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Article

Branched-Chain Amino Acids and Risk of Breast Cancer

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Abstract

Background: Circulating branched-chain amino acid (BCAA) levels reflect metabolic health and dietary intake. However, associations with breast cancer are unclear. **Methods:** We evaluated circulating BCAA levels and breast cancer risk within the Nurses' Health Study (NHS) and NHSII (1997 cases and 1997 controls). A total of 592 NHS women donated 2 blood samples 10 years apart. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer risk in multivariable logistic regression models. We conducted an external validation in 1765 cases in the Women's Health Study (WHS). All statistical tests were 2-sided. **Results:** Among NHSII participants (predominantly premenopausal at blood collection), elevated circulating BCAA levels were associated with lower breast cancer risk (eg, isoleucine highest vs lowest quartile, multivariable OR = 0.86, 95% CI = 0.65 to 1.13, P_{trend} = .20), with statistically significant linear trends among fasting samples (eg, isoleucine OR = 0.74, 95% CI = 0.53 to 1.05, P_{trend} = .05). In contrast, among postmenopausal women, proximate measures (<10 years from blood draw) were associated with increased breast cancer risk (eg, isoleucine OR = 1.63, 95% CI = 1.12 to 2.39, P_{trend} = .01), with stronger associations among fasting samples (OR = 1.73, 95% CI = 1.15 to 2.61, P_{trend} = .01). Distant measures (10-20 years since blood draw) were not associated with risk. In the WHS, a positive association was observed for distant measures of leucine among postmenopausal women (OR = 1.23, 95% CI = 0.96 to 1.58, P_{trend} = .04). **Conclusions:** No statistically significant associations between BCAA levels and breast cancer risk were consistent across NHS and WHS or NHSII and WHS. Elevated circulating BCAA levels were associated with lower breast cancer risk among predominantly premenopausal NHSII women and higher risk among postmenopausal women in NHS but not in the WHS. Additional studies are needed to understand this complex relationship.

Breast cancer is the most common malignancy in women, with more than 250 000 diagnoses annually in the United States (1). Epidemiologic studies have identified modifiable risk factors, including increased body mass index (BMI) and low physical activity in postmenopausal women (2). However, BMI is inversely associated with premenopausal breast cancer (3). These findings indicate that poor metabolic health may be associated with breast cancer, although mechanisms and explanations for the variation by menopausal status remain unclear.

The branched-chain amino acids (BCAA) leucine, valine, and isoleucine are essential amino acids obtained from diet and are important metabolites involved in cell-signaling pathways and muscle protein synthesis (4). Elevated plasma BCAA concentrations are strongly positively correlated with BMI and insulin resistance and are a marker of dysfunctional metabolism (5). Whether elevated BCAAs are associated with breast cancer incidence, and whether this differs by menopausal status, remains unknown.

To date, few studies have evaluated BCAAs with breast cancer risk, with inconsistent results, and only 1 assessed menopausal status (6-10). We conducted a nested case-control study within the Nurses' Health Study (NHS) and NHSII to investigate the association between plasma BCAA levels and breast cancer risk. In secondary analyses, we conducted a validation analysis in the Women's Health Study (WHS).

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Methods

Study Population

In 1976, 121 701 female registered nurses aged 30-55 years enrolled in the NHS with the return of a mailed questionnaire (11). Participants have been followed biennially with questionnaires on reproductive history, lifestyle factors, diet, medication use, and new disease diagnoses. The NHSII began in 1989 with 116 429 female registered nurses aged 25-42 years, with biennial follow-up using similar questionnaires as NHS.

In 1989-1990, 32 826 NHS participants aged 43-69 years contributed blood samples, as previously described (12). In 2000-2002, 18 473 of these women aged 53-80 years donated a second sample using a similar protocol. In the NHSII, 29 611 women aged 32-54 years donated blood samples in 1996-1999. Follow-up in the blood subcohorts is high (NHS 97% in 2010; NHSII 96% in 2011). Detailed information on sample collection, covariates, and selection of cases and controls in NHS/NHSII and WHS is in the Supplementary Methods (available online).

The study protocol 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. The return of the self-administered questionnaire and blood sample was considered to imply consent.

Laboratory Assays

In the NHS and NHSII, BCAAs were assayed through a metabolomic profiling platform at the Broad Institute using a liquid chromatography mass spectrometry method designed to measure polar metabolites such as amino acids, amino acid derivatives, dipeptides, and other cationic metabolites (13-15). BCAAs were identified by matching measured chromatographic retention times and mass-to-charge ratios with authentic reference standards. The relative abundance of each BCAA was determined by integration of liquid chromatography tandem mass spectrometry peak areas, which are unitless numbers directly proportional to metabolite concentrations. A detailed description of the laboratory assays used to measure BCAAs, gene expression, estradiol, and C-peptide is included in the Supplementary Methods (available online).

Statistical Analysis

BCAA values were log transformed and standardized (mean = 0; SD = 1) within each cohort and blood collection separately (based on the distribution in all samples, including both cases and controls). To estimate the association between BCAAs as a group and risk of breast cancer, we calculated the sum of all 3 BCAAs (total BCAAs) and considered it an exposure in our analyses.

We estimated within-person stability over 10 years by calculating intra-class correlation (ICC) using mixed liner models among participants who donated 2 blood samples 10 years apart.

We used linear regression models of probit-transformed circulating BCAA levels to estimate beta coefficients for potential predictors, such as dietary BCAA intake, fasting status, BMI, age, and other lifestyle factors among NHS and NHSII (N = 9112) women.

Conditional logistic regression was used to evaluate the associations between BCAAs and breast cancer risk in each cohort separately. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) across quartiles (based on the control distribution) of BCAA levels and used quartile medians (based on the control distribution) to estimate linear trend *P* values. In a sensitivity analysis, we compared conditional with unconditional logistic regression adjusted for matching factors and obtained similar results (data not shown). Thus, we used unconditional logistic regression in analyses stratified by BMI and estrogen receptor (ER) status.

In multivariable models, we adjusted for established breast cancer risk factors: BMI at age 18 years, weight change from age 18 years to blood draw, age at menarche, parity and age at first birth, family history of breast cancer, history of benign breast disease, physical activity, alcohol consumption, exogenous hormone use, and breastfeeding history. In a separate analysis among NHS participants, we cross-classified participants based on the median BCAA levels among controls at the 2 blood collections. In the WHS, we used Cox proportional hazards regression models with follow-up from the date of random assignment to date of first invasive cancer diagnosis, death, or December 31, 2018. The Cox proportional hazard assumption was tested through the inclusion of a cross product term for BCAA and time (years from baseline blood draw); this assumption was met, with no indication for a violation. We assessed heterogeneity between NHS and WHS, and between NHSII and WHS using the DerSimonian-Laird estimator (16), and based on these findings, meta-analyzed individual cohort results using a fixed or random effects approach.

We used Correlation Adjusted Mean Rank analysis on tumor gene expression data to explore functional enrichment of biological pathways associated with BCAAs (Supplementary Methods, available online) (17).

We conducted sensitivity analyses restricting to fasting samples (>8 hours since last meal), restricting to premenopausal or postmenopausal women at blood collection, adjusting for BMI at the time of the blood collection instead of BMI at age 18 years and weight change between age 18 years and blood collection, and adjusting for plasma C-peptide (a marker of insulin production) and estradiol in individual models.

All statistical tests were 2-sided, and a *P* value of less than .05 was considered statistically significant. Analyses were conducted using R version 3.6.0, R version 3.1.4 and SAS Version 9.3 software (SAS Institute, Cary, NC).

Results

In total, 1997 matched case-control pairs were included (Table 1; Figure 1). NHSII women (1057 cases, 1057 controls) were predominantly premenopausal (80.2% cases, 79.7% controls) at blood collection (mean age = 45 years). NHS participants included 940 cases and their matched controls with a blood sample during the first collection (1989-1990, distant); of these, 592 cases and their matched controls had a second sample (2000-2002, proximate). NHS participants were predominantly postmenopausal (first collection = 61.9%; second collection = 98.1%), with a mean age of 55 years at distant and 66 years at proximate collections. Mean times between blood collection and diagnosis were: NHSII, 8 years; NHS distant measure, 15 years; and NHS proximate measure, 4 years.

WHS (N = 1765 cases) included 54.0% postmenopausal and 46.0% premenopausal women at blood collection. Mean time to diagnosis was similar to NHS and NHSII: 6 years for postmenopausal cases with proximate samples, 16 years for postmenopausal cases with distant samples, and 5 years for premenopausal

Table 1. Characteristics of breast cancer cases and matched controls in the NHSs

Participant characteristics	NHSII		NHS distant collection[a]		NHS proximate collection[b]	
	Cases (N = 1057)	Controls (N = 1057)	Cases (N = 940)	Controls (N = 940)	Cases (N = 592)	Controls (N = 592)
Mean age at blood collection[c] (SD), y	44.7 (4.5)	44.8 (4.4)	55.5 (6.9)	55.6 (6.9)	66.4 (6.9)	66.5 (6.8)
Mean time between blood collection and diagnosis (SD), y	8.0 (4.4)	—	14.6 (3.0)	—	4.0 (2.6)	—
Mean age at menarche (SD), y	12.4 (1.3)	12.5 (1.4)	12.5 (1.4)	12.6 (1.4)	12.5 (1.4)	12.6 (1.4)
Parity and age at first birth, %						
Nulliparous	21.1	18.4	9.6	8.0	8.6	5.9
1-2 children <25 y	14.7	15.9	13.5	14.1	13.0	15.9
1-2 children ≥25 y	39.2	34.9	20.1	20.6	20.4	19.3
3+ children <25 y	11.3	16.6	35.5	35.5	36.5	38.3
3+ children ≥25 y	13.8	14.2	21.3	21.7	21.5	20.6
Family history of breast cancer, %	17.4	10.8	14.6	10.7	22.5	14.2
Personal history of benign breast disease, %	22.1	15.6	45.9	37.8	62.5	54.7
BMI at age 18, kg/m^2	20.8 (2.9)	21.1 (3.1)	21.1 (2.7)	21.3 (3.0)	21.0 (2.6)	21.3 (3.0)
Mean weight change between age 18 y and blood collection (SD), kg	11.6 (12.0)	12.6 (13.2)	12.3 (10.9)	10.6 (11.2)	15.5 (12.7)	13.8 (12.7)
Mean physical activity (SD), MET-h/wk	18.0 (15.3)	18.1 (15.5)	15.4 (18.8)	15.9 (17.6)	17.7 (14.8)	19.0 (17.7)
Mean alcohol consumption (SD), g/d	3.8 (6.9)	3.3 (5.6)	6.9 (9.9)	5.9 (8.2)	6.7 (9.2)	5.8 (7.7)
Past/current exogenous hormone use[c,d], %	86.3	86.7	68.1	68.2	80.6	81.2
Ever breastfed, %	63.1	65.0	64.3	62.0	67.4	64.4
Menopausal status at blood collection[c], %						
Premenopausal	80.2	79.7	25.4	25.5	0.5	0.8
Postmenopausal	12.7	13.1	61.9	61.9	98.1	98.1
Unknown	7.1	7.3	12.7	12.6	1.4	1.0
Menopausal status at diagnosis[c], %						
Premenopausal	42.0	42.2	1.3	1.3	1.4	1.0
Postmenopausal	46.4	47.1	97.3	98.1	97.8	98.3
Unknown	11.6	10.7	1.4	0.6	0.8	0.7
Fasting (>8 h) at blood collection[c], %	68.7	74.7	66.6	72.7	87.0	92.4
Caucasian[c], %	97.2	98.4	98.3	98.8	98.6	99.5

[a]NHS first blood collection. —Data available for cases only; BMI = body mass index; MET = metabolic equivalent task; NHS = Nurses' Health Study.
[b]NHS second blood collection.
[c]Matching factor.
[d]Oral contraceptive or menopausal hormone therapy.

women at blood collection. Demographics were similar to NHS; exceptions included lower family history of breast cancer (Table 2).

BCAA levels were reasonably stable over 10 years among women with repeated measures (N = 592; ICC isoleucine = 0.45, leucine = 0.44, valine = 0.48). Dietary intake of BCAAs, BMI, and nonfasting blood collection were statistically significantly positively associated with BCAA levels, and Asian Americans had higher levels than Caucasians (Table 3). Alcohol consumption and diet quality were statistically significantly inversely associated with BCAA levels.

Among predominantly premenopausal women at blood collection (1057 cases), BCAAs were inversely associated with risk of breast cancer (simple model) (eg, isoleucine highest vs lowest quartile OR = 0.76, 95% CI = 0.59 to 0.99, P_{trend} = .02; Table 4), with statistically significant linear trends. These associations were attenuated and no longer statistically significant with adjustment for breast cancer risk factors (eg, isoleucine highest vs lowest quartile OR = 0.86, 95% CI = 0.65 to 1.13, P_{trend} = .20). Associations were similar for leucine (OR = 0.77, 95% CI = 0.58 to 1.01) and valine (OR = 0.82, 95% CI = 0.62 to 1.08). We observed stronger associations among fasting samples only (715 cases; top vs bottom quartile OR = isoleucine = 0.74, 95% CI = 0.53 to 1.05, P_{trend} = .05; leucine = 0.66, 95% CI = 0.47 to 0.94, P_{trend} = .04; valine = 0.74, 95% CI = 0.53 to 1.04, P_{trend} = .08). Associations with total BCAAs followed a similar pattern but were attenuated compared with individual BCAAs (OR = 0.79, 95% CI = 0.56 to 1.11, P_{trend} = .12). We observed similar associations when we further restricted to premenopausal women at blood collection (541 cases; OR: leucine = 0.61, 95% CI = 0.40 to 0.92, P_{trend} = .04; data not shown) and when we restricted to women premenopausal at diagnosis (255 cases; data not shown).

Among postmenopausal women, we observed positive associations between distant (10-20 years before diagnosis; 940 cases) measures of isoleucine and leucine and breast cancer risk in the simple model; however, these were attenuated and no longer statistically significant with multivariable adjustment (eg, isoleucine OR = 1.15, 95% CI = 0.87 to 1.52, P_{trend} = .35). BCAAs from proximate samples (592 cases) were positively associated with breast cancer risk and similar between the simple and multivariable models (eg, isoleucine multivariable OR = 1.63, 95% CI = 1.12 to 2.39, P_{trend} = .01). Weaker associations were observed for leucine (OR = 1.26, 95% CI = 0.87 to 1.83, P_{trend} = .17) and valine (OR = 1.34, 95% CI = 0.93 to 1.94, P_{trend} = .12). Associations were stronger, with statistically significant linear trends (except for leucine), when restricted to fasting samples (513 cases; isoleucine OR = 1.73, 95% CI = 1.15 to 2.61, P_{trend} = .01; leucine OR = 1.31, 95% CI = 0.87 to 1.98, P_{trend} = .12; valine OR = 1.64, 95% CI = 1.11 to 2.43, P_{trend} = .04). Association with total BCAAs followed the same pattern as individual

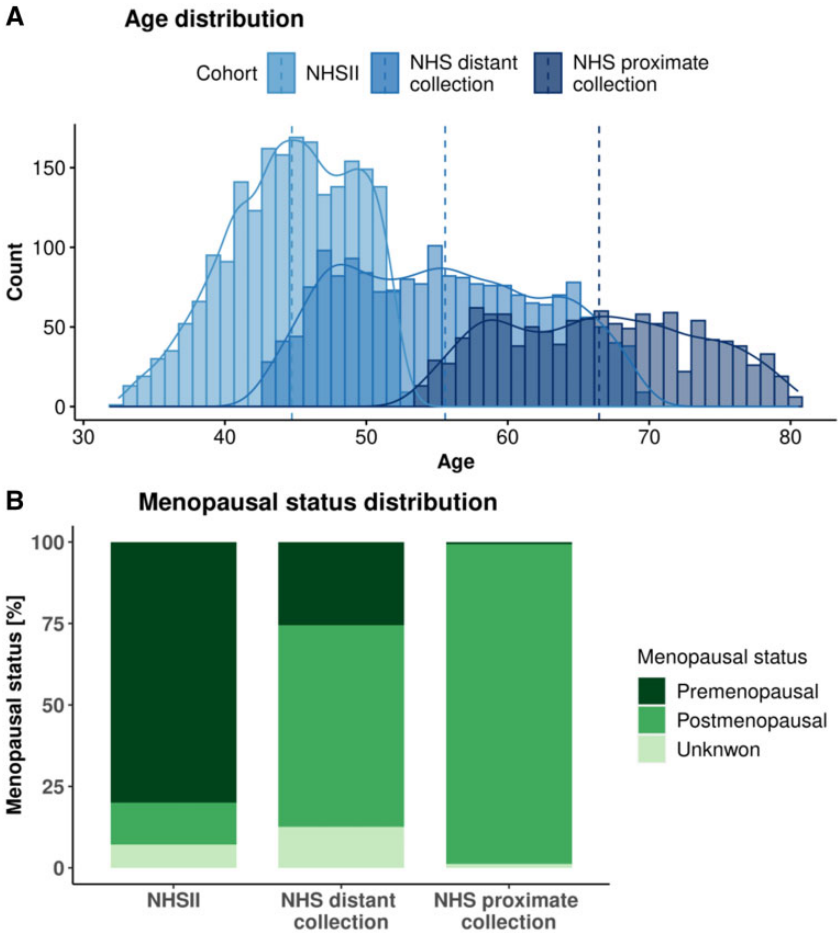

Figure 1. Age and menopausal status distribution at blood collection. Panel **A** shows the age distribution in the 3 datasets: Nurses' Health Study (NHS) distant collection in **blue**, NHS proximate collection in **dark blue**, and NHSII in **light blue**. Median age is marked by **vertical dashed lines**. Panel **B** shows distribution of menopausal status in the 3 datasets: premenopausal status is shown in **dark green**, postmenopausal status is shown in **green**, and unknown status is shown in **light green.**

BCAAs (eg, fasting samples, multivariable OR = 1.56, 95% CI = 1.04 to 2.34, P_{trend} = .06). A statistically significant interaction with menopausal status at blood collection ($P < .004$) was observed when we pooled NHSII and NHS women with proximate measures in the multivariable model.

Individual and total BCAAs were not associated with breast cancer risk among WHS premenopausal at blood collection (763 cases) or postmenopausal women with distant (515 cases) or proximate (487 cases) blood collections. For example, among postmenopausal women with proximate measures, the multivariable odds ratio for isoleucine was 0.97 (95% CI = 0.75 to 1.26, P_{trend} = .85) (Table 5). A suggestive positive association was observed for leucine and risk among postmenopausal women with distant sample collection (multivariable OR = 1.23, 95% CI = 0.96 to 1.58, P_{trend} = .04). Results were similar when restricted to fasting samples (70.1%-73.8%). There were too few women premenopausal at diagnosis to examine these associations in WHS (n = 36). We did not observe statistically significant heterogeneity between the cohorts except for isoleucine among postmenopausal women with proximate blood collection. We observed no statistically significant associations between individual and total BCAA levels and breast cancer risk when meta-analyzing NHS and WHS or NHSII and WHS results.

Results among NHS and NHSII women did not change in sensitivity analyses (data not shown), among pre- and postmenopausal women separately, in which we adjusted for BMI at blood collection instead of BMI at age 18 years and weight change between age 18 years and blood collection. Among women with previously measured plasma C-peptide (n = 579 NHSII, 244 NHS proximate, 407 NHS distant) and estradiol (n = 558 luteal and 532 follicular NHSII, 234 NHS proximate, 288 NHS distant), the associations with BCAAs were unchanged with additional adjustment for C-peptide or estradiol levels.

No associations were observed for individual and total BCAAs when we cross-classified BCAA levels 10 years apart. However, we observed a threefold increase in breast cancer risk for NHS participants with low isoleucine levels in the first sample but high isoleucine levels in the second sample (low/high) compared with participants who had low isoleucine levels in both timepoints (low/low; Table 6).

Interactions with BMI were not statistically significant (Supplementary Table 1, available online). There were no statistically significant associations between BCAA levels and breast cancer risk by estrogen receptor (ER) status (Supplementary Table 2, available online).

In breast tumor gene expression analyses, similar pathway activity was observed for each of the individual BCAAs. Circulating BCAA levels were associated with upregulation of mTOR signaling, interferon response, MYC targets, E2F targets, G2M targets, and DNA repair among NHSII women (73.2%

Table 2. Characteristics of the WHS[a]

Participant characteristics	WHS premenopausal at blood collection	WHS postmenopausal at blood collection
Total, No. (%)	12 413 (46.0)	14 587 (54.0)
Mean age at blood collection (SD), y	50.2 (3.5)	58.5 (7.1)
Mean age at menarche (SD), y	12.4 (1.4)	12.5 (1.5)
Parity and age at first birth, %:		
Nulliparous	22.4	22.8
1-2 children <30 y	27.2	18.0
3+ children <30 y	28.7	33.6
1-2 children ≥30 y	5.8	3.8
3+ children ≥30 y	1.6	2.2
Unknown	14.4	19.7
Family history of breast cancer, %	5.7	6.5
Personal history of benign breast disease, %	32.5	27.6
Mean BMI at blood draw (SD), kg/m^2	26.0 (5.2)	25.8 (4.8)
Mean physical activity (SD), MET-h/wk	14.8 (18.6)	14.8 (18.3)
Alcohol consumption, frequency of intake, %		
Rarely/never	42.6	45.0
1-3/mo	13.7	13.0
1-6/wk	34.3	30.8
1+/d	9.3	11.3
Past/current exogenous hormone use, %	29.7	69.9
Fasting (>8 h) at blood collection, %	70.1	73.8
Caucasian, %	94.4	94.6

[a]BMI = body mass index; MET = metabolic equivalent task; WHS = Women's Health Study.

premenopausal at blood collection) but with upregulation of estrogen response among NHS participants (all postmenopausal women; Supplementary Table 3, available online).

Discussion

In this prospective analysis, elevated circulating BCAA levels were associated with lower breast cancer risk among premenopausal women at blood collection but higher breast cancer risk among postmenopausal women at blood collection with proximate (<10 years before diagnosis) assessments, independent of adiposity measures. Associations were similar across individual and total BCAAs. Both inverse and positive associations were slightly stronger with statistically significant linear trends among fasting women (statistically significant predictor of circulating BCAA levels), which may better reflect underlying metabolic dysregulation compared with samples collected shortly after meals, when BCAA levels may be more likely to reflect recent dietary intake than long-term metabolic state (18). Statistically significant associations generally were not observed when assessing distant measures of BCAAs among postmenopausal women. We did not observe interactions with BMI or heterogeneity by ER status. Associations did not validate in WHS.

BCAAs are essential nutrients acquired from food or biosynthesized by the microbiome (19). Several studies found a weak positive correlation between dietary BCAA intake and circulating BCAAs ($r = 0.11$-0.14) (20-23). Similarly, we observed that dietary intake was a statistically significant but fairly weak predictor of circulating levels. Diets high in animal protein, especially red meat, are associated with increased BCAA levels compared with those with predominately plant sources of protein (23-26), and higher intake of red meat is associated with increased risk of pre- and postmenopausal breast cancer (27,28). However, BCAAs were not identified as markers of dietary patterns (29) or dietary intake, suggesting the role of BCAAs in breast cancer etiology may reflect mechanisms beyond their dietary intake.

The role of obesity in postmenopausal breast cancer is well established (30,31), and diabetes and insulin resistance have been associated with breast cancer risk (32). Elevated levels of circulating BCAAs are associated with obesity and insulin resistance in cross-sectional studies (5,33,34) and with incident Type II diabetes (23,35). Adiposity and insulin resistance have a causal effect on serum BCAA levels (36-38), and circulating BCAAs play a causal role in the development of Type II diabetes (20). Together, these findings emphasize that elevated BCAA levels are indicative of dysregulated metabolism. Further, dietary BCAAs in experimental and human studies cause impaired insulin activity through upregulation of the mTOR pathway (39,40), which has been implicated in breast carcinogenesis (41).

Our observed opposite associations between plasma BCAAs and breast cancer risk by menopausal status parallel the associations between BMI and breast cancer, though associations with BCAAs persisted even with adjustment for different adiposity measures and was independent of plasma estradiol levels. We also observed differential associations by menopausal status between circulating BCAAs and breast tumor gene expression, with mTOR and interferon signaling and DNA repair among premenopausal women at blood collection, but estrogen response among postmenopausal women. These findings suggest that BCAAs play a role in breast carcinogenesis beyond their role in obesity.

Few epidemiologic studies have investigated the association of circulating BCAA levels with breast cancer risk, and only one assessed this relationship by menopausal status. No statistically significant association was observed between BCAAs and breast cancer risk (7) in a case-cohort analysis in the European Prospective Investigation into Cancer and Nutrition (EPIC) Heidelberg cohort (114 pre- and 248 postmenopausal cases) or in a larger study (6) in EPIC (434 pre-, 318 peri-, and 872 postmenopausal cases). Higher levels of valine were associated with increased breast cancer risk among pre- and postmenopausal women within the "SUpplementation en VItamines et

Table 3. Effect estimates for predictors of probit transformed circulating BCAA levels from multivariable linear regression among 9112 NHS and NHSII women

Predictors	No.	Isoleucine β (95% CI)	Leucine β (95% CI)	Valine β (95% CI)
Dietary intake[a], mg/d				
Q1	1999-2010	Ref	Ref	Ref
Q2	2016-2026	0.10 (0.03 to 0.16)	0.09 (0.03 to 0.16)	0.11 (0.05 to 0.17)
Q3	2014-2030	0.13 (0.06 to 0.20)	0.18 (0.11 to 0.25)	0.26 (0.20 to 0.33)
Q4	2011-2034	0.16 (0.08 to 0.24)	0.21 (0.13 to 0.28)	0.31 (0.23 to 0.39)
Q5	2025-2034	0.21 (0.11 to 0.31)	0.28 (0.18 to 0.37)	0.42 (0.32 to 0.51)
P_{trend}		<.001	<.001	<.001
Fasting status				
Fasting (>8 h)	7836	Ref	Ref	Ref
Nonfasting	2771	0.20 (0.16 to 0.25)	0.11 (0.07 to 0.15)	0.10 (0.06 to 0.15)
Age at blood collection, y				
<40	574	Ref	Ref	Ref
40-50	3829	0.00 (−0.09 to 0.09)	−0.04 (−0.13 to 0.05)	0.04 (−0.05 to 0.13)
50-60	3541	0.00 (−0.11 to 0.11)	−0.03 (−0.14 to 0.08)	0.12 (0.01 to 0.23)
>60	2665	0.02 (−0.10 to 0.14)	−0.03 (−0.15 to 0.09)	0.12 (<0.01 to 0.23)
P_{trend}		.47	.95	.04
Race				
Caucasian	10 248	Ref	Ref	Ref
Black	264	−0.11 (−0.26 to 0.04)	−0.04 (−0.19 to 0.11)	−0.19 (−0.33 to −0.04)
Asian	68	0.28 (0.03 to 0.53)	0.26 (0.01 to 0.51)	0.34 (0.09 to 0.58)
Other	29	0.03 (−0.34 to 0.40)	0.06 (−0.31 to 0.43)	−0.01 (−0.37 to 0.35)
Smoking status				
Never	5602	Ref	Ref	Ref
Past	3722	−0.01 (−0.05 to 0.04)	0.01 (−0.03 to 0.06)	0.01 (−0.04 to 0.05)
Current	1263	0.01 (−0.06 to 0.07)	0.00 (−0.06 to 0.07)	−0.02 (−0.09 to 0.04)
BMI, kg/m^2				
<25	5601	Ref	Ref	Ref
25-30	3154	0.34 (0.3 to 0.38)	0.34 (0.3 to 0.39)	0.40 (0.36 to 0.45)
≥30	1822	0.70 (0.65 to 0.76)	0.68 (0.62 to 0.74)	0.82 (0.77 to 0.88)
P_{trend}		<.001	<.001	<.001
Physical activity, MET-h/wk				
<9	4734	Ref		
9-27	3718	−0.05 (−0.09 to 0.00)	−0.05 (−0.09 to 0.00)	−0.04 (−0.09 to 0.00)
≥27	1946	−0.01 (−0.06 to 0.05)	0.01 (−0.05 to 0.07)	0.01 (−0.04 to 0.06)
P_{trend}		.62	.88	.88
Alcohol consumption, g/d				
0	3531	Ref	Ref	Ref
0.88-10	4309	−0.08 (−0.13 to −0.04)	−0.07 (−0.11 to −0.02)	−0.04 (−0.09 to 0.00)
10-20	1099	−0.12 (−0.19 to −0.06)	−0.07 (−0.14 to −0.01)	−0.07 (−0.14 to −0.01)
≥20	632	−0.26 (−0.34 to −0.17)	−0.16 (−0.25 to −0.08)	−0.18 (−0.26 to −0.10)
P_{trend}		<.001	<.001	<.001
Alternative Healthy Eating Index[b]				
<37.9	1909	Ref	Ref	Ref
[37.9-43.5)	1906	−0.04 (−0.10 to 0.02)	−0.01 (−0.07 to 0.05)	−0.01(−0.05 to 0.07)
[43.5,49)	1910	−0.07 (−0.13 to −0.01)	−0.04 (−0.10 to 0.03)	−0.04 (−0.10 to 0.02)
[49,55.6)	1908	−0.10 (−0.16 to −0.03)	−0.06 (−0.13 to 0.00)	−0.04 (−0.10 to 0.02)
≥55.6	1909	−0.08 (−0.15 to −0.02)	−0.04 (−0.10 to 0.03)	−0.02 (−0.08 to 0.04)
P_{trend}		.001	.07	.20
Menopausal status and PMH use				
Premenopausal	4337	Ref	Ref	Ref
Postmenopausal PMH	2447	0.05 (−0.02 to 0.11)	0.06 (−0.01 to 0.12)	0.12 (0.06 to 0.18)
Postmenopausal no PMH	3189	0.10 (0.04 to 0.17)	0.15 (0.08 to 0.22)	0.17 (0.11 to 0.24)
Unknown	649	0.08 (−0.01 to 0.17)	0.06 (−0.04 to 0.15)	0.10 (0.00 to 0.19)

[a]Number and cutpoints vary by BCAA: isoleucine dietary intake quintile cutpoints [mg/d]: <2.86; [2.86,3.47); [3.47,4.06); [4.06,4.82); ≥4.82. Leucine dietary intake quintile cutpoints [mg/d]: <5.33; [5.33,6.49); [6.49,7.58); [7.58,9.05); ≥9.05. Valine dietary intake quintile cutpoints [mg/d]: <3.22; [3.22,3.93); [3.93,4.59); [4.59,5.47); ≥5.47. BCAA = branched-chain amino acid; CI = confidence interval; MET = metabolic equivalent task; NHS = Nurses' Health Study; NHSII = Nurses' Health Study II; PMH = postmenopausal hormone therapy; Q = quintile.

[b]Calculated without alcohol intake.

Table 4. OR of breast cancer according to quartiles of plasma BCAA among premenopausal and postmenopausal women

BCAA	Q1	Q2	Q3	Q4	P_{trend}
Premenopausal[a] women at blood collection in NHSII (N = 1057 cases/controls)					
Isoleucine					
All samples					
No. of cases/controls	300/265	282/264	239/264	236/264	
Simple[b] OR (95% CI)	Ref	0.93 (0.73 to 1.18)	0.79 (0.61 to 1.01)	0.76 (0.59 to 0.99)	.02
Multivariable[c] OR (95% CI)	Ref	0.99 (0.77 to 1.27)	0.87 (0.67 to 1.13)	0.86 (0.65 to 1.13)	.20
Fasting samples					
No. of cases/controls	216/179	201/179	149/178	149/179	
Multivariable[c] OR (95% CI)	Ref	0.97 (0.72 to 1.30)	0.77 (0.56 to 1.05)	0.74 (0.53 to 1.05)	.05
Leucine					
All samples					
No. of cases/controls	296/265	268/264	278/264	215/264	
Simple[b] OR (95% CI)	Ref	0.90 (0.70 to 1.14)	0.93 (0.72 to 1.19)	0.71 (0.55 to 0.92)	.02
Multivariable[c] OR (95% CI)	Ref	0.92 (0.72 to 1.18)	1.00 (0.77 to 1.30)	0.77 (0.58 to 1.01)	.11
Fasting samples					
No. of cases/controls	209/179	184/179	190/178	132/179	
Multivariable[c] OR (95% CI)	Ref	0.88 (0.66 to 1.18)	0.94 (0.68 to 1.29)	0.66 (0.47 to 0.94)	.04
Valine					
All samples					
No. of cases/controls	293/265	262/264	283/264	219/264	
Simple[b] OR (95% CI)	Ref	0.89 (0.69 to 1.13)	0.95 (0.75 to 1.20)	0.74 (0.58 to 0.95)	.04
Multivariable[c] OR (95% CI)	Ref	0.91 (0.71 to 1.18)	1.02 (0.80 to 1.31)	0.82 (0.62 to 1.08)	.28
Fasting samples					
No. of cases/controls	217/179	181/179	170/178	147/179	
Multivariable[c] OR (95% CI)	Ref	0.86 (0.63 to 1.16)	0.81 (0.60 to 1.10)	0.74 (0.53 to 1.04)	.08
Total BCAA					
All samples					
No. of cases/controls	278/265	293/264	257/264	229/264	
Simple[b] OR (95% CI)	Ref	1.05 (0.83 to 1.34)	0.92 (0.72 to 1.18)	0.81 (0.63 to 1.05)	.07
Multivariable[c] OR (95% CI)	Ref	1.10 (0.86 to 1.41)	0.99 (0.77 to 1.28)	0.91 (0.69 to 1.19)	.41
Fasting samples					
No. of cases/controls	206/179	198/179	166/178	145/179	
Multivariable[c] OR (95% CI)	Ref	1.02 (0.76 to 1.38)	0.85 (0.62 to 1.16)	0.79 (0.56 to 1.11)	.12
Postmenopausal[d] women in NHS, distant sample collection (10-20 y before diagnosis, N = 940 cases/controls)					
Isoleucine					
All samples					
No. of cases/controls	226/235	220/235	205/235	289/235	
Simple[b] OR (95% CI)	Ref	0.98 (0.75 to 1.26)	0.92 (0.70 to 1.19)	1.29 (1.00 to 1.67)	.05
Multivariable[c] OR (95% CI)	Ref	0.95 (0.73 to 1.24)	0.83 (0.63 to 1.09)	1.15 (0.87 to 1.52)	.35
Fasting samples					
No. of cases/controls	157/156	132/156	150/155	184/156	
Multivariable[c] OR (95% CI)	Ref	0.83 (0.60 to 1.15)	0.84 (0.60 to 1.18)	0.98 (0.69 to 1.39)	.97
Leucine					
All samples					
No. of cases/controls	220/235	217/235	215/235	288/235	
Simple[b] OR (95% CI)	Ref	0.98 (0.75 to 1.29)	0.98 (0.76 to 1.28)	1.32 (1.02 to 1.72)	.03
Multivariable[c] OR (95% CI)	Ref	0.95 (0.72 to 1.25)	0.90 (0.68 to 1.18)	1.19 (0.90 to 1.58)	.24
Fasting samples					
No. of cases/controls	147/156	145/156	141/155	190/156	
Multivariable[c] OR (95% CI)	Ref	0.95 (0.68 to 1.33)	0.83 (0.59 to 1.17)	1.08 (0.75 to 1.56)	.86
Valine					
All samples					
No. of cases/controls	215/235	236/235	233/235	256/235	
Simple[b] OR (95% CI)	Ref	1.10 (0.85 to 1.42)	1.08 (0.84 to 1.40)	1.20 (0.92 to 1.55)	.20
Multivariable[c] OR (95% CI)	Ref	1.03 (0.79 to 1.34)	0.99 (0.76 to 1.29)	1.05 (0.80 to 1.40)	.77
Fasting samples					
No. of cases/controls	146/156	161/156	137/155	179/156	
Multivariable[c] OR (95% CI)	Ref	1.01 (0.72 to 1.41)	0.82 (0.58 to 1.15)	1.03 (0.72 to 1.47)	.90

(continued)

Table 4. (continued)

BCAA	Q1	Q2	Q3	Q4	P_{trend}
Total BCAA					
All samples					
No. of cases/controls	217/235	225/235	217/235	281/235	
Simple[b] OR (95% CI)	Ref	1.04 (0.80 to 1.35)	1.00 (0.78 to 1.29)	1.32 (1.02 to 1.70)	.05
Multivariable[c] OR (95% CI)	Ref	0.99 (0.75 to 1.30)	0.91 (0.69 to 1.19)	1.17 (0.88 to 1.55)	.33
Fasting samples					
No. of cases/controls	142/156	148/156	150/155	183/156	
Multivariable[c] OR (95% CI)	Ref	1.00 (0.72 to 1.40)	0.91 (0.65 to 1.28)	1.06 (0.74 to 1.52)	.88
Postmenopausal[d] women in NHS, proximate sample collection (<10 y before diagnosis, N = 592 cases/controls)					
Isoleucine					
All samples					
No. of cases/controls	112/148	146/148	154/148	180/148	
Simple[b] OR (95% CI)	Ref	1.30 (0.94 to 1.81)	1.39 (1.00 to 1.95)	1.63 (1.17 to 2.29)	.01
Multivariable[c] OR (95% CI)	Ref	1.29 (0.91 to 1.83)	1.45 (1.01 to 2.09)	1.63 (1.12 to 2.39)	.01
Fasting samples					
No. of cases/controls	91/129	130/128	136/128	156/128	
Multivariable[c] OR (95% CI)	Ref	1.49 (1.02 to 2.17)	1.59 (1.06 to 2.37)	1.73 (1.15 to 2.61)	.01
Leucine					
All samples					
No. of cases/controls	123/148	144/148	164/148	161/148	
Simple[b] OR (95% CI)	Ref	1.17 (0.83 to 1.63)	1.33 (0.96 to 1.84)	1.32 (0.94 to 1.84)	.08
Multivariable[c] OR (95% CI)	Ref	1.20 (0.84 to 1.71)	1.43 (1.01 to 2.03)	1.26 (0.87 to 1.83)	.17
Fasting samples					
No. of cases/controls	103/129	123/128	147/128	140/128	
Multivariable[c] OR (95% CI)	Ref	1.29 (0.88 to 1.90)	1.58 (1.08 to 2.31)	1.31 (0.87 to 1.98)	.12
Valine					
All samples					
No. of cases/controls	119/148	146/148	158/148	169/148	
Simple[b] OR (95% CI)	Ref	1.21 (0.87 to 1.68)	1.31 (0.95 to 1.80)	1.42 (1.02 to 1.98)	.03
Multivariable[c] OR (95% CI)	Ref	1.23 (0.87 to 1.73)	1.33 (0.94 to 1.88)	1.34 (0.93 to 1.94)	.12
Fasting samples					
No. of cases/controls	99/129	134/128	111/128	169/128	
Multivariable[c] OR (95% CI)	Ref	1.45 (1.00 to 2.10)	1.13 (0.76 to 1.67)	1.64 (1.11 to 2.43)	.04
Total BCAA					
All samples					
No. of cases/controls	119/148	149/148	148/148	176/148	
Simple[b] OR (95% CI)	Ref	1.25 (0.90 to 1.74)	1.25 (0.90 to 1.74)	1.49 (1.07 to 2.08)	.02
Multivariable[c] OR (95% CI)	Ref	1.30 (0.92 to 1.85)	1.35 (0.94 to 1.93)	1.45 (1.00 to 2.09)	.06
Fasting samples					
No. of cases/controls	101/129	129/128	123/128	160/128	
Multivariable[c] OR (95% CI)	Ref	1.41 (0.96 to 2.08)	1.35 (0.92 to 1.98)	1.56 (1.04 to 2.34)	.06

[a]Predominantly premenopausal (see Table 1 and Figure 1 for details). BCAA = branched-chain amino acids; CI = confidence interval; NHS = Nurses' Health Study; NHSII = Nurses' Health Study II; OR = odds ratios; Q = quartile.
[b]Simple model: no adjustment factors were included.
[c]Multivariable model: BMI at age 18 years, weight change from age 18 years to time of blood draw, age at menarche, parity and age at first birth, family history of breast cancer, history of benign breast disease, physical activity, alcohol consumption, exogenous hormone use, and breastfeeding history.
[d]Predominantly postmenopausal women (see Table 1 and Figure 1 for details).

MinéralAntioXydants" (SU.VI.MAX) study (129 pre- and 82 postmenopausal cases) (8). Given the mix of menopausal status, it is difficult to compare these results with our findings. Consistent with our results, in an examination of BMI-correlated metabolites in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO), which included valine and allo-isoleucine (N = 621 postmenopausal cases), higher levels of allo-isoleucine, a byproduct of isoleucine transamination (42), were associated with increased postmenopausal breast cancer risk (9). Notably, 2 other metabolites involved in alternative isoleucine and leucine degradation, 2-methylbutyrylcarnitine and 3-methylglutarylcarnitine, were positively associated with risk (9). Sensitivity analyses adjusting for insulin resistance–related metabolites resulted in slight attenuation of the associations. Similarly, we observed no changes when adjusting for C-peptide, a measure of insulin production, suggesting that the role of BCAAs in postmenopausal breast cancer etiology may be independent of insulin resistance. In summary, results from PLCO, NHS, and NHSII suggest that isoleucine and leucine may play a role in postmenopausal breast cancer, although findings from WHS were not consistent. However, to what extent individual BCAAs contribute to breast cancer and how this relationship is modulated by menopausal status is not clear. Additional prospective studies are needed to confirm these relationships.

Table 5. ORs of breast cancer according to quartiles of plasma BCAA among premenopausal and postmenopausal women in WHS

BCAA	Q1	Q2	Q3	Q4	P_{trend}
Premenopausal women at blood collection in WHS (N = 763 cases)					
Isoleucine					
No. of cases/noncases	191/2873	188/2906	190/2891	194/2980	
Multivariable[a] OR (95% CI)	Ref	0.98 (0.80 to 1.20)	0.99 (0.81 to 1.21)	0.99 (0.80 to 1.20)	.93
Leucine					
No. of cases/noncases	183/3000	187/2903	213/2798	180/2949	
Multivariable[a] OR (95% CI)	Ref	1.06 (0.87 to 1.31)	1.22 (1.00 to 1.49)	1.00 (0.81 to 1.24)	.62
Valine					
No. of cases/noncases	206/3081	187/2849	179/2836	191/2884	
Multivariable[a] OR (95% CI)	Ref	0.98 (0.80 to 1.20)	0.95 (0.77 to 1.16)	0.97 (0.79 to 1.20)	.76
Total BCAA					
No. of cases/noncases	196/3058	181/2850	193/2780	193/2962	
Multivariable[a] OR (95% CI)	Ref	0.98 (0.80 to 1.21)	1.07 (0.88 to 1.32)	1.01 (0.82 to 1.25)	.76
Postmenopausal women in WHS, distant sample collection (10-20 y before diagnosis, N = 515 cases)					
Isoleucine					
No. of cases/noncases	125/3561	118/3538	144/3525	128/3448	
Multivariable[a] OR (95% CI)	Ref	0.94 (0.73 to 1.21)	1.16 (0.91 to 1.47)	1.11 (0.86 to 1.43)	.25
Leucine					
No. of cases/noncases	121/3446	105/3555	146/3593	143/3478	
Multivariable[a] OR (95% CI)	Ref	0.85 (0.65 to 1.10)	1.15 (0.90 to 1.47)	1.23 (0.96 to 1.58)	.04
Valine					
No. of cases/noncases	127/3336	127/3587	129/3606	132/3543	
Multivariable[a] OR (95% CI)	Ref	0.94 (0.73 to 1.21)	0.95 (0.74 to 1.22)	0.99 (0.76 to 1.29)	.95
Total BCAA					
No. of cases/noncases	127/3369	121/3598	135/3642	132/3463	
Multivariable[a] OR (95% CI)	Ref	0.88 (0.69 to 1.13)	0.98 (0.76 to 1.25)	1.05 (0.81 to 1.36)	.60
Postmenopausal women in WHS, proximate sample collection (<10 y before diagnosis, N = 487 cases)					
Isoleucine					
No. of cases/noncases	136/3550	116/3540	120/3549	115/3461	
Multivariable[a] OR (95% CI)	Ref	0.87 (0.68 to 1.12)	0.93 (0.73 to 1.19)	0.97 (0.75 to 1.26)	.85
Leucine					
No. of cases/noncases	126/3441	115/3545	123/3616	123/3498	
Multivariable[a] OR (95% CI)	Ref	0.91 (0.70 to 1.17)	0.97 (0.76 to 1.25)	1.05 (0.81 to 1.36)	.68
Valine					
No. of cases/noncases	119/3344	133/3581	128/3607	107/3568	
Multivariable[a] OR (95% CI)	Ref	1.09 (0.85 to 1.39)	1.04 (0.81 to 1.34)	0.96 (0.73 to 1.26)	.75
Total BCAA					
No. of cases/noncases	128/3368	119/3600	126/3651	114/3481	
Multivariable[a] OR (95% CI)	Ref	0.89 (0.69 to 1.15)	0.97 (0.75 to 1.24)	0.98 (0.75 to 1.27)	.98

[a]Multivariable model is adjusted for age, randomized treatment assignment, BMI, age at menarche, parity and age at first birth, family history of breast cancer, history of benign breast disease, physical activity, alcohol consumption, HRT, menopausal status, fasting status and race. BCAA = branched-chain amino acids; BMI = body mass index; CI = confidence interval; HRT = hormone replacement therapy; OR = odds ratios; Q = quartile; WHS = Women's Health Study.

Table 6. ORs of breast cancer according to 10-year change[a] in plasma BCAA in postmenopausal women in NHS

BCAA	Low/low	Low/high	High/low	High/high
Isoleucine				
No. of cases/controls	118/96	55/69	55/50	118/131
Multivariable[b] OR (95% CI)	1.00 (ref)	3.00 (1.45 to 6.20)	0.87 (0.41 to 1.83)	1.45 (0.77 to 2.71)
Leucine				
No. of cases/controls	116/104	57/62	57/50	116/130
Multivariable[b] OR (95% CI)	1.00 (ref)	1.49 (0.72 to 3.08)	0.70 (0.32 to 1.50)	1.22 (0.64 to 2.33)
Valine				
No. of cases/controls	114/104	59/60	59/65	114/117
Multivariable[b] OR (95% CI)	1.00 (ref)	1.15 (0.58 to 2.28)	1.54 (0.76 to 3.11)	0.90 (0.48 to 1.69)
Total BCAA				
No. of cases/controls	120/107	53/57	53/55	120/127
Multivariable[b] OR (95% CI)	1.00 (ref)	1.42 (0.69 to 2.93)	1.13 (0.55 to 2.33)	0.99 (0.53 to 1.85)

[a]Cross-classified by median in distant or proximate sample collections. BCAA = branched-chain amino acids; CI = confidence interval; NHS = Nurses' Health Study; OR = odds ratio.

[b]Multivariable model: body mass index at age 18 years, weight change from age 18 years to time of blood draw, age at menarche, parity and age at first birth, family history of breast cancer, history of benign breast disease, physical activity, alcohol consumption, exogenous hormone use, and breastfeeding history.

Our study has several strengths and limitations. We measured prediagnostic plasma BCAAs among a large number of pre- and postmenopausal women. We had detailed information on breast cancer risk factors, including measures of adiposity. We had limited statistical power in analyses of ER− tumors. Although we had some participants with 2 blood samples, our main findings are based on 1-point-in-time blood samples. However, BCAAs showed good within-person stability over 1-2 years (ICC $\geq$ 0.55) (43) as well as good within-person stability over 10 years (ICC > 0.4). Metabolomics platforms differed between NHS and NHSII and WHS; nuclear magnetic resonance (NMR) approaches may be more limited in measuring BCAA levels (44). However, others showed good correlations and consistent associations with diabetes between the platforms (45).

In summary, elevated circulating BCAA levels were associated with higher risk of postmenopausal breast cancer in NHS when assessed within 10 years of diagnosis, independent of established risk factors, including adiposity, though this finding was not replicated among predominantly postmenopausal WHS women. Whether circulating BCAAs levels are inversely associated with breast cancer risk among premenopausal women warrants further investigation.

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Data Availability

Data access must be approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health. Inquiries are encouraged through http://www.nurseshealthstudy.org/researchers.

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