HHS Public Access

Author manuscript

Int J Cancer. Author manuscript; available in PMC 2022 June 19.

Published in final edited form as:

Int J Cancer. 2021 May 15; 148(10): 2471–2480. doi:10.1002/ijc.33449.

Dietary intake of branched-chain amino acids and survival after colorectal cancer diagnosis

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Conflict of Interest Disclosures: No conflicts were reported.

Abstract

Background: Branched chain amino acids (BCAAs), including leucine, isoleucine, and valine, may potentially influence cancer progression by various mechanisms including its role in insulin resistance. However, the association of BCAAs with survival among patients with established colorectal cancer (CRC) remains unclear.

Methods: We evaluated the associations between postdiagnostic BCAA intake with CRC-specific and overall-mortality among 1,674 patients with nonmetastatic CRC in the Nurses' Health Study and the Health Professionals Follow-up Study. Patients completed a validated food frequency questionnaire. Multivariable hazard ratios (HRs) were calculated using Cox proportional hazards regression model after adjustment for tumor characteristics and potential confounding factors.

Results: Comparing the highest with the lowest quartile intake of postdiagnostic total BCAA, the multivariable HRs were 1.18 [95% confidence interval (CI), 0.75–1.85, Ptrend=0.46 across quartiles] for CRC-specific mortality and 1.30 (95% CI, 1.01–1.69, Ptrend=0.04) for all-cause mortality. No statistically significant associations with each of the BCAA intake were observed for CRC-specific mortality (all Ptrend>0.30). However, the multivariable HRs (the highest vs. the lowest quartile) for all-cause mortality were 1.33 (95% CI, 1.03–1.73, Ptrend=0.02) for valine, 1.28 (95% CI, 0.99–1.66, Ptrend=0.05) for leucine, and 1.25 (95% CI, 0.96–1.61, Ptrend=0.06) for isoleucine.

Conclusion: Our findings suggest a positive associations between higher intake of dietary BCAAs and risk of all-cause mortality in CRC patients. These findings need to be confirmed and potential mechanisms underlying this association need to be elucidated.

Introduction

Colorectal cancer (CRC) is the third leading cause of cancer related death in the United States, with approximately 51,020 cases dying from this cancer in 2019[1]. Environmental and lifestyle factors, including diet, have been associated with the risk of developing CRC[2, 3]. However, research that defines the benefits of dietary factors among CRC survivors is limited[4].

Branched chain amino acids (BCAAs), including leucine, isoleucine, and valine, are essential amino acids. BCAAs play important roles in insulin metabolism as well as protein synthesis[5]. Prospective studies have reported positive associations between higher consumption or plasma levels of BCAAs and risk of metabolic diseases, such as type 2 diabetes mellitus[6] and cardiovascular disease[7]. These studies have drawn attention to the potential adverse effects of BCAAs on metabolic diseases, which may share common risk factors with CRC[8]. Emerging evidence shows that BCAAs are essential nutrients for tumor growth and are used as energy sources by cancer[9]. Additionally, BCAAs appear to potentially drive cancer progression by various mechanisms[10, 11]. For example, the overexpression of the enzymes, especially branched chain amino acid transaminase 1 (BCAT1), catalyzing the first step in BCAA degradation, correlates with enhanced cancer growth, whereas suppression of BCAT1 limits proliferation[10, 12, 13]. In light of these

evidence, we hypothesized that higher post-diagnostic BCAA intake was associated with higher mortality among patients with CRC.

To our knowledge, no study has yet examined the association between BCAA intake and survival of CRC patients. We used data from two large prospective cohorts in the United States, the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS), to evaluate the associations between intake of BCAAs and mortality among patients with established CRC.

Methods

Study population

The Nurses' Health Study (NHS) enrolled 121,700 registered female nurses who were aged 30 to 55 years in 1976. The Health Professionals Follow-up Study (HPFS) enrolled 51,529 male health professionals who were aged 40 to 75 years in 1986. Details about these two cohorts have been reported previously[14–17]. Questionnaires were administered at baseline and updated information were collected biennially on lifestyle practices and medical history. This study was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health, and those of participating registries as required.

In this analysis, the study population was those who were diagnosed with a first primary incident CRC in these two cohort studies. These participants reported a diagnosis of CRC in the biennial follow-up questionnaires. Medical records and pathologic reports were obtained with permission and were reviewed by physicians who confirmed a diagnosis of CRC (International Classification of Diseases-9 codes of 153 and 154). Data on age at diagnosis, year of diagnosis, stage, grade, and subsite were also extracted. The main outcomes of this study were CRC-specific death and overall death. Most death were identified through review of the National Death Index, and family members or the postal system in response to the follow-up questionnaires. Over 98% of deaths in each cohort have been identified[18, 19].

We used 1980 for the NHS and 1986 for the HPFS as baseline, when we first collected detailed data on dietary intake. By the end of June 1, 2012 for the NHS, and January 31, 2012 for the HPFS, 3,936 cases of CRC were identified (2510 in the NHS, 1426 in the HPFS). We applied the following exclusion criteria: diagnosis of stage IV CRC (398 in the NHS, 208 in the HPFS), death in baseline or earlier (26 in the NHS, 0 in the HPFS), cancer diagnosis before baseline or after cutoff (188 in the NHS, 5 in the HPFS), diagnosis after death (42 in the NHS, 31 in the HPFS), missing data on post-diagnostic and pre-diagnostic BCAA intake (406 in the NHS, 245 in the HPFS), no food frequency questionnaires (FFQs) (0 in the NHS, 1 in the HPFS), post-diagnostic dietary assessment after more than four years of diagnosis (424 in the NHS, 288 in the HPFS). After these exclusions, 1,674 participants (1026 in the NHS, 648 in the HPFS) remained in the final analysis.

Assessment of dietary intake

Dietary intake was collected and updated using validated FFQs for almost every 4 years. We asked participants how often they consumed a standard portion size of each food on

average during the previous year with nine categories, ranging from “never or less than once per month” to “six or more times per day”. The average daily intake for each nutrient was calculated by multiplying the reported frequency of consumption of each food by its nutrient content and summing across from all foods. All nutrient intakes were adjusted for total energy intake using the residual method[20]. Detailed description of BCAA intake assessment has been reported previously[6, 21]. The total BCAAs were defined as the sum of energy-adjusted dietary valine, leucine and isoleucine. AHEI-2010 score was developed based on 11 dietary components that were shown to be associated with lower risk of chronic disease[22]. Emphasizes a higher consumption of whole grains, nuts and legumes, vegetables, fruits, polyunsaturated fatty acids, long-chain omega-3 fatty acids and a lower consumption of red and processed meat, sugar-sweetened beverages, trans fat and moderate alcohol, as captured by the FFQ. Each of the components was scored from 0 to 10 points based on predefined criteria. A higher total score was considered to represent a healthier diet. Data on glycemic index (GI), glycemic load (GL)[23–25] as well as the insulin index (II) and insulin load (IL)[26] were also available in these cohorts.

Assessment of other covariates

Updated information on age, body weight, smoking status, physical activity and regular use of aspirin was collected in each biennial questionnaire. Height was ascertained on the 1976 enrolment questionnaire in NHS, and the 1986 enrolment questionnaire in HPFS. Physical activity was calculated by summing the products of time spent on a variety of activities with the average metabolic equivalent for that activity. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

Statistical Analysis

Dietary intake reported on the first FFQ at least 6 months but no more than 4 years after diagnosis was used for post-diagnostic intake to avoid assessment during the period of active treatment[27]. Pre-diagnostic intake assessment was based on the last FFQ reported before CRC diagnosis. Person-years of follow-up were calculated from the return date of the FFQ that was used for post-diagnostic assessment to death, or the end of the study period (June 1, 2012 for the NHS, January 31, 2012 for the HPFS), whichever came first. In the CRC-specific mortality analysis, death from CRC was the primary end point, and deaths from other causes were censored. In the cardiovascular disease specific mortality analysis, death from cardiovascular disease was the primary end point, and deaths from other causes were censored. In the overall mortality analysis, death from any cause was the end point.

Cox proportional hazards regression models were used to calculate hazard ratios (HRs) of death with time since diagnosis as the time scale, adjusted for tumor stage, differentiation, anatomic subsites, pre-diagnostic BCAA intake, post-diagnostic BMI, smoking, physical activity, regular use of aspirin, intake of alcohol, and AHEI-2010 scores without alcohol (categorizations of these variables see the footnote of Table 2). In sensitivity analyses, we additionally adjusted for the intake of total calcium, vitamin D, folate, omega-3 fatty acids, fiber, GI, GL, II, and IL. None of them changed the results much. So, we decided not to include these factors in the final multivariable models. We tested proportional hazards assumption by including the interaction term between BCAA intake and time into the

model, and did not observe statistical evidence for violation of the proportional hazard assumption.

We categorized BCAA intake into quartile categories based on the distribution of each cohort. Considering that there was no statistically significant heterogeneity between sex (P-heterogeneity>0.05), we combined the data from the two cohorts into a single dataset for all analyses and controlled for cohort. The trend tests were conducted using the median of each category of BCAA intake as a continuous variable, and *P* value for trend was calculated using a Wald test. Consistent with our previous study[21], we presented the HR of mortality per 1-standarad category of BCAA intake. We also conduct a priori stratified analyses by lifestyle and clinicopathological factors (study, age, smoking, alcohol consumption, BMI, physical activity, regular aspirin use, pre-existing type 2 diabetes, cancer subsite and cancer stage). Test of interaction was conducted using the likelihood ratio test by comparing the model with product terms between stratified covariate and BCAA intake to that without these terms. As red meat, processed meat, turkey and chicken, milk are main sources of BCAAs in this study, for distinguishing between associations of intakes of BCAAs, vegetable and animal protein on CRC survival, we calculated the Spearman rank correlation coefficients between dietary BCAAs and dietary intakes of animal protein and vegetable protein. And we also examined the associations with cancer mortality in relation to post-diagnostic intakes of red meat, processed meat, turkey and chicken, milk as well as energy-adjusted intakes of total protein, animal protein, vegetable protein, all of which were categorized into quartiles.

We used SAS 9.4 for all analyses (SAS Institute, Cary, NC). All statistical tests were two-sided.

Results

During a median follow-up of 10.6 years, we documented a total of 1,674 patients with CRC throughout follow-up and completed the FFQ after diagnosis. Among them, 991 deaths were identified, including 206 CRC-specific deaths and 143 cardiovascular disease specific deaths.

Participants with higher total BCAA intake were slightly younger, have higher BMI and AHEI-2010 score, lower dietary glycemic load and index, higher proportion of type 2 diabetes and more likely to use aspirin regularly, and consume folate, vitamin D, calcium, red meat, turkey and chicken, milk, total and animal protein. (Table 1). The characteristics of patients with higher BCAA intake in these two studies were consistent with those in pooled study (Supplementary table 1).

Higher post-diagnostic intake of BCAAs appeared to be associated with higher risk of all-cause mortality (top vs. bottom quartile, 1.30, 95% CI: 1.01–1.69; *P* for trend=0.04), but not associated with CRC-specific mortality (*P* for trend=0.46; Table 2). Positive associations with all-cause mortality and cardiovascular disease specific mortality appeared to be primarily observed among men (Supplementary Table 2) but not among women

(Supplementary Table 3). But no statistically significant heterogeneity between sex (data not shown).

In an exploratory analysis, we examined the associations of post-diagnostic BCAA intake with mortality across strata of some a priori potential predictors of cancer mortality (Table 3). No statistically significant interactions between these factors and BCAA intake were found. We also performed a sensitivity analysis by excluding 210 CRC patients with unspecified stage. The results were essentially unchanged (data not shown).

The main food sources of BCAAs were meat (chicken, beef, and pork; ~37%), milk (~12%) and fish (~8%) in this study. The spearman rank correlation coefficient of overall BCAA with animal protein is 0.92 (P<0.001), and that with vegetable protein is 0.24 (P<0.001). Participants with the higher intake of animal protein had 64% increased risk of CRC-specific mortality and 47% increased risk of all-cause mortality (P for trend=0.03 and 0.001, respectively; Supplementary Table 4). By contrast, vegetable protein intake was associated with lower risk of CRC-specific and all-cause mortality (P for trend=0.03 and 0.009, respectively; Supplementary Table 4).

Discussion

In this study using data from two prospective cohorts of US health professionals, we found a suggestive positive association between BCAA intake and risk of all-cause mortality among CRC patients. Our findings provide initial evidence for the potential negative influence of dietary BCAA consumption on CRC patients.

BCAAs are essential amino acids and diet is their only source. To date, the studies on BCAAs and CRC are limited. We are aware one study on BCAA intake and CRC risk. This study was conducted in the same NHS/HPFS cohort studies and reported null associations[21]. Another cross-sectional study in Japan reported an inverse association of total plasma BCAA levels with risk of colorectal adenoma in men, but not in women[28]. Furthermore, only one study in Germany reported a non-statistically significant positive association between concentrations of urine valine and isoleucine and risk of death in stage I-III CRC patients based on 31 death and 24 months of follow-up[29].

In contrast to limited epidemiologic research on BCAA and CRC, there are biologically plausible mechanisms for the adverse effects of high intakes of BCAAs on CRC development and prognosis. The progression of CRC is related to the essential change of amino acid metabolism due to the needs of tumor and its interaction with host[30]. The proliferation and growth of tumor cells need to obtain essential nutrients from the tumor microenvironment. Even in the condition of the poor supply of nutrient and oxygen, tumor cells can also use them to maintain survival[31, 32]. BCAAs, as essential nutrients for cancer growth, are utilized by tumor in various biosynthetic pathways and as an energy source of tumor cells[32]. In particular, tumor cells distant from the vasculature have diminished accessibility to nutrients and oxygen and may engage in alternative forms of metabolism including oxidation of BCAAs to support cell viability[32]. BCAA metabolism and expression of BCAAs associated with metabolic enzymes are closely related to oncogenic mutations

and cancer tissue-of-origin[5]. The BCAT1, one BCAAs metabolic enzyme which are overexpressed in many cancers, was reported to be correlates with enhanced cancer growth, whereas suppression of BCAT1 limits proliferation[10, 12, 13]. And Inhibition of BCAT1 activity was considered to be useful therapeutic strategy in the treatment of several cancers[10, 13]. In addition, BCAT1 also plays an important role in cancer diagnosis as an prognostic marker of CRC[33, 34], glioblastoma[35], chronic myelogenous leukemia[36], ovarian cancer[37], hepatocellular carcinoma[38], and breast cancer[39].

Our results lend support to potentially adverse, rather than beneficial effects of high consumption of BCAAs on CRC survival. Although BCAA intake was not associated with CRC-specific mortality in our study, we noted a potential adverse effect of BCAA intake for overall mortality and CVD specific mortality, particularly in men. This might be partly due to the higher proportion of pre-existing type 2 diabetes in men than in women (17.6% vs. 9.1%) in our study, which may increase cardiovascular disease risk because of the common risk factors associated with the insulin-resistance syndrome (“common soil” hypothesis)[40]. In addition, 80% dietary BCAAs reach blood circulation and higher levels of BCAAs may increase CVD risk through the promotion of insulin resistance-mediated atherosclerosis[41]. Laboratory and epidemiologic evidence of the relationship between BCAAs and metabolic diseases began to accumulate these years[42]. At the molecular level, a consequence of increased BCAA levels is the activation of the mTOR/p70S6K pathway and phosphorylation of IRS-1 on multiple serine sites[43], which inhibits insulin signaling and insulin-stimulated glucose transport in muscle[44] and fat[45]. Findings from both animal and human intervention studies suggest that high circulating levels of BCAAs or associated genetic markers were associated with insulin resistance, impaired fasting glucose, elevated blood pressure, dyslipidemia, and indicators of coronary artery disease[41, 46–49]. Some prospective studies also have reported that higher diet and plasma BCAA metabolite levels were associated with an elevated risk of T2DM[50, 51] and CVD[7, 52], which may share etiological pathways with CRC[8]. Considering circulating levels of BCAAs are not only determined by BCAAs intake and the complex relationship between plasma BCAA and insulin metabolism, further studies are needed to elucidate the relation between plasma BCAA and CRC survival.

It worth noting that the BCAA-disease associations might also depend food source. For example, two previous studies[6, 53], in which the major food contributors to BCAAs were different, reported different results on the relationship between BCAA intake and type 2 diabetes. The major contributors to BCAA intake in the Japanese diet were cereals, potatoes, and starches (23–25%), fish and shellfish (21–23%), and meats (14–15%). But the major food contributors were meat (chicken, beef, and pork; ~37%), milk (~12%) and fish (~8%) in NHS and HPFS cohort. In our study, dietary BCAAs are highly correlated with intake of total protein and animal protein intake, but not much correlated with plant protein. We observed that patients with higher intake of animal protein demonstrated a substantial higher risk of all-cause mortality and a moderate higher risk of CRC-specific mortality than those with the lowest intake, which supported previous studies[54, 55]. As the major different make up of animal and plant protein is that animal protein is higher in essential amino acids, including BCAAs. BCAAs may partly explain the effect of animal protein on all-cause mortality among CRC patients.

Our study has some limitations. First, as an observational study, residual confounding cannot be completely excluded, although our detailed data resources enable us to adjust for a wide range of potential confounders. Second, in our participants, meat, milk and fish are main contributors of total BCAA intake. Considering the high correlations between the major food sources of BCAAs and CRC survival, we cannot completely exclude that the observed associations may be due to the intake of other components in BCAA-rich foods, although the association of BCAA and all-cause mortality of CRC remained after adjusting for these BCAA-rich foods. Third, only a fraction of whites, US health professionals with post-diagnosis data were included in our study. Therefore, both the statistical power and generalizability of our findings were limited. Lastly, detailed data on cancer treatment and recurrence are not collected in the cohort. However, more than 60% of patients had stage I or II disease in the analysis, in which surgery alone would generally be the standard of care. In addition, adjuvant therapy was largely standardized and related to disease stage. We have adjusted for stage in this study.

In conclusion, we observed suggestive positive association between higher dietary intakes of BCAAs after diagnosis and the risk of all-cause mortality among CRC patients. More studies are warranted to confirm these findings and elucidate the potential mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

The HPFS and NHS were supported by the NCI at the NIH (grant numbers UM1 CA186107, P01 CA087969, P01 CA055075, UM1 CA167552, and U01 CA167552). This work was supported by NIH grants (R01 CA248857 to S.O., R35 CA197735 to S.O., R01 CA151993 to S.O., R01 CA137178 to A.T.C., K24 DK098311 to A.T.C., K07 CA188126 to X.Z., and R21 CA238651 to X.Z.). A.T.C. is a Stuart and Suzanne Steele MGH Research Scholar. X.Z. is also supported by the American Cancer Society Research Scholar Grant (RSG NEC-130476), Dana-Farber Harvard Cancer Center (DF/HCC) GI SPORE Developmental Research Project Award (P50CA127003), and DF/HCC Cancer Center Support Grant (CCSG, 5P30CA006516-55), Harvard T.H. Chan School of Public Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of NIH. The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript. We would like to thank the participants and staff of the Nurses' Health Study and the Health Professionals Follow-up Study for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data.

Abbreviations:

AHEI	alternative healthy eating index
BCAAs	branched chain amino acids
BCAT1	branched chain amino acid transaminase 1
BMI	body mass index
CI	confidence interval
CRC	colorectal cancer

FFQ	food frequency questionnaire
GI	glycemic index
GL	glycemic load
HPFS	Health Professionals Follow-up Study
HR	hazard ratio
II	insulin index
IL	insulin load
IQR	inter-quartile range
NHS	the Nurses' Health Study

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Table 1

Characteristics of colorectal cancer patients according to postdiagnosis total branched-chain amino acids (BCAA) intake

	Quartile categories of post-diagnosis total BCAA intake			
	Quartile 1 (n=418)	**Quartile 2 (n=419)**	**Quartile 3 (n=418)**	**Quartile 4 (n=419)**
Age, years *	70.0 (8.7)	68.5 (9.6)	67.2 (9.0)	66.8 (9.3)
White, %	97.3	97.8	97.8	98.0
Body mass index, kg/m^2	25.4 (4.1)	26.3 (4.8)	26.4 (5.0)	27.0 (5.0)
Activity, MET-hrs/week	19.0 (24.7)	20.7 (24.0)	21.4 (25.4)	17.5 (21.8)
Pack years, smoking	18.5 (23.6)	16.4 (22.3)	16.5 (22.3)	15.2 (21.3)
Current smoking, %	13.4	9.8	8.2	7.8
Multivitamin use, %	55.3	59.1	58.6	58.6
Regular aspirin use (2 or more tablets/wk), %	37.5	35.5	35.4	33.9
Family history of colorectal cancer, %	19.2	21.3	22.2	21.8
Alcohol, g/day	12.3 (17.1)	7.1 (11.6)	7.3 (11.2)	4.7 (7.9)
Pre-existing type 2 diabetes, %	4.8	9.2	13.8	18.7
AHEI-2010 score	49.0 (12.1)	50.7 (12.1)	51.6 (11.6)	53.3 (12.6)
Dietary glycemic load	128 (28.6)	124 (24.7)	118 (23.6)	112 (21.9)
Dietary glycemic index	54.0 (3.7)	53.6 (3.3)	52.9 (3.3)	51.9 (3.5)
Dietary insulin load	754 (130)	757 (118)	747 (122)	737 (108)
Dietary insulin index	43.1 (7.0)	43.3 (5.4)	42.4 (5.2)	41.9 (4.9)
Dietary intake				
Post-diagnosis total BCAA intake, g/d	9.9 (1.7)	12.2 (1.5)	13.8 (1.6)	16.6 (2.5)
Post-diagnosis valine intake, g/d	2.9 (0.5)	3.6 (0.4)	4.1 (0.5)	4.9 (0.7)
Post-diagnosis isoleucine intake, g/d	2.6 (0.4)	3.2 (0.4)	3.6 (0.4)	4.4 (0.7)
Post-diagnosis leucine intake, g/d	4.4 (0.8)	5.4 (0.7)	6.1 (0.7)	7.3 (1.1)
Pre-diagnosis total BCAA intake, g/d	11.7 (2.7)	13.0 (2.5)	13.7 (2.6)	15.2 (2.8)
Total carbohydrate, g/d	237 (46.9)	231 (41.6)	224 (40.8)	216 (38.3)
Total energy, kcal/d	1740 (606)	1824 (589)	1876 (604)	1803 (597)
Total folate, ug/d	642 (356)	664 (334)	670 (328)	703 (353)
Total vitamin D, IU/d	454 (395)	483 (371)	562 (429)	622 (401)
Total fiber, g/d	20.5 (7.3)	21.4 (6.6)	21.3 (6.2)	22.1 (7.2)

	Quartile categories of post-diagnosis total BCAA intake			
	Quartile 1 (n=418)	**Quartile 2 (n=419)**	**Quartile 3 (n=418)**	**Quartile 4 (n=419)**
Total calcium, mg/d	998 (531)	1129 (534)	1182 (549)	1280 (551)
Processed meat, servings/w	2.0 (2.6)	2.0 (2.3)	2.2 (2.4)	2.1 (3.5)
Red meat, servings/w	2.4 (1.9)	3.3 (2.4)	3.8 (2.7)	3.9 (2.9)
Turkey and chicken, servings/w	1.5 (1.3)	2.1 (1.8)	2.7 (1.9)	3.9 (2.9)
Milk, servings/w	20.3 (24.8)	35.7 (34.6)	45.5 (40.7)	60.4 (51.8)
Total protein, g/d	58.5 (9.9)	70.6 (8.9)	78.8 (9.3)	93.4 (14.2)
Animal protein, g/d	34.8 (9.2)	45.6 (7.8)	54.0 (8.9)	68.9 (13.5)
Vegetable protein, g/d	23.8 (7.0)	25.0 (6.6)	24.8 (6.9)	24.5 (6.5)
Tumor subsite, %				
Proximal colon	39.0	41.6	40.9	41.8
Distal colon	30.4	34.0	31.1	31.1
Rectum	25.4	17.3	22.5	22.8
Unspecified	5.2	7.2	5.5	4.3
Tumor grade, %				
Grade 1 well differentiated	14.9	13.0	18.1	14.4
Grade 2 moderately differentiated	58.3	60.4	54.3	54.7
Grade 3 poorly differentiated	11.9	11.1	13.1	15.1
Unspecified	14.9	15.6	14.6	15.8
Tumor Stage, %				
Stage I	30.3	35.2	37.5	33.2
Stage II	32.6	27.6	27.9	29.9
Stage III	23.7	23.1	21.9	25.7
Unspecified	13.4	14.1	12.7	11.2

Values are means(SD) or percentages and are standardized to the age distribution of the study population.

*Value is not age adjusted.

Int J Cancer. Author manuscript; available in PMC 2022 June 19.

Int J Cancer. Author manuscript; available in PMC 2022 June 19.

Table 2.

Postdiagnostic BCAA intake and mortality among colorectal cancer patients [a]

	Quartile category 1	Quartile category 2	Quartile category 3	Quartile category 4	Per SD g/day	*P*-trend
Colorectal cancer specific mortality (n=206)						
Total BCAA, median[b], g/day						
No. of events	52	42	48	64		
Model 1 [c] HR (95% CI)	1 (Referent)	0.79 (0.52–1.20)	0.92 (0.62–1.37)	1.10 (0.75–1.61)	1.05 (0.91–1.21)	0.52
Model 2 [d] HR (95% CI)	1 (Referent)	0.82 (0.53–1.26)	0.94 (0.61–1.46)	1.18 (0.75–1.85)	1.09 (0.92–1.29)	0.46
Valine, median[b], g/day						
No. of events	54	38	51	63		
Model 1 [c] HR (95% CI)	1 (Referent)	0.69 (0.45–1.05)	0.96 (0.65–1.42)	1.10 (0.75–1.60)	1.05 (0.91–1.21)	0.42
Model 2 [d] HR (95% CI)	1 (Referent)	0.73 (0.47–1.13)	0.98 (0.64–1.50)	1.21 (0.77–1.91)	1.09 (0.92–1.29)	0.34
leucine, median[b], g/day						
No. of events	52	44	48	62		
Model 1 [c] HR (95% CI)	1 (Referent)	0.80 (0.53–1.21)	0.89 (0.60–1.34)	1.10 (0.75–1.62)	1.04 (0.91–1.20)	0.55
Model 2 [d] HR (95% CI)	1 (Referent)	0.83 (0.54–1.28)	0.93 (0.60–1.45)	1.17 (0.75–1.84)	1.08 (0.91–1.28)	0.47
Isoleucine, median[b], g/day						
No. of events	51	44	47	64		
Model 1 [c] HR (95% CI)	1 (Referent)	0.87 (0.58–1.32)	0.89 (0.59–1.34)	1.11 (0.76–1.64)	1.06 (0.92–1.22)	0.59
Model 2 [d] HR (95% CI)	1 (Referent)	0.84 (0.55–1.29)	0.86 (0.55–1.33)	1.13 (0.72–1.77)	1.09 (0.92–1.29)	0.63
All-cause mortality (n=647)						
Total BCAA						
No. of events	159	149	152	187		
Model 1 [c] HR (95% CI)	1 (Referent)	0.98 (0.78–1.23)	1.02 (0.81–1.28)	1.21 (0.97–1.51)	1.08 (1.00–1.17)	0.07
Model 2 [d] HR (95% CI)	1 (Referent)	1.00 (0.79–1.28)	1.05 (0.82–1.35)	1.30 (1.01–1.69)	1.12 (1.02–1.23)	0.04
Valine						
No. of events	161	146	153	187		
Model 1 [c] HR (95% CI)	1 (Referent)	0.89 (0.71–1.12)	1.04 (0.83–1.31)	1.21 (0.97–1.51)	1.08 (1.00–1.17)	0.04

	Quartile category 1	Quartile category 2	Quartile category 3	Quartile category 4	Per SD g/day	*P*-trend
Model 2 [d] HR (95% CI)	1 (Referent)	0.93 (0.73–1.18)	1.07 (0.84–1.38)	1.33 (1.03–1.73)	1.12 (1.02–1.24)	0.02
leucine						
No. of events	162	150	153	182		
Model 1 [c] HR (95% CI)	1 (Referent)	0.97 (0.77–1.22)	1.01 (0.80–1.27)	1.21 (0.97–1.51)	1.12 (1.01–1.24)	0.07
Model 2 [d] HR (95% CI)	1 (Referent)	1.00 (0.78–1.27)	1.07 (0.83–1.38)	1.28 (0.99–1.66)	1.13 (1.02–1.25)	0.05
Isoleucine						
No. of events	156	144	156	191		
Model 1 [c] HR (95% CI)	1 (Referent)	0.94 (0.75–1.19)	1.03 (0.81–1.29)	1.19 (0.96–1.49)	1.08 (1.00–1.17)	0.07
Model 2 [d] HR (95% CI)	1 (Referent)	0.96 (0.75–1.22)	1.03 (0.80–1.33)	1.25 (0.96–1.61)	1.12 (1.02–1.23)	0.06

Abbreviations: HR, hazard ratio; CI, confidence interval.

The medians for quartile category 1 were 9.83 g/day for total BCAA, 2.92 g/day for valine, 4.38 g/day for leucine and 2.53 g/day for isoleucine.

The medians for quartile category 2 were 11.61 g/day for total BCAA, 3.43 g/day for valine, 5.15 g/day for leucine and 3.01 g/day for isoleucine.

The medians for quartile category 3 were 13.11 g/day for total BCAA, 3.88 g/day for valine, 5.84 g/day for leucine and 3.42 g/day for isoleucine.

The medians for quartile category 4 were 16.14 g/day for total BCAA, 4.72 g/day for valine, 7.16 g/day for leucine and 4.25 g/day for isoleucine.

[a] Postdiagnostic intake at least 6 months but no more than 4 years after diagnosis to avoid potential impact of active treatment.

[b] Medians of each quartile categories of postdiagnostic BCAA intake for all colorectal cancer patients.

[c] Cox model stratified by age at diagnosis (<55, 55 to 59, 60 to 64, 65 to 69, 70 to 74, and ≥75 years), cancer stage (I, II, III, and unspecified) and study (NHS and HPFS).

[d] Model 1 + tumor characteristics (tumor stage, grade, and subsite), year of diagnoses (continuous), prediagnostic BCAA intake (in quartiles), postdiagnostic BMI (<23, 23 to 24.9, 25 to 27.4, 27.5 to 29.9, ≥30 kg/m^2), postdiagnostic physical activity (<3, 3 to 8.9, 9 to 11.9, 12 to 17.9, ≥18 METS-hours/week), postdiagnostic regular use of aspirin (yes or no), and postdiagnostic smoking (0, 1 to 9, 10 to 19, 20 to 40, ≥40 pack years), postdiagnostic alcohol consumption (<5, 5 to 14.9, ≥15 g/d), AHEI score without alcohol (in quartile).

Table 3.

Stratified analyses of postdiagnostic total BCAA intake with mortality among colorectal cancer patients[a]

	Colorectal cancer specific mortality			All-cause mortality		
	No. of events/patients	**Per SD HR (95% CI)**	***P* for interaction**	**No. of events/patients**	**Per SD HR (95% CI)**	***P* for interaction**
Age			0.03			0.36
<70 years	125/938	1.10 (1.00–1.21)		327/938	1.07 (1.00–1.13)	
≥ 70 years	81/736	0.95 (0.85–1.06)		320/736	1.03 (0.97–1.09)	
Smoking			0.34			0.25
Never	5/439	0.85 (0.58–1.26)		33/439	0.95 (0.82–1.11)	
Ever	201/1235	1.03 (0.96–1.11)		614/1235	1.04 (1.00–1.09)	
Alcohol consumption			0.63			0.40
< 10 g/day	157/1224	1.04 (0.95–1.13)		477/1224	1.05 (1.00–1.10)	
≥ 10 g/day	49/450	1.00 (0.89–1.14)		170/450	1.02 (0.95–1.09)	
Body mass index			0.88			0.73
< 25 kg/m^2	102/705	1.04 (0.95–1.15)		281/705	1.05 (0.99–1.11)	
≥ 25 kg/m^2	96/922	1.03 (0.94–1.14)		334/922	1.04 (0.99–1.09)	
Physical activity			0.87			0.12
< 9 METS-hours/week	103/748	1.03 (0.94–1.13)		332/748	1.02 (0.97–1.07)	
≥ 9 METS-hours/week	88/872	1.02 (0.92–1.13)		293/872	1.07 (1.01–1.13)	
Regular aspirin use			0.62			0.19
No	149/1087	1.04 (0.96–1.13)		425/1087	1.03 (0.98–1.08)	
Yes	57/587	1.01 (0.89–1.13)		222/587	1.08 (1.01–1.15)	
Pre-existing type 2 diabetes			0.05			0.71
No	139/1300	1.18 (0.96–1.45)		377/1300	1.10 (0.97–1.25)	
Yes	16/163	0.59 (0.30–1.15)		77/163	1.13 (0.88–1.45)	
Cancer subsite			0.28			0.82
Proximal colon	62/685	0.90 (0.77–1.05)		257/685	0.97 (0.90–1.05)	
Distal colon	79/528	1.21 (1.06–1.39)		213/528	1.16 (1.06–1.26)	
Rectum	58/370	1.05 (0.87–1.27)		139/370	1.08 (0.98–1.20)	
Cancer stage			0.99			0.25
I to II	80/1065	1.03 (0.93–1.14)		389/1065	1.05 (1.00–1.10)	

	Colorectal cancer specific mortality			All-cause mortality		
	No. of events/patients	**Per SD HR (95% CI)**	***P* for interaction**	**No. of events/patients**	**Per SD HR (95% CI)**	***P* for interaction**
III	92/399	1.03 (0.93–1.13)		162/399	1.00 (0.94–1.07)	

Abbreviation: HR, hazard ratio; CI, confidence interval.

[a]Postdiagnostic intake at least 6 months but no more than 4 years after diagnosis to avoid potential impact of active treatment.

Cox model stratified by age at diagnosis (<55, 55 to 59, 60 to 64, 65 to 69, 70 to 74, and ≥75 years) and cancer stage (I, II, III, and unspecified), with additional adjustment for age at diagnosis (continuous), tumor grade of differentiation (well differentiated, moderately differentiated, poorly differentiated, and unspecified), tumor subsite (proximal colon, distal colon, rectum and unspecified), prediagnostic total BCAA intake (in quartiles),postdiagnostic BMI (<23, 23 to 24.9, 25 to 27.4, 27.5 to 29.9, ≥30 kg/m2), postdiagnostic physical activity (<3, 3 to 8.9, 9 to 11.9, 12 to 17.9, ≥18 METS-hours/week), postdiagnostic regular use of aspirin (yes or no), postdiagnostic smoking (0, 1 to 9, 10 to 19, 20 to 40, ≥40 pack years), year of diagnosis (continuous), postdiagnostic alcohol consumption (<5, 5 to 14.9, ≥15 g/d) and AHEI scores without ahcohol (all in quartiles). Variables examined in this table were not adjusted for.