest) n = 18,633
Energy-adjusted gluten intake, g/d	2.7 (0.6)	4.7 (0.2)	7.5 (1.4)	3.4 (0.7)	5.9 (0.3)	9.3 (1.5)
Age, years	53.1 (6.9)	52.2 (7.2)	52.6 (7.3)	36.2 (4.8)	36.2 (4.6)	36.2 (4.6)
Body mass index, kg/m ²	25.4 (4.9)	25.4 (4.8)	25.1 (4.8)	25.2 (5.7)	24.7 (5.3)	23.9 (4.9)
Whole grains, g/d	9.1 (11.3)	12.7 (10.4)	24.0 (17.9)	15.4 (14.2)	19.3 (13.3)	28.4 (19.3)
Refined grains, g/d	31.9 (12.5)	47.1 (10.9)	61.6 (18.6)	42.4 (18.4)	60.9 (12.3)	84.1 (20.3)
Alternate Healthy Eating Index score	51.9 (11.9)	51.3 (10.9)	52.5 (11.0)	47.7 (11.4)	48.1 (10.7)	50.5 (11.0)
All physical activity, MET-hours/week	15.1 (22.3)	14.0 (19.4)	13.7 (20.3)	21.1 (28.3)	20.3 (25.8)	21.9 (29.0)
Smoking status, %						
Never	40%	45%	47%	66%	69%	69%
Past smoker	34%	35%	36%	21%	23%	25%
Current smoker	26%	20%	17%	13%	8.6%	6.1%
Pack-years among ever-smokers	25.3 (27.3)	22.7 (27.4)	21.5 (18.5)	8.6 (60.5)	7.8 (62.9)	6.2 (51.4)
History of diabetes, %	3.9%	3.5%	4.4%	1.2%	0.9%	1.0%
Regular NSAIDs use ² , %	36%	37%	36%	24%	25%	22%
Pre-menopausal, %	31%	33%	34%	90%	93%	94%
Current menopausal hormone therapy ³ , %	27%	27%	27%	43%	44%	44%

¹ All values other than age are standardized to the age distribution of the study population. Values are mean (standard deviation) unless stated otherwise.

² NSAIDs use in Year 1990 for NHS, Year 1995 for NHS II

³ Among post-menopausal participants

Table 2.**Dietary Gluten Intake and Risk of Microscopic Colitis**

	Quintiles of Energy-adjusted Gluten Intake					P _{trend}
	1 (lowest)	2	3	4	5 (highest)	
Pooled Analysis (NHS + NHS II)						
No. of events	40	37	54	45	48	
Person Years	701,209	744,876	756,408	758,476	754,976	
Age-adjusted HR (95% CI)	1 (Ref)	0.83 (0.53-1.30)	1.19 (0.79-1.79)	0.99 (0.65-1.52)	1.09 (0.71-1.65)	0.65
Multivariable-adjusted HR (95% CI) [/]	1 (Ref)	0.82 (0.52-1.28)	1.17 (0.78-1.76)	0.98 (0.64-1.51)	1.08 (0.71-1.64)	0.69
Additionally adjusted for refined grains	1 (Ref)	0.84 (0.53-1.32)	1.21 (0.78-1.87)	1.03 (0.64-1.67)	1.16 (0.67-1.98)	0.64
Additionally adjusted for whole grains	1 (Ref)	0.81 (0.52-1.27)	1.15 (0.76-1.74)	0.96 (0.62-1.48)	1.03 (0.66-1.61)	0.92

CI, confidence interval, HR, hazard ratio, NHS, Nurses' Health Study

[/] Additionally adjusted for smoking status (never, past, current), menopausal hormone therapy (never, past, current, premenopausal), Alternate Healthy Eating Index score (quintiles), physical activities (quintiles), current use of non-steroid anti-inflammatory drugs, and body mass index (<25, 25-30, ≥30 kg/m²).

Table 3.

Gluten Intake and Risk of Collagenous Colitis and Lymphocytic Colitis

	Quintiles of Energy-adjusted Gluten Intake					P _{trend}	P _{heterogeneity}
	1 (lowest)	2	3	4	5 (highest)		
Collagenous Colitis							
No. of events	18	19	20	30	20		0.60
Person Years	701,209	744,876	756,408	758,476	754,976		
Age-adjusted HR (95% CI)	1 (Ref)	0.94 (0.49-1.79)	0.97 (0.51-1.84)	1.46 (0.81-2.62)	1.01 (0.53-1.92)	0.66	
Multivariable-adjusted HR (95% CI) [/]	1 (Ref)	0.95 (0.50-1.81)	0.99 (0.52-1.88)	1.50 (0.83-2.70)	1.05 (0.55-2.00)	0.58	
Lymphocytic Colitis							
No. of events	21	14	30	12	28		
Person Years	701,209	744,876	756,408	758,476	754,976		
Age-adjusted HR (95% CI)	1 (Ref)	0.60 (0.31-1.18)	1.25 (0.72-2.19)	0.51 (0.25-1.03)	1.19 (0.68-2.10)	0.63	
Multivariable-adjusted HR (95% CI) [/]	1 (Ref)	0.58 (0.30-1.15)	1.20 (0.69-2.10)	0.48 (0.24-0.98)	1.14 (0.64-2.01)	0.76	

CI, confidence interval, HR, hazard ratio

[/] Additionally adjusted for smoking status (never, past, current), menopausal hormone therapy (never, past, current, premenopausal), Alternate Healthy Eating Index score (quintiles), physical activities (quintiles), current use of non-steroid anti-inflammatory drugs, and body mass index (<25, 25-30, ≥30 kg/m²).

Table 4.
Gluten Intake and Risk of Microscopic Colitis according to Age, Body Mass Index, and Smoking Status

	Quintiles of Energy-adjusted Gluten Intake					P _{trend}	P _{Interaction}
	1 (lowest)	2	3	4	5 (highest)		
Age < 60 years							
No. of events	12	8	18	21	10		0.96
Person Years	468,803	499,274	508,854	512,126	509,304		
Age-adjusted HR (95% CI)	1 (Ref)	0.62 (0.25-1.51)	1.36 (0.66-2.83)	1.57 (0.77-3.20)	0.77 (0.33-1.77)	0.99	
Multivariable-adjusted HR (95% CI) [/]	1 (Ref)	0.61 (0.25-1.49)	1.35 (0.65-2.81)	1.55 (0.76-3.18)	0.76 (0.32-1.77)	0.96	
Age 60 years							
No. of events	28	29	36	24	38		
Person Years	232,406	245,602	247,554	246,350	245,672		
Age-adjusted HR (95% CI)	1 (Ref)	0.92 (0.55-1.55)	1.11 (0.68-1.83)	0.75 (0.44-1.30)	1.22 (0.75-1.99)	0.56	
Multivariable-adjusted HR (95% CI) [/]	1 (Ref)	0.91 (0.54-1.52)	1.10 (0.67-1.81)	0.75 (0.43-1.29)	1.23 (0.75-2.00)	0.55	
Body Mass Index < 25 kg/m ²							
No. of events	19	21	38	25	33		0.25
Person Years	364,124	390,314	403,970	422,026	449,846		
Age-adjusted HR (95% CI)	1 (Ref)	1.00 (0.54-1.87)	1.75 (1.01-3.03)	1.11 (0.61-2.02)	1.38 (0.78-2.43)	0.38	
Multivariable-adjusted HR (95% CI) [/]	1 (Ref)	0.98 (0.53-1.83)	1.70 (0.98-2.96)	1.10 (0.60-2.00)	1.41 (0.80-2.48)	0.34	
Body Mass Index ≥ 25 kg/m ²							
No. of events	21	16	16	20	15		
Person Years	337,085	354,562	352,438	336,450	305,130		
Age-adjusted HR (95% CI)	1 (Ref)	0.69 (0.36-1.32)	0.69 (0.36-1.33)	0.90 (0.49-1.67)	0.76 (0.39-1.47)	0.55	
Multivariable-adjusted HR (95% CI) [/]	1 (Ref)	0.68 (0.36-1.30)	0.69 (0.36-1.32)	0.90 (0.48-1.66)	0.79 (0.40-1.53)	0.61	
Never Smoker							
No. of events	16	12	21	25	21		0.55
Person Years	372,184	414,818	429,861	436,528	442,622		
Age-adjusted HR (95% CI)	1 (Ref)	0.64 (0.30-1.36)	1.09 (0.57-2.08)	1.29 (0.69-2.41)	1.07 (0.56-2.06)	0.23	
Multivariable-adjusted HR (95% CI) [/]	1 (Ref)	0.61 (0.29-1.30)	1.02 (0.53-1.96)	1.21 (0.64-2.26)	0.99 (0.51-1.90)	0.35	

	Quintiles of Energy-adjusted Gluten Intake					P _{trend}	P _{interaction}
	1 (lowest)	2	3	4	5 (highest)		
Past or current smoker							
No. of events	24	25	33	20	27		
Person Years	329,025	330,058	326,547	321,948	312,354		
Age-adjusted HR (95% CI)	1 (Ref)	0.99 (0.56-1.73)	1.30 (0.77-2.20)	0.80 (0.44-1.45)	1.14 (0.65-1.97)	0.76	
Multivariable-adjusted HR (95% CI) [/]	1 (Ref)	0.95 (0.54-1.67)	1.25 (0.74-2.11)	0.76 (0.42-1.39)	1.10 (0.63-1.91)	0.65	

[/] Additionally adjusted for smoking status (never, past, current), menopausal hormone therapy (never, past, current, premenopausal), Alternate Healthy Eating Index score (quintiles), physical activities (quintiles), current use of non-steroid anti-inflammatory drugs, and body mass index (<25, 25-30, 30 kg/m²).