Original Article

Baseline Dietary Glutamic Acid Intake and the Risk of Colorectal Cancer: The Rotterdam Study

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BACKGROUND: Animal studies have shown that glutamine supplementation may decrease colon carcinogenesis, but any relation with glutamine or its precursors has not been studied in humans. The primary aim of this study was to assess whether dietary glutamic acid intake was associated with colorectal cancer (CRC) risk in community-dwelling adults. A secondary aim was to evaluate whether the association could be modified by the body mass index (BMI). **METHODS:** This study was embedded in the Rotterdam study, which included a prospective cohort from 1990 onward that consisted of 5362 subjects who were 55 years old or older and were free of CRC at the baseline. Glutamic acid was calculated as a percentage of the total protein intake with a validated food frequency questionnaire at the baseline. Incident cases of CRC were pathology-based. **RESULTS:** During follow-up, 242 subjects developed CRC. Baseline dietary glutamic acid intake was significantly associated with a lower risk of developing CRC (hazard ratio [HR] per percent increase in glutamic acid of protein, 0.78; 95% confidence interval [CI], 0.62-0.99). After stratification for BMI, the risk reduction for CRC by dietary glutamic acid was 42% for participants with a BMI $\leq 25\,kg/m^2$ (HR per percent increase in glutamic acid of protein, 0.58; 95% CI, 0.40-0.85), whereas no association was found in participants with a BMI $> 25\,kg/m^2$ (HR per percent increase in glutamic acid of protein, 0.97; 95% CI, 0.73-1.31). **CONCLUSIONS:** Our data suggest that baseline dietary glutamic acid intake is associated with a lower risk of developing CRC, but this association may be mainly present in nonoverweight subjects. ***Cancer*** **2016;122:899-907.**
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KEYWORDS: colorectal cancer, epidemiology, glutamic acid, glutamine.

INTRODUCTION

Colorectal cancer (CRC) is a major public health problem: globally, nearly 1.2 million new cases of CRC are diagnosed every year, and the majority occur in Western countries.[1,2]

A large body of evidence has shown that poor diet and lifestyle habits increase the risk of CRC.[3,4] For example, dietary intake of red and processed meat,[5,6] alcohol intake,[3] and body fatness have been linked to an increased risk of developing CRC.[7,8]

In contrast, dietary protein intake has been associated with a decreased risk of CRC in some studies,[9] but results are still inconsistent and may depend on differences in food sources rich in protein, such as meat versus fish.[9] The mechanisms linking protein intake to CRC are largely unknown, but one of the hypotheses is that it may be explained by specific amino acids.

One of the amino acids that may be of interest in CRC etiology is glutamic acid. Foods sources that include glutamic acid are plant and animal protein sources such as beef, pork, poultry, milk, soy, cheese, spinach, and cabbage.[10] Glutamic acid can form glutamine and is one of the most common amino acids in plasma.[11] Glutamine has been ascribed different roles in the gastrointestinal tract. For instance, it stimulates crypt cell proliferation in the gut[12-14] and acts as a trophic factor and as a protective factor for the intestinal mucosa.[12,14] Also, it serves as a precursor for nucleotide synthesis in

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Gilson Gabriel Viana Veloso and Jessica C. Kiefte-de Jong analyzed the data and wrote the manuscript. Oscar H. Franco, Jessica C. Kiefte-de Jong, Albert Hofman, and Bruno C. Stricker designed the study. Rikje Ruiter, Catherina E. de Keyser, and Bruno C. Stricker collected data on cancer incidence. Jessica C. Kiefte-de Jong had primary responsibility for the final content. All authors read and approved the final manuscript.

The authors thank Wieke Altorf-van der Kuil (Department of Human Nutrition, Wageningen University, Wageningen, the Netherlands) for her contribution to the calculations of dietary amino acid intake.

DOI: 10.1002/cncr.29862, **Received:** August 13, 2015; **Revised:** December 2, 2015; **Accepted:** December 7, 2015, **Published online** December 30, 2015 in Wiley Online Library (wileyonlinelibrary.com)

enterocytes.[15] In addition, a previous study found that glutamine supplementation may reduce chronic bowel inflammation through suppression of cytokines in mouse models with colitis-associated tumors.[16]

Another potential pathway through which glutamic acid may play a role in the etiology of CRC is body weight control. In addition, obesity and weight gain have been found to be associated with CRC risk,[8,17] but supplementation with a polymer of glutamic acid or glutamine has been reported to be involved in weight control in humans.[18,19] However, whether glutamic acid may also have a role in CRC prevention in healthy human individuals is not well established. Therefore, the primary aim was to study the association between dietary glutamic acid intake and CRC risk in a population-based cohort study of adults who were 55 years old or older. A secondary aim was to assess to what extent the association could be modified by the body mass index (BMI).

MATERIALS AND METHODS

Rotterdam Study

This study was embedded in the Rotterdam study, a population-based, prospective cohort of subjects aged 45 years or older and living in Ommoord, a suburb in Rotterdam, the Netherlands.[20] The Rotterdam study comprises 3 cohorts: the first cohort included subjects who were 55 years old or older and started in 1990, the second cohort started in 2000 and included subjects who were 50 years old, and the third cohort started in 2007 and included subjects who were 45 years old or older. For the current study, the first Rotterdam study cohort (n = 7983) that started in 1990 and included subjects who were 55 years old or older was used because this cohort included sufficient follow-up on cancer and had dietary data available for the baseline.

Participants were interviewed at home and were physically examined at the research center every 3 to 4 years. The medical ethics committee of the Erasmus Medical Center (Rotterdam, the Netherlands) approved the study, and all participants provided written informed consent.[21]

Dietary Data Collection

A food frequency questionnaire (FFQ) was used to collect dietary data from participants at the baseline. The FFQ consisted of 170 food items, and participants were asked to complete the questionnaire with information about the frequency of consumption of each food and the number of servings as described in detail previously.[22] To minimize loss of information, the dietary assessment included a 2-step protocol: a self-administered questionnaire that included a checklist of foods that the subjects had consumed at least twice per month in the previous year and then a standardized interview based on the checklist with a trained dietician using the computerized, validated FFQ. For each item, the frequency was recorded as times per day, week, or month. The number of servings per frequency was expressed in natural units (eg, slice of bread), household measures (eg, cup), or grams (eg, cooked vegetables). Data collected from the FFQ were then analyzed to calculate macronutrient intake according to the Dutch Food Composition Table.[23] To calculate the dietary amino acid content, the Dutch Food Composition Table of 1993 was extended by the addition of data on amino acid content from McCance and Widdowson's food composition table, which is based on chemically analyzed amino acids of 150 foods,[10] as described in detail previously.[24]

For this study, the following dietary variables were taken into account in the analyses: energy intake (kcal/day), total amino acid and glutamic acid intake (g/d), total monounsaturated fat intake (g/d), total polyunsaturated fat intake (g/d), total saturated fat intake (g/d), total processed meat intake (g/d), total unprocessed meat intake (g/d), total magnesium intake (g/d), total dietary fiber intake (g/d), and total polysaccharide intake (g/d).

Because dietary glutamic acid can be closely correlated with other amino acids, dietary data on glutamic acid intake were analyzed first through the determination of the glutamic acid intake percentage of the total amino acid intake. Subsequently, this percentage was adjusted for the total energy intake by the residual method.[25] Because dietary data from the FFQ are best suited for ranking individuals instead of analyzing absolute values alone,[22] the energy-adjusted glutamic acid intake was categorized into quartiles; the quartile that included the median intake (quartile 2) was used as a reference for further analyses.

Protein intake from the FFQ was validated against urea excretion within a representative sample (n = 80). The Spearman correlation coefficient between protein intake estimated from the FFQ and urea excretion was 0.67.[22] Other macronutrients and micronutrients were validated against multiple food records and showed a correlation between 0.4 and 0.8 after adjustments for age, sex, total energy intake, and within-person variation.[22]

CRC

Cancer cases were ascertained through 4 yearly follow-up rounds in which all available data per participant were collected from the general practitioners. In addition, the

Rotterdam study is linked to Pathan, a local pathology and cytology laboratory in Rotterdam that provides pathology services to the hospitals in the Rotterdam area. Last, the study is linked to the information database for admissions as collected by Dutch Hospital Data. Two research physicians independently assessed the diagnoses of CRC on the basis of pathology data and medical records. All events were classified according to *International Classification of Diseases, Tenth Revision*.[26] In the case of a discrepancy, consensus was sought, or an oncologist decided. Only cases confirmed by pathology were considered in the analyses. The index date was defined as the earliest date found in the pathology reports. The CRC status was ascertained from the baseline until December 31, 2011. Any CRC diagnosis before the baseline measurement was excluded.

Covariates

Several other medical and lifestyle variables were considered as potential confounders or mediators. For lifestyle habits, we considered alcohol intake, smoking status, BMI, and physical activity levels. Alcohol intake (g/d) was analyzed continuously. The smoking status was divided into 2 categories: never or former smokers and current smokers. BMI (kg/m^2) was analyzed continuously and was also categorized on the basis of cutoffs for overweight ($\geq$25.0 kg/m^2) and obesity ($\geq$30.0 kg/m^2). Any use of antidiabetic medication was defined on the basis of Anatomical Therapeutic Chemical code A010.

Physical activity was assessed only during the third visit to the research center by means of an adapted version of the Zutphen Physical Activity Questionnaire.[27] The questionnaire consisted of questions on walking, cycling, gardening, diverse sports, hobbies, and housekeeping. The total time spent on physical activity was calculated as the sum of minutes per week for each type of activity; thus, the weekly duration of physical activity was obtained.

For sociodemographic variables, the financial status and the education level were assessed. The financial status was divided into 2 categories: low to middle income (net income $<$ €1090 per month) and middle to high income (net income $\geq$ €1090 per month). The education level was divided into 2 categories as well: primary education solely and secondary education or higher.

Population of Analysis

From the first Rotterdam study cohort (n = 7983), subjects with a history of CRC (n = 2) as well as subjects without dietary data (n = 2619 or 32.8%) were excluded. Dietary data were missing when individuals participated in the pilot phase of the Rotterdam study cohort (between 1989 and 1990), individuals were institutionalized, or the research dietician considered the dietary data unreliable (eg, when subjects had difficulties with recall of their food intake or when dementia was suspected). No difference in CRC risk over time was observed in subjects with and without dietary data (hazard ratio [HR], 1.23; 95% confidence interval [CI], 0.92-1.67). The final population of the analysis consisted of 5362 subjects.

Statistical Analysis

Cox regression was performed with quartiles of energy-adjusted glutamic acid intake as the independent variable and CRC diagnosis as the dependent variable. As the underlying timescale, the follow-up time (in years) was used. Participants were followed until a CRC diagnosis, death, or the end of follow-up (December 31, 2011), whichever occurred first.

Crude (age- and sex-adjusted) and multivariate Cox proportional hazards analyses were performed to evaluate the association between glutamic acid intake and CRC risk. Potential confounders were added stepwise to the crude model. The selection of potential confounders for the multivariate models was based on previous literature as well as changes in the effect estimate of more than 10%.[28] In addition, the presence of effect modification by BMI was evaluated by the addition of a product term of glutamic acid times BMI to the crude and multivariate models, and an analysis stratified by BMI was performed when effect modification was present.

Nonlinear relations between glutamic acid intake and CRC risk were assessed by the inclusion of a quadratic term of glutamic acid intake.

Several sensitivity analyses were performed. To assess potential reverse causality, we excluded CRC cases in which the diagnosis occurred within the first 2 and 5 years of follow-up. To assess whether the association was different for colon cancer versus rectal cancer, we reran analyses by splitting colon cancer and rectal cancer (including the rectum and rectosigmoid junction).

To reduce a potential attrition bias, we performed a multiple imputation procedure for missing covariates (5 imputations; Tables 1 and 2).[29] Results of the Cox regression models are presented as HRs and 95% CIs. A P value $\leq$ .05 was considered to be statistically significant. All analyses were performed with IBM SPSS Statistics (SPSS, version 21.0; SPSS Inc, Chicago, Ill).

TABLE 1. Details of the Multiple Imputation Procedure

Software	SPSS 21.0 for Windows
Imputation method	Custom, fully conditional specification (Markov chain Monte Carlo); 10 maximum interactions
No. of imputations	5
Variables with missing data included in the imputation procedure	Smoking status, body mass index, family history of cancer, education level, financial status, physical activity
Variables without missing data but added as predictors for the imputation to improve the missing-at-random assumption	Age at start, sex, total energy intake, total glutamic acid intake, total amino acid intake minus glutamic acid intake, total processed meat intake, total unprocessed meat intake, total monounsaturated fat intake, total polyunsaturated fat intake, total saturated fat intake, total dietary fiber intake, total dietary magnesium intake, total polysaccharide intake
Treatment of continuous variables	Predictive mean matching
Treatment of categorical variables	Logistic regression

TABLE 2. Comparison of Variables With Missing Data Before and After the Imputation Procedure

Variable	Original Data (n = 5362), Valid No. (%)	After Multiple Imputation Procedure (n = 5362), No. (%)
Smoking status		
Never or former	4080 (76.1)	4103 (76.5)
Current	1250 (23.3)	1259 (23.5)
Missing	32 (0.6)	—
Body mass index (kg/m^2)		
Valid No.	5328 (99.4)	5362 (100)
Missing	34 (0.6)	—
Family history of cancer		
Yes	2218 (41.4)	2759 (51.4)
No	2074 (38.6)	2603 (48.6)
Missing	1070 (20)	—
Education level		
Primary	2782 (51.9)	2796 (52.1)
Secondary or higher	2553 (47.6)	2566 (47.9)
Missing	27 (0.5)	—
Financial status		
Low to middle income	130 (2.4)	145 (2.7)
Middle to high income	4716 (88)	5217 (97.3)
Missing	516 (9.6)	—
Physical activity (h/wk)		
Below the median	1924 (35.9)	2844 (53)
Above the median	1920 (35.8)	2518 (47)
Missing	1518 (28.3)	—

RESULTS

Population Characteristics

Baseline characteristics are shown in Table 3. During the median follow-up period of 16 years (range, 21 years), 242 subjects developed CRC (104 men and 138 women); 120 (50%) had colon cancer, 117 (48%) had rectal cancer, and 5 (2%) had other types of CRC.

The median intake of glutamic acid intake was 16 g/d (range, 4-46 g/d), which was a median of 20% (range, 17%-27%) of the total amino acid intake.

The main food sources of dietary glutamic acid were dairy, fish, meat, and grains; this explained 57% of the total variance in glutamic acid of the population.

Participants with a high intake of glutamic acid had a lower intake of alcohol and a lower intake of other amino acids and were less often smokers (Table 3).

Dietary Glutamic Acid Intake and CRC Risk

Associations between the baseline dietary glutamic acid intake and the CRC risk are presented in Table 4. A higher glutamic acid level was associated with a lower risk of CRC (HR per percent increase in glutamic acid of protein, 0.79; 95% CI, 0.62-0.99; Table 4) after adjustments for dietary, lifestyle, and socioeconomic confounders. Additional adjustments for BMI did not alter this result (Table 4). With respect to the median glutamic acid intake, subjects with a high intake of glutamic acid had a 50% lower risk of CRC (HR, 0.51; 95% CI, 0.30-0.87; Table 4). No evidence for a nonlinear relation was found ($P_{\text{quadratic term}} > .05$).

Subgroup and Sensitivity Analysis

The association between baseline dietary glutamic acid and CRC risk was modified by BMI ($P_{\text{interaction}} = .05$). After stratification by BMI, a significant association between dietary glutamic acid intake and CRC was more prominent in subjects with a normal BMI (Table 5) but was not in subjects who were overweight with a BMI > 25 kg/m^2 (Table 5).

HRs did not differ significantly after the exclusion of subjects from the analyses who developed CRC after less than 2 (n = 25) and 5 years of follow-up (n = 62; Table 6). Separating the outcome by colon cancer and rectal cancer revealed similar associations for colon and rectal cancer (HR for colon cancer, 0.85; 95% CI, 0.74-0.97; HR for rectal cancer, 0.70; 95% CI, 0.61-0.80 [per percent increase in glutamic acid of protein after adjustments for age, sex, alcohol intake, physical activity, smoking, family history of cancer, dietary fiber intake, dietary magnesium intake, unprocessed meat intake, dietary

TABLE 3. Baseline Characteristics

	Energy-Adjusted Glutamic Acid Intake			
	First Quartile (Median, 19% of Protein; n = 1340)	Second Quartile (Median, 20% of Protein; n = 1341)	Third Quartile (Median, 21% of Protein; n = 1341)	Fourth Quartile (Median, 22% of Protein; n = 1340)
Age at start, mean (SD), y	67 (8)	67 (8)	67 (8)	68 (8)
Sex, No. (%)				
Male	596 (44)	553 (41)	534 (40)	523 (39)
Female	744 (56)	788 (59)	807 (60)	817 (61)
Alcohol intake, median (range), g/d	9 (145)	5 (99)	2 (105)	1 (97)
Smoking status, No. (%)				
Never or former	946 (70.5)	1023 (76.2)	1052 (78.4)	1059 (79)
Current	387 (29)	307 (23)	281 (21)	275 (20.5)
Missing	7 (0.5)	11 (0.8)	8 (0.6)	6 (0.5)
Body mass index, mean (SD), kg/m^2	27 (3)	26 (4)	26 (3)	26 (4)
Missing, No. (%)	11 (0.2)	9 (0.2)	4 (0.1)	10 (0.2)
Family history of cancer, No. (%)				
Yes	515 (38)	561 (42)	591 (44)	551 (41)
No	559 (42)	523 (39)	498 (37)	494 (37)
Missing	266 (20)	257 (19)	252 (19)	295 (22)
Education level, No. (%)				
Primary	688 (51.6)	676 (50.4)	709 (53)	709 (53)
Secondary or higher	647 (48)	654 (48.8)	626 (47.6)	626 (46.6)
Missing	5 (0.4)	11 (0.8)	6 (0.4)	5 (0.4)
Financial status, No. (%)				
Low to middle income	34 (2.5)	28 (2)	34 (2.5)	34 (2.5)
Middle to high income	1180 (88)	1180 (88)	1181 (88)	1175 (88)
Missing	126 (9.5)	133 (10)	126 (9.5)	131 (9.5)
Physical activity levels,[a] No. (%)				
Below the median	487 (36)	477 (36)	477 (36)	483 (36)
Above the median	474 (35)	508 (38)	500 (37)	438 (33)
Missing	379 (29)	356 (26)	364 (27)	419 (31)
Use of antidiabetic medication, No. (%)				
Yes	46 (3.4).	53 (4)	54 (4.0)	54 (4)
No	1293 (96.5)	1288 (96)	1286 (95.9)	1284 (95.8)
Missing	1 (0.)	—	1 (0.1)	2 (0.1)
Total energy intake, mean (SD), kcal/day	1965 (556)	1965 (472)	2010 (506)	1972 (467)
Total amino acid intake minus glutamic acid, mean (SD), g/d	86 (23)	84 (19)	84 (19)	78 (18)
Polyunsaturated fat intake, mean (SD), g/d	15 (8)	15 (7)	16 (8)	15 (7)
Monounsaturated fat intake, mean (SD), g/d	29 (12)	28 (10)	27 (10)	26 (9)
Saturated fat intake, mean (SD), g/d	32 (13)	32 (11)	32 (12)	31 (11)
Total fiber intake, mean (SD), g/d	16 (6)	17 (5)	17 (5)	16 (5)
Processed meat intake, mean (SD), servings/d	1.5 (1.2)	1.5 (1.2)	1.5 (1.2)	1.5 (1.4)
Unprocessed meat intake, mean (SD), servings/d	1.0 (0.6)	0.8 (0.4)	0.7 (0.3)	0.5 (0.3)
Total polysaccharide intake, mean (SD), g/d	91 (30)	100 (27)	111 (30)	118 (30)
Total magnesium intake, mean (SD), g/d	300 (82)	310 (71)	315 (75)	299 (70)

Abbreviation: SD, standard deviation.
[a] Measured during the third visit at the research center (not baseline)

polysaccharide intake, financial status, smoking status, and BMI]).

DISCUSSION

This study showed that a low intake of baseline glutamic acid was associated with a higher risk of developing CRC among community-dwelling adults. This association was modified by BMI and differed for those with a higher BMI ($>25\,kg/m^2$) versus those with a normal BMI ($\leq 25\,kg/m^2$).

This is the first population-based study evaluating the potential role of glutamic acid in the risk of developing CRC. Previous animal and laboratory studies have provided some evidence showing that glutamic acid may play a role in carcinogenesis and subsequent prevention of CRC.

The amino acid glutamic acid is closely related to glutamine. The human body is able to produce L-glutamine itself from L-glutamic acid.[30] Glutamic acid and glutamine are considered to be the most abundant amino

TABLE 4. Dietary Glutamic Acid Intake and Colorectal Cancer Risk

	Hazard Ratio (95% Confidence Interval)		
	Crude[a]	Multivariate 1[b]	Multivariate 2[c]
Energy-adjusted glutamic acid (continuously per % of protein)	0.87 (0.76-0.99)[d]	0.79 (0.62-0.99)[d]	0.78 (0.62-0.99)[d]
Energy-adjusted glutamic acid (categorical)			
Quartile 1	0.84 (0.60-1.18)	0.85 (0.54-1.33)	0.84 (0.53-1.30)
Quartile 2	Reference	Reference	Reference
Quartile 3	0.77 (0.54-1.08)	0.81 (0.52-1.26)	0.80 (0.52-1.24)
Quartile 4	0.67 (0.47-0.96)[d]	0.52 (0.31-0.88)[d]	0.51 (0.30-0.87)[d]

[a] The crude model was adjusted for age (continuously) and sex (male and female).
[b] This model included the crude model plus additional adjustments for the following: alcohol intake (continuously), physical activity (continuously), family history of cancer (yes vs no), dietary fiber intake (continuously), dietary magnesium intake (continuously), unprocessed meat intake (continuously), dietary polysaccharide intake (continuously), smoking status (current vs former/no), and financial status (low-middle vs middle-high). Additional adjustments for education level, saturated fat intake, monounsaturated fat, polyunsaturated fat, processed meat intake, and use of antidiabetic medication did not change the results by more than 10%.
[c] This model included multivariate model 1 plus an additional adjustment for the body mass index (continuously).
[d] $P < .05$.

acids in the body.[31] Glutamine has been shown to play a role in protecting cells from inflammation and oxidative stress,[32] and it is preferred as fuel for many cells, including enterocytes[33] and colonocytes.[34]

For the intestinal mucosa, the role of glutamine as a trophic factor is well described.[35] Glutamine repairs the epithelial layer by preserving mucosal integrity and maintains bowel barrier functions by reducing permeability[36] and bacterial translocation.[37] Moreover, glutamine has been shown to have several anti-inflammatory activities.[32] In addition, the relation between inflammation and carcinogenesis of the colon has been hypothesized previously by studies showing neoplastic transformation in subjects with inflammatory bowel disease.[38]

If we combine these latter mechanisms, it can be hypothesized that glutamic acid may have a protective role through glutamine against the development of CRC. Previous mouse models have shown that glutamine prevents the progression of colitis-associated CRC.[16] Also, it has been recently demonstrated that glutaminase, an enzyme that converts glutamine to glutamate, suppresses proliferation and induces apoptosis in cell lines of colorectal adenomas.[39]

In our study, we evaluated the interaction between glutamic acid intake and BMI with respect to CRC risk because BMI is an established risk factor for CRC.[8] We demonstrated that the protective effect of glutamic acid against CRC risk may exist only for participants with a BMI $\leq 25\ kg/m^2$. It may be speculated that being overweight dilutes any potentially protective effect of glutamic acid on CRC risk. Another explanation might be that the association between glutamic acid and CRC is mediated by body weight control. In addition, it has been shown that both glutamic acid and glutamine may play a role in weight reduction.[18,19]

It can be argued that the association between dietary glutamic acid and CRC risk may be explained by an increased demand for glutamine due to early malignant processes in the colon. In addition, tumor progression is associated with an increased demand for glutamine from tumor cells.[40] As a result, depression of the activity of other immune cells may occur because of decreased glutathione concentrations.[41] To account for this potential reverse causality, we excluded CRC cases at the baseline. Also, the exclusion of CRC cases after 2 and 5 years of follow-up did not show different effect estimates; this suggests that the influence of reverse causality in the relation between glutamic acid and CRC may be limited.

The main strengths of our study are the population-based setting, which strengthens the generalizability of our results, and the prospective study design, which minimizes the recall bias potentially associated with CRC. Moreover, this is the first study on glutamic acid and CRC in a healthy population. Most studies have been performed with animals, cell lines, or CRC patients, all of which may have limited generalizability.

To appreciate the findings of this study, limitations must be taken into consideration. Although our FFQ did have fairly good agreement with protein intake assessed from 24-hour urine testing ($r = 0.67$), we were not able to validate the FFQ for glutamic acid specifically. Hence, our FFQ could still be prone to measurement error.[42] By adjusting for the total energy intake, we aimed to reduce the magnitude of the systematic measurement error.

TABLE 5. Dietary Glutamic Acid Intake and Colorectal Cancer Risk Stratified by the Weight Status

	Hazard Ratio (95% Confidence Interval)	
	Crude[a]	Multivariate[b]
BMI < 25 kg/m^2		
Energy-adjusted glutamic acid (continuously per % of protein)	0.69 (0.55-0.86)[11]	0.58 (0.40-0.84)[11]
Energy-adjusted glutamic acid (categorical)	1.11 (0.65-1.91)	1.16 (0.55-2.47)
Quartile 1	Reference	Reference
Quartile 2	0.64 (0.36-1.16)	0.73 (0.35-1.54)
Quartile 3	0.50 (0.27-0.92)[11]	0.38 (0.15-0.93)[11]
Quartile 4		
BMI ≥ 25 kg/m^2		
Energy-adjusted glutamic acid (continuously per % of protein)	1.01 (0.85-1.20)	0.97 (0.73-1.31)
Energy-adjusted glutamic acid (categorical)	0.71 (0.46-1.10)	0.68 (0.38-1.19)
Quartile 1	Reference	Reference
Quartile 2	0.85 (0.56-1.31)	0.84 (0.49-1.45)
Quartile 3	0.80 (0.51-1.25)	0.60 (0.31-1.16)
Quartile 4		

[a] The crude model was adjusted for age (continuously) and sex (male and female).
[b] This model included the crude model plus additional adjustments for the following: alcohol intake (continuously), physical activity (continuously), family history of cancer (yes vs no), dietary fiber intake (continuously), dietary magnesium intake (continuously), unprocessed meat intake (continuously), dietary polysaccharide intake (continuously), smoking status (current vs former/no), and financial status (low-middle vs middle-high). Additional adjustments for education level, saturated fat intake, monounsaturated fat, polyunsaturated fat, processed meat intake, and use of antidiabetic medication did not change the results by more than 10%.
[c] $P < .05$.

TABLE 6. Sensitivity Analysis

	Hazard Ratio (95% Confidence Interval)		
	Crude[a]	Multivariate 1[b]	Multivariate 2[c]
>2 y of follow-up: glutamic acid % of protein (continuously)	0.85 (0.74-0.98)[d]	0.77 (0.61-0.99)[d]	0.77 (0.61-0.98)[d]
>5 y of follow-up: glutamic acid % of protein (continuously)	0.86 (0.73-1.00)	0.76 (0.63-0.93)[d]	0.76 (0.59-0.97)[d]

Cases with less than 2 and 5 years of follow-up were excluded.
[a] The crude model was adjusted for age (continuously) and sex (male and female).
[b] This model included the crude model plus additional adjustments for the following: alcohol intake (continuously), physical activity (continuously), family history of cancer (yes vs no), dietary fiber intake (continuously), dietary magnesium intake (continuously), unprocessed meat intake (continuously), dietary polysaccharide intake (continuously), smoking status (current vs former/no), and financial status (low-middle vs middle-high). Additional adjustments for education level, saturated fat intake, monounsaturated fat, polyunsaturated fat, processed meat intake, and use of antidiabetic medication did not change the results by more than 10%.<zaq;12>
[c] This model included multivariate model 1 plus an additional adjustment for the body mass index (continuously).
[d] $P < .05$.

However, a random measurement error still may have led to an underestimation of dietary intake. Although it has been reported that diet-disease associations are biased toward the null when a random error of dietary intake is present,[42] residual confounding by other dietary variables due to measurement error may still have been present and may have led to an overestimation of our associations. We had only baseline dietary measurements available for our study. However, in studies of diet and cancer, the dietary assessment should address long-term intake because a long latency period may be involved. We used baseline dietary data under the assumption that dietary habits remain similar over time. Indeed, the latter has been confirmed by another study using 5 annual repeated FFQs similar to the one used in our study; it showed that the ranking of subjects according to dietary intake remained fairly similar over time.[43] This suggests that our single FFQ measurement may be relevant for reflecting recent dietary habits not only at the baseline but also over a longer period.[43] Nonetheless, repeated measurement of diet could reduce the magnitude of any random error, and this could lead to stronger or weaker associations than 1 dietary measurement at the baseline or the most recent dietary intake.[44]

Dietary data were not collected for those institutionalized and for those with recall difficulties. As a result, subjects without dietary data reflect a more diseased and vulnerable study group. Although the CRC risk was not different for subjects with dietary data and subjects without dietary data and this decision was made a priori to maintain the quality of the dietary data assessment, this selection may have affected the generalizability of the study results.

This study was observational in design, so conclusions regarding the causality of the observed association should be made with caution. In addition, although we had data on the family history of cancer, we did not have specific data on the family history of CRC or polyposis. Individuals with a family history of CRC and polyposis are at increased risk for developing CRC.[45] Although we adjusted for any self-reported family history of cancer, residual confounding by a family history of CRC and polyposis may still be present. Also, we did not have any data on chronic inflammatory bowel disease at the baseline, and this has been found to be associated with a higher risk of CRC[46]; this may have influenced our results.

At last, physical activity levels were assessed only during the third visit to the research center and not at the

baseline. Hence, residual confounding by physical activity levels may still be partly present because it has been suggested that physical activity may also play a role in CRC risk.[3] At last, although we did not find any indication for reverse causality by excluding 2 and 5 years of follow-up, there is evidence that CRC may develop over a 10-year interval,[47] and this still may have influenced our results.

We were not able to study the association between glutamic acid intake and right-sided colon cancer versus left-sided colon cancer, although it has been suggested that diet as well as BMI may have different effects on left-sided lesions versus right-sided lesions.[48] This suggests different etiologies for left-sided and right-sided colon cancer.

In conclusion, this is the first study showing in a population-based setting that a high baseline intake of dietary glutamic acid might be associated with a lower risk for CRC. This relation may be modified by BMI and needs further replication in other population-based studies.

FUNDING SUPPORT

No specific funding was disclosed.

CONFLICT OF INTEREST DISCLOSURES

Gilson G. Viana Veloso, Jessica C. Kiefte-de Jong, and Oscar H. Franco work for ErasmusAGE, a center for aging research across the course of life that is funded by Nestle Nutrition (Nestec, Ltd), Metagenics, Inc, and AXA. These funding sources had no role in the design or conduct of the study; in the collection, management, analysis, or interpretation of the data; or in the preparation, review, or approval of this article.

REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide. Lyon, France: International Agency for Research on Cancer; 2013. IARC CancerBase; No. 11
2. Boyle P, Leon ME. Epidemiology of colorectal cancer. *Br Med Bull*. 2002;64:1-25.
3. World Cancer Research Fund, American Institute for Cancer Research. Colorectal Cancer 2011 Report: Food, Nutrition, Physical Activity, and the Prevention of Colorectal Cancer. http://www.wcrf.org/sites/default/files/Colorectal-Cancer-2011-Report.pdf. Accessed August 12, 2015.
4. Huxley RR, Ansary-Moghaddam A, Clifton P, Czernichow S, Parr CL, Woodward M. The impact of dietary and lifestyle risk factors on risk of colorectal cancer: a quantitative overview of the epidemiological evidence. *Int J Cancer*. 2009;125:171-180.
5. Corpet DE. Red meat and colon cancer: should we become vegetarians, or can we make meat safer? *Meat Sci*. 2011;89:310-316.
6. Kim E, Coelho D, Blachier F. Review of the association between meat consumption and risk of colorectal cancer. *Nutr Res*. 2013;33:983-994.
7. Giovannucci E. Obesity, gender, and colon cancer. *Gut*. 2002;51:147.
8. Larsson SC, Wolk A. Obesity and colon and rectal cancer risk: a meta-analysis of prospective studies. *Am J Clin Nutr*. 2007;86:556-565.
9. Williams CD, Satia JA, Adair LS, et al. Associations of red meat, fat, and protein intake with distal colorectal cancer risk. *Nutr Cancer*. 2010;62:701-709.
10. Paul AA, Southgate DAT, Russell J. First Supplement to McCance and Widdowson's the Composition of Foods: Amino Acid Composition (mg per 100 g Food) and Fatty Acid Composition (g per 100 g Food). Amsterdam, the Netherlands: Elsevier/North-Holland Biomedical Press; 1980.
11. Buchman AL. Glutamine: commercially essential or conditionally essential?. A critical appraisal of the human data. *Am J Clin Nutr*. 2001;74:25-32.
12. Turowski GA, Rashid Z, Hong F, Madri JA, Basson MD. Glutamine modulates phenotype and stimulates proliferation in human colon cancer cell lines. *Cancer Res*. 1994;54:5974-5980.
13. Scheppach W, Loges C, Bartram P, et al. Effect of free glutamine and alanyl-glutamine dipeptide on mucosal proliferation of the human ileum and colon. *Gastroenterology*. 1994;107:429-434.
14. DeBerardinis RJ, Cheng T. Q's next: the diverse functions of glutamine in metabolism, cell biology and cancer. *Oncogene*. 2010;29:313-324.
15. Watford M. Glutamine metabolism and function in relation to proline synthesis and the safety of glutamine and proline supplementation. *J Nutr*. 2008;138:2003S-2007S.
16. Tian Y, Wang K, Wang Z, Li N, Ji G. Chemopreventive effect of dietary glutamine on colitis-associated colon tumorigenesis in mice. *Carcinogenesis*. 2013;34:1593-1600.
17. Schlesinger S, Lieb W, Koch M, et al. Body weight gain and risk of colorectal cancer: a systematic review and meta-analysis of observational studies. *Obes Rev*. 2015;16:607-619.
18. Park JH, Choi JC, Sung MH, Kang JH, Chang MJ. High molecular weight poly-gamma-glutamic acid regulates lipid metabolism in rats fed a high-fat diet and humans. *J Microbiol Biotechnol*. 2011;21:766-775.
19. Laviano A, Molfino A, Lacaria MT, et al. Glutamine supplementation favors weight loss in nondieting obese female patients. A pilot study. *Eur J Clin Nutr*. 2014;68:1264-1266.
20. Hofman A, Brusselle GG, Darwish Murad S, et al. The Rotterdam study: 2016 objectives and design update. *Eur J Epidemiol*. 2015;30:661-708.
21. Hofman A, Darwish Murad S, van Duijn CM, et al. The Rotterdam study: 2014 objectives and design update. *Eur J Epidemiol*. 2013;28:889-926.
22. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA, et al. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. *Eur J Clin Nutr*. 1998;52:588-596.
23. Dutch Food Composition Table (NEVO) [in Dutch]. The Hague, the Netherlands: Voorlichtingsbureau Voor de Voeding; 1996.
24. Altorf-van der Kuil W, Engberink MF, De Neve M, et al. Dietary amino acids and the risk of hypertension in a Dutch older population: the Rotterdam study. *Am J Clin Nutr*. 2013;97:403-410.
25. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr*. 1997;65:1220S-1228S.
26. World Health Organization. International Statistical Classification of Diseases and Related Health Problems. Vol 1. 10th rev. Geneva, Switzerland: World Health Organization; 1992.
27. Caspersen CJ, Bloemberg BP, Saris WH, Merritt RK, Kromhout D. The prevalence of selected physical activities and their relation with coronary heart disease risk factors in elderly men: the Zutphen study, 1985. *Am J Epidemiol*. 1991;133:1078-1092.
28. Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. *Am J Epidemiol*. 1989;129:125-137.
29. Sterne JA, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ*. 2009;338:b2393.
30. Wallace C, Keast D. Glutamine and macrophage function. *Metabolism*. 1992;41:1016-1020.
31. Tapiero H, Mathe G, Couvreur P, Tew KD. II. Glutamine and glutamate. *Biomed Pharmacother*. 2002;56:446-457.
32. Cruzat VF, Bittencourt A, Scomazzon SP, Leite JS, de Bittencourt PI Jr, Tirapegui J. Oral free and dipeptide forms of glutamine supplementation attenuate oxidative stress and inflammation induced by endotoxemia. *Nutrition*. 2014;30:602-611.

33. Oba M, Baldwin RL IV, Bequette BJ. Oxidation of glucose, glutamate, and glutamine by isolated ovine enterocytes in vitro is decreased by the presence of other metabolic fuels. *J Anim Sci*. 2004;82:479-486.
34. Blachier F, Boutry C, Bos C, Tome D. Metabolism and functions of L-glutamate in the epithelial cells of the small and large intestines. *Am J Clin Nutr*. 2009;90:814S-821S.
35. Wang WW, Qiao SY, Li DF. Amino acids and gut function. *Amino Acids*. 2009;37:105-110.
36. Wang B, Wu G, Zhou Z, et al. Glutamine and intestinal barrier function. *Amino Acids*. 2015;47:2143-2154.
37. Ren W, Duan J, Yin J, et al. Dietary L-glutamine supplementation modulates microbial community and activates innate immunity in the mouse intestine. *Amino Acids*. 2014;46:2403-2413.
38. Rutter MD, Riddell RH. Colorectal dysplasia in inflammatory bowel disease: a clinicopathologic perspective. *Clin Gastroenterol Hepatol*. 2014;12:359-367.
39. Huang F, Zhang Q, Ma H, Lv Q, Zhang T. Expression of glutaminase is upregulated in colorectal cancer and of clinical significance. *Int J Clin Exp Pathol*. 2014;7:1093-1100.
40. Medina MA. Glutamine and cancer. *J Nutr*. 2001;131:2539S-2542S.
41. Gamcsik MP, Kasibhatla MS, Teeter SD, Colvin OM. Glutathione levels in human tumors. *Biomarkers*. 2012;17:671-691.
42. Freedman LS, Schatzkin A, Midthune D, Kipnis V. Dealing with dietary measurement error in nutritional cohort studies. *J Natl Cancer Inst*. 2011;103:1086-1092.
43. Goldbohm RA, van't Veer P, van den Brandt PA, et al. Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. *Eur J Clin Nutr*. 1995;49:420-429.
44. Hu FB, Stampfer MJ, Rimm E, et al. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. *Am J Epidemiol*. 1999;149:531-540.
45. Amersi F, Agustin M, Ko CY. Colorectal cancer: epidemiology, risk factors, and health services. *Clin Colon Rectal Surg*. 2005;18:133-140.
46. Triantafillidis JK, Nasioulas G, Kosmidis PA. Colorectal cancer and inflammatory bowel disease: epidemiology, risk factors, mechanisms of carcinogenesis and prevention strategies. *Anticancer Res*. 2009;29:2727-2737.
47. Lieberman DA, Rex DK, Winawer SJ, et al. Guidelines for colonoscopy surveillance after screening and polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology*. 2012;143:844-857.
48. Wallace K, Grau MV, Ahnen D, et al. The association of lifestyle and dietary factors with the risk for serrated polyps of the colorectum. *Cancer Epidemiol Biomarkers Prev*. 2009;18:2310-2317.