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 Nurses’ Health Study (NHS) and NHSII (1,997 cases and 1,997 controls). 592 NHS women donated two blood samples 10 years apart. We estimate 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 two-sided.

**Results:** Among NHSII participants (predominantly premenopausal at blood collection), elevated circulating BCAA levels were associated with lower breast cancer risk (e.g., isoleucine highest vs. lowest quartile, multivariable OR=0.86, 95% CI = 0.65-1.13, $P_{\text{trend}}$=0.20), with statistically significant linear trends among fasting samples (e.g., isoleucine OR=0.74, 95% CI = 0.53-1.05, $P_{\text{trend}}$=0.05). In contrast, among postmenopausal women, proximate measures (<10 years from blood draw) were associated with increased breast cancer risk (e.g., isoleucine OR=1.63, 95% CI = 1.12-2.39, $P_{\text{trend}}$=0.01), with stronger associations among fasting samples (OR=1.73, 95% CI = 1.15-2.61, $P_{\text{trend}}$=0.01). Distant measures (10-20y 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 = 0.96-1.58, $P_{\text{trend}}$=0.04).
Conclusion: No statistically significant associations between BCAA levels and breast cancer risk were consistent across NHS/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 >250,000 diagnoses annually in the US\textsuperscript{1}. Epidemiologic studies have identified modifiable risk factors, including increased BMI and low physical activity in postmenopausal women\textsuperscript{2}. However, BMI is inversely associated with premenopausal breast cancer\textsuperscript{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 important metabolites involved in cell-signaling pathways and muscle protein synthesis\textsuperscript{4}. Elevated plasma BCAA concentrations are strongly positively correlated with BMI and insulin resistance, and are a marker of dysfunctional metabolism\textsuperscript{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 one assessed menopausal status\textsuperscript{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 with breast cancer risk. In secondary analyses, we conducted a validation analysis in the Women’s Health Study (WHS).
Methods

Study Population

In 1976, 121,701 female registered nurses aged 30-55y enrolled in the NHS with the return of a mailed questionnaire\textsuperscript{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 25-42y, with biennial follow-up using similar questionnaires as NHS.

In 1989-1990, 32,826 NHS participants aged 43-69y contributed blood samples, as previously described\textsuperscript{12}. In 2000-2002, 18,473 of these women aged 53-80y donated a second sample using a similar protocol. In the NHSII, 29,611 women aged 32-54y 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.

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/NHSII, BCAAs were assayed through a metabolomic profiling platform at the Broad Institute using a liquid chromatography tandem mass
spectrometry (LC-MS) method designed to measure polar metabolites such as amino acids, amino acids derivatives, dipeptides, and other cationic metabolites. 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 LC-MS peak areas, which are unitless numbers directly proportional to metabolite concentrations. Detailed description of the laboratory assays used to measure BCAAs, gene expression, estradiol and C-peptide is included in the **Supplementary Methods**.

**Statistical Analysis**

BCAA values were log transformed and standardized (mean=0; standard deviation [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 three 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 two 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/NHSII (N=9,112) 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 to 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 ER status.

In multivariable models, we adjusted for established breast cancer risk factors: BMI at age 18, weight change from age 18 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 two blood collections. In the WHS, we used Cox proportional hazards regression models with follow-up from the date of randomization 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/NHSII and WHS using the DerSimonian-Laird estimator\(^\text{16}\), and based on these findings, meta-analyzed individual cohort results using a fixed or random effects approach.
We used Correlation Adjusted Mean Rank (CAMERA) on tumor gene expression data to explore functional enrichment of biological pathways associated with BCAAs (Supplementary Methods)\textsuperscript{17}.

We conducted sensitivity analyses restricting to fasting samples (>8h 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 and weight change between age 18 and blood collection, and adjusting for plasma C-peptide (a marker of insulin production) and estradiol in individual models.

All statistical tests were two-sided and a P value of less than 0.05 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 (N=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/NHSII: 6 years for postmenopausal cases with proximate samples, 16 years for
postmenopausal cases with distant samples and 5 years for premenopausal women at
blood collection. Demographics were similar to NHS; exceptions include 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 non-fasting 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) (e.g.,
isoleucine highest vs. lowest quartile OR=0.76, 95% CI = 0.59-0.99, $P_{\text{trend}}=0.02$; Table
4), with statistically significant linear trends. These associations were attenuated and no
longer statistically significant with adjustment for breast cancer risk factors (e.g.,
isoleucine highest vs. lowest quartile OR=0.86, 95% CI = 0.65-1.13, $P_{\text{trend}}=0.20$).
Associations were similar for leucine (OR=0.77, 95% CI = 0.58-1.01) and valine
(OR=0.82, 95% CI = 0.62-1.08). We observed stronger associations among fasting
samples only (715 cases; top vs. bottom quartile OR: isoleucine=0.74, 95% CI = 0.53-
1.05, $P_{\text{trend}} = 0.05$; leucine=0.66, 95% CI = 0.47-0.94, $P_{\text{trend}} = 0.04$; valine=0.74, 95% CI = 0.53-1.04, $P_{\text{trend}} = 0.08$). Associations with total BCAAs followed a similar pattern, but were attenuated compared to individual BCAAs: OR=0.79, 95% CI = 0.56-1.11, $P_{\text{trend}} = 0.12$. We observed similar associations when we further restricted to premenopausal women at blood collection (541 cases; OR; leucine=0.61, 95% CI = 0.40-0.92, $P_{\text{trend}} = 0.04$; 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 (10-20y 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 (e.g., isoleucine OR=1.15, 95% CI = 0.87-1.52, $P_{\text{trend}} = 0.35$). BCAAs from proximate samples (592 cases) were positively associated with breast cancer risk and similar between the simple and multivariable models (e.g., isoleucine multivariable OR=1.63, 95% CI = 1.12-2.39, $P_{\text{trend}} = 0.01$). Weaker associations were observed for leucine (OR=1.26, 95% CI = 0.87-1.83, $P_{\text{trend}} = 0.17$) and valine (OR = 1.34, 95% CI = 0.93-1.94, $P_{\text{trend}} = 0.12$). Associations were stronger, with statistically significant linear trends (except for leucine), when restricted to fasting samples (513 cases; isoleucine OR=1.73, 95% CI = 1.15-2.61, $P_{\text{trend}} = 0.01$; leucine OR=1.31, 95% CI = 0.87-1.98, $P_{\text{trend}} = 0.12$; valine OR=1.64, 95% CI = 1.11-2.43, $P_{\text{trend}} = 0.04$). Association with total BCAAs followed the same pattern as individual BCAAs (e.g., fasting samples, multivariable OR=1.56, 95% CI = 1.04-2.34, $P_{\text{trend}} = 0.06$). A statistically significant interaction with menopausal status at blood collection.
(P<0.004) 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 OR for isoleucine was 0.97 (95% CI = 0.75-1.26, Pr\text{\_trend} =0.85) (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 = 0.96-1.58, Pr\text{\_trend} =0.04). 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/NHSII and WHS results.

Results among NHS/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 and weight change between age 18 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 3-fold 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 to participants who had low isoleucine levels in both timepoints (low/low; Table 6).

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

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, E2F and G2M targets, and DNA repair among NHSII women (73.2% premenopausal at blood collection) but with upregulation of estrogen response among NHS participants (all postmenopausal women; Supplementary Table 3).

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 (<10y 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. 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. Several studies found a weak positive correlation between dietary BCAA intake and circulating BCAAs ($r=0.11-0.14$). 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 to those with predominately plant sources of protein, while higher intake of red meat is associated with increased risk of pre- and postmenopausal breast cancer. However, BCAAs were not identified as markers of dietary patterns 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, and diabetes and insulin resistance have been associated with breast cancer risk. Elevated levels of circulating BCAAs are associated with obesity and insulin resistance in cross-sectional studies, and associated with incident Type II Diabetes (T2D). Adiposity and insulin resistance have a causal effect on serum BCAA levels, and circulating BCAAs play a causal role in the development of T2D. 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\textsuperscript{40,41}, which has been implicated in breast carcinogenesis\textsuperscript{42}.

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\textsuperscript{7} in a case-cohort analysis in the EPIC-Heidelberg cohort (114 pre- and 248 postmenopausal cases) or in a larger study\textsuperscript{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 SU.VI.MAX study (129 pre- and 82 postmenopausal cases)\textsuperscript{8}. Given the mix of menopausal status, it is difficult to compare these results to our findings. Consistent with our results, in an examination of BMI-correlated metabolites in the PLCO cohort, which included valine and allo-isoleucine, (N=621 postmenopausal cases), higher levels of allo-isoleucine, a byproduct
of isoleucine transamination\textsuperscript{43}, were associated with increased postmenopausal breast cancer risk\textsuperscript{9}. Notably, two other metabolites involved in alternative isoleucine and leucine degradation, 2-methylbutyrylcarnitine and 3-methylglutaryl carnitine, were positively associated with risk\textsuperscript{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 and NHS/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.

Our study has several strengths and limitations. We measured pre-diagnostic 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 two blood samples, our main findings are based on one-point-in-time blood samples. However, BCAAs showed good within-person stability over 1-2 years (ICC\textgeq0.55)\textsuperscript{44} as well as good within-person stability over 10 years (ICC\textgt0.4). Metabolomics platforms differed between NHS/NHSII and WHS; NMR approaches may be more limited in measuring BCAA levels\textsuperscript{45}. However, others showed good correlations and consistent associations with diabetes between the platforms\textsuperscript{46}. 
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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### Tables

**Table 1: Characteristics of breast cancer cases and matched controls in the Nurses’ Health Studies**

| Participant characteristics | NHSII Cases (N=1057) | NHS II Controls (N=1057) | NHS distant collection Cases (N=940) | NHS distant collection Controls (N=940) | NHS proximate collection Cases (N=592) | NHS proximate collection Controls (N=592) |
|----------------------------|----------------------|--------------------------|-----------------------------------|---------------------------------------|--------------------------------------|----------------------------------------|
| Mean age at blood collectionc (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 <25y | 14.7 | 15.9 | 13.5 | 14.1 | 13.0 | 15.9 |
| 1-2 children ≥25y | 39.2 | 34.9 | 20.1 | 20.6 | 20.4 | 19.3 |
| 3+ children <25y | 11.3 | 16.6 | 35.5 | 35.5 | 36.5 | 38.3 |
| 3+ children ≥25y | 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/m² | 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 years and blood collection (SD), kg | 11.6 (12.0) | 12.6 | 12.3 | 10.6 | 15.5 | 13.8 |
| Mean physical activity (SD), MET-hrs/wk | 18.0 (15.3) | 18.1 | 15.4 | 15.9 | 17.7 | 19.0 |
| Mean alcohol consumption (SD), g/day | 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 usec,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 collectionc, % | 80.2 | 79.7 | 25.4 | 25.5 | 0.5 | 0.8 |
| Premenopausal | 12.7 | 13.1 | 61.9 | 61.9 | 98.1 | 98.1 |
| Postmenopausal | 7.1 | 7.3 | 12.7 | 12.6 | 1.4 | 1.0 |
| Unknown |  |  |  |  |  |  |
| Menopausal status at diagnosisc, % | 42.0 | 42.2 | 1.3 | 1.3 | 1.4 | 1.0 |
| Premenopausal | 46.4 | 47.1 | 97.3 | 98.1 | 97.8 | 98.3 |
| Postmenopausal | 11.6 | 10.7 | 1.4 | 0.6 | 0.8 | 0.7 |
| Fasting (>8h) at blood collectionc, % | 68.7 | 74.7 | 66.6 | 72.7 | 87.0 | 92.4 |
| Caucasianc, % | 97.2 | 98.4 | 98.3 | 98.8 | 98.6 | 99.5 |

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*a NHS first blood collection. BMI = body mass index.*
b NHS second blood collection
c matching factor
d oral contraceptive or menopausal hormone therapy
— data available for cases only
Table 2: Characteristics of the Women’s Health Study

| Participant characteristics | WHS Premenopausal at blood collection | WHS Postmenopausal at blood collection |
|----------------------------|--------------------------------------|---------------------------------------|
| Total, No. (%)             | 12413 (46.0)                         | 14587 (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 <30y          | 27.2                                 | 18.0                                 |
| 3+ children <30y           | 28.7                                 | 33.6                                 |
| 1-2 children ≥30y          | 5.8                                  | 3.8                                  |
| 3+ children ≥30y           | 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/m² | 26.0 (5.2)                          | 25.8 (4.8)                           |
| Mean physical activity (SD), MET-hrs/wk | 14.8 (18.6)                       | 14.8 (18.3)                          |
| Alcohol consumption, frequency of intake, % |                                      |                                       |
| Rarely/never               | 42.6                                 | 45.0                                 |
| 1-3/months                 | 13.7                                 | 13.0                                 |
| 1-6/week                   | 34.3                                 | 30.8                                 |
| 1+/day                     | 9.3                                  | 11.3                                 |
| Past/current exogenous hormone use, % | 29.7                                | 69.9                                 |
| Fasting (>8h) at blood collection, % | 70.1                                | 73.8                                 |
| Caucasian, %               | 94.4                                 | 94.6                                 |

\(^a\) BMI = body mass index; WHS = Women’s Health Study
Table 3: Effect estimates for predictors of probit transformed circulating BCAA levels from multivariable linear regression among 9,112 NHS/NHSII women.

| Predictors                      | No.   | Isoleucine β (95% CI) | Leucine β (95% CI) | Valine β (95% CI) |
|---------------------------------|-------|------------------------|--------------------|-------------------|
| **Dietary intake**, mg/d        |       |                        |                    |                   |
| Q1                              | 1999-2010 | ref                     | ref                | ref               |
| Q2                              | 2016-2026 | 0.10 (0.03; 0.16)       | 0.09 (0.03; 0.16)  | 0.11 (0.05; 0.17) |
| Q3                              | 2014-2030 | 0.13 (0.06; 0.20)       | 0.18 (0.11; 0.25)  | 0.26 (0.20; 0.33) |
| Q4                              | 2011-2034 | 0.16 (0.08; 0.24)       | 0.21 (0.13; 0.28)  | 0.31 (0.23; 0.39) |
| Q5                              | 2025-2034 | 0.21 (0.11; 0.31)       | 0.28 (0.18; 0.37)  | 0.42 (0.32; 0.51) |
| $P_{trend}$                     |        | <0.001                 | <0.001             | <0.001            |
| **Fasting status**              |       |                        |                    |                   |
| fasting (>8h)                   | 7836  | ref                     | ref                | ref               |
| non-fasting                     | 2771  | 0.20 (0.16; 0.25)       | 0.11 (0.07; 0.15)  | 0.10 (0.06; 0.15) |
| **Age at blood collection, y**  |       |                        |                    |                   |
| <40                             | 574   | ref                     | ref                | ref               |
| 40-50                           | 3829  | 0.00 (-0.09; 0.09)      | -0.04 (-0.13; 0.05) | 0.04 (-0.05; 0.13) |
| 50-60                           | 3541  | 0.00 (-0.11; 0.11)      | -0.03 (-0.14; 0.08) | 0.12 (0.01; 0.23) |
| >60                             | 2665  | 0.02 (-0.10; 0.14)      | -0.03 (-0.15; 0.09) | 0.12 (<0.01; 0.23) |
| $P_{trend}$                     | 0.47  | 0.95                    | 0.04               |
| **Race**                        |       |                        |                    |                   |
| Caucasian                       | 10248 | ref                     | ref                | ref               |
| Black                           | 264   | -0.11 (-0.26; 0.04)     | -0.04 (-0.19; 0.11) | -0.19 (-0.33; -0.04) |
| Asian                           | 68    | 0.28 (0.03; 0.53)       | 0.26 (0.01; 0.51)  | 0.34 (0.09; 0.58) |
| Other                           | 29    | 0.03 (-0.34; 0.40)      | 0.06 (-0.31; 0.43) | -0.01 (-0.37; 0.35) |
| **Smoking status**              |       |                        |                    |                   |
| Never                           | 5602  | ref                     | ref                | ref               |
| Past                            | 3722  | -0.01 (-0.05; 0.04)     | 0.01 (-0.03; 0.06) | 0.01 (-0.04; 0.05) |
| Current                         | 1263  | 0.01 (-0.06; 0.07)      | 0.00 (-0.06; 0.07) | -0.02 (-0.09; 0.04) |
| **BMI, kg/m2**                  |       |                        |                    |                   |
| <25                             | 5601  | ref                     | ref                | ref               |
| [25,30)                         | 3154  | 0.34 (0.3; 0.38)        | 0.34 (0.3; 0.39)   | 0.40 (0.36; 0.45) |
| >30                             | 1822  | 0.70 (0.65; 0.76)       | 0.68 (0.62; 0.74)  | 0.82 (0.77; 0.88) |
| $P_{trend}$                     | <0.001| <0.001                  | <0.001             |
| **Physical activity, MET-hr/wk**|       |                        |                    |                   |
| <9                              | 4734  | ref                     | ref                | ref               |
| 9-27                            | 3718  | -0.05 (-0.09; 0.00)     | -0.05 (-0.09; 0.00) | -0.04 (-0.09; 0.00) |
| ≥27                             | 1946  | -0.01 (-0.06; 0.05)     | 0.01 (-0.05; 0.07) | 0.01 (-0.04; 0.06) |
| $P_{trend}$                     | 0.62  | 0.88                    | 0.88               |
| **Alcohol consumption, g/day**  |       |                        |                    |                   |
| 0                               | 3531  | ref                     | ref                | ref               |
| 0.88-10                         | 4309  | -0.08 (-0.13; -0.04)    | -0.07 (-0.11; -0.02) | -0.04 (-0.09; 0.00) |
| 10-20                           | 1099  | -0.12 (-0.19; -0.06)    | -0.07 (-0.14; -0.01) | -0.07 (-0.14; -0.01) |
| Category | Number | \( P_{\text{trend}} \) |
|----------|--------|----------------|
| \( \geq 20 \) | 632 | 0.001 | 0.001 | 0.001 |

**Alternative Healthy Eating Index**

| Category | Number | \( P_{\text{trend}} \) |
|----------|--------|----------------|
| \(< 43.5\) | 1909 | ref | ref | ref |
| \([37.9, 43.5)\) | 1906 | -0.04 (-0.10; 0.02) | -0.01 (-0.07; 0.05) | 0.01 (-0.05; 0.07) |
| \([43.5, 49)\) | 1910 | -0.07 (-0.13; -0.01) | -0.04 (-0.10; 0.03) | -0.04 (-0.10; 0.02) |
| \([49, 55.6)\) | 1908 | -0.10 (-0.16; -0.03) | -0.06 (-0.13; 0.00) | -0.04 (-0.10; 0.02) |
| \(\geq 55.6\) | 1909 | -0.08 (-0.15; -0.02) | -0.04 (-0.10; 0.03) | -0.02 (-0.08; 0.04) |

**Menopausal status and PMH use**

| Category | Number | \( P_{\text{trend}} \) |
|----------|--------|----------------|
| Premenopausal | 4337 | ref | ref | ref |
| Postmenopausal PMH | 2447 | 0.05 (-0.02; 0.11) | 0.06 (-0.01; 0.12) | 0.12 (0.06; 0.18) |
| Postmenopausal no PMH | 3189 | 0.10 (0.04; 0.17) | 0.15 (0.08; 0.22) | 0.17 (0.11; 0.24) |
| Unknown | 649 | 0.08 (-0.01; 0.17) | 0.06 (-0.04; 0.15) | 0.10 (0.00; 0.19) |

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\(a\) 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]; \(\geq 4.82\). Leucine dietary intake quintile cutpoints [mg/d]: <5.33; [5.33, 6.49]; [6.49, 7.58]; [7.58, 9.05]; \(\geq 9.05\). Valine dietary intake quintile cutpoints [mg/d]: <3.22; [3.22, 3.93]; [3.93, 4.59]; [4.59, 5.47]; \(\geq 5.47\). CI = confidence interval; NHS = Nurses' Health Study; NHSII = Nurses' Health Study II; PMH = postmenopausal hormone therapy.

\(b\) calculated without alcohol intake
Table 4: Odds ratios (OR) of breast cancer according to quartiles of plasma branched-chain amino acids among premenopausal and postmenopausal women

| BCAAs      | Q1     | Q2     | Q3     | Q4     | \( P_{\text{trend}} \) |
|------------|--------|--------|--------|--------|--------------------------|
| **Isoleucine** |        |        |        |        |                          |
| All Samples |        |        |        |        |                          |
| No. of cases/controls | 300/265 | 282/264 | 239/264 | 236/264 |                          |
| Simple OR (95% CI) | ref 0.93 (0.73-1.18) | 0.79 (0.61-1.01) | 0.76 (0.59-0.99) | 0.02 |
| Multivariable OR (95% CI) | ref 0.99 (0.77-1.27) | 0.87 (0.67-1.13) | 0.86 (0.65-1.13) | 0.20 |
| Fasting Samples |        |        |        |        |                          |
| No. of cases/controls | 216/179 | 201/179 | 149/178 | 149/179 |                          |
| Multivariable OR (95% CI) | ref 0.97 (0.72-1.30) | 0.77 (0.56-1.05) | 0.74 (0.53-1.05) | 0.05 |
| **Leucine** |        |        |        |        |                          |
| All Samples |        |        |        |        |                          |
| No. of cases/controls | 296/265 | 268/264 | 278/264 | 215/264 |                          |
| Simple OR (95% CI) | ref 0.90 (0.70-1.14) | 0.93 (0.72-1.19) | 0.71 (0.55-0.92) | 0.02 |
| Multivariable OR (95% CI) | ref 0.92 (0.72-1.18) | 1.00 (0.77-1.30) | 0.77 (0.58-1.01) | 0.11 |
| Fasting Samples |        |        |        |        |                          |
| No. of cases/controls | 209/179 | 184/179 | 190/178 | 132/179 |                          |
| Multivariable OR (95% CI) | ref 0.88 (0.66-1.18) | 0.94 (0.68-1.29) | 0.66 (0.47-0.94) | 0.04 |
| **Valine** |        |        |        |        |                          |
| All Samples |        |        |        |        |                          |
| No. of cases/controls | 293/265 | 262/264 | 283/264 | 219/264 |                          |
| Simple OR (95% CI) | ref 0.89 (0.69-1.13) | 0.95 (0.75-1.20) | 0.74 (0.58-0.95) | 0.04 |
| Multivariable OR (95% CI) | ref 0.91 (0.71-1.18) | 1.02 (0.80-1.31) | 0.82 (0.62-1.08) | 0.28 |
| Fasting Samples |        |        |        |        |                          |
| No. of cases/controls | 217/179 | 181/179 | 170/178 | 147/179 |                          |
| Multivariable OR (95% CI) | ref 0.86 (0.63-1.16) | 0.81 (0.60-1.10) | 0.74 (0.53-1.04) | 0.08 |
| **Total BCAAs** |        |        |        |        |                          |
| All Samples |        |        |        |        |                          |
|                  | Simple OR (95% CI) | Multivariable OR (95% CI) |
|------------------|--------------------|---------------------------|
| **Fasting Samples** |                    |                           |
| No. of cases/controls | 278/265           | 293/264                   |
| Simple OR (95% CI)  | ref                | 1.05 (0.83-1.34)          |
| Multivariable OR (95% CI) | ref          | 1.10 (0.86-1.41)          |
| Postmenopausal women in NHS, distant sample collection (10-20y before diagnosis, N=940 cases/controls) | | |
| Isoleucine        |                    |                           |
| All Samples       |                    |                           |
| No. of cases/controls | 226/235           | 220/235                   |
| Simple OR (95% CI)  | ref                | 0.98 (0.75-1.26)          |
| Multivariable OR (95% CI) | ref          | 0.95 (0.73-1.24)          |
| Fasting Samples   |                    |                           |
| No. of cases/controls | 157/156           | 132/156                   |
| Multivariable OR (95% CI) | ref          | 0.83 (0.60-1.15)          |
| Leucine           |                    |                           |
| All Samples       |                    |                           |
| No. of cases/controls | 220/235           | 217/235                   |
| Simple OR (95% CI)  | ref                | 0.98 (0.75-1.29)          |
| Multivariable OR (95% CI) | ref          | 0.95 (0.72-1.25)          |
| Fasting Samples   |                    |                           |
| No. of cases/controls | 147/156           | 145/156                   |
| Multivariable OR (95% CI) | ref          | 0.95 (0.68-1.33)          |
| Valine            |                    |                           |
| All Samples       |                    |                           |
| No. of cases/controls | 215/235           | 236/235                   |
| Simple OR (95% CI)  | ref                | 1.10 (0.85-1.42)          |
| Multivariable OR (95% CI) | ref          | 1.03 (0.79-1.34)          |
|                | All Samples | Fasting Samples | Postmenopausal women in NHS, proximate sample collection (<10y before diagnosis, N=592 cases/controls) |
|----------------|-------------|-----------------|--------------------------------------------------------------------------------------------------|
| **Total BCAAs**|             |                 |                                                                                                  |
| No. of cases/controls | 217/235 | 225/235 | 217/235 | 281/235 | 146/156 | 161/156 | 137/155 | 179/156 |
| Simple OR (95% CI) | ref | 1.00 (0.72-1.40) | 0.91 (0.65-1.28) | 1.06 (0.74-1.52) | 0.90 |
| Multivariable OR (95% CI) | ref | 1.04 (0.80-1.35) | 1.32 (1.02-1.70) | 1.17 (0.88-1.55) | 0.05 |

**Isoleucine**

|                | All Samples | Fasting Samples | Postmenopausal women in NHS, proximate sample collection (<10y before diagnosis, N=592 cases/controls) |
|----------------|-------------|-----------------|--------------------------------------------------------------------------------------------------|
| No. of cases/controls | 112/148 | 146/148 | 154/148 | 180/148 | 91/129 | 130/128 | 136/128 | 156/128 |
| Simple OR (95% CI) | ref | 1.30 (0.94-1.81) | 1.63 (1.17-2.29) | 1.63 (1.12-2.39) | 0.01 |
| Multivariable OR (95% CI) | ref | 1.29 (0.91-1.83) | 1.45 (1.01-2.09) | 1.63 (1.12-2.39) | 0.01 |

**Leucine**

|                | All Samples | Fasting Samples | Postmenopausal women in NHS, proximate sample collection (<10y before diagnosis, N=592 cases/controls) |
|----------------|-------------|-----------------|--------------------------------------------------------------------------------------------------|
| No. of cases/controls | 123/148 | 144/148 | 164/148 | 161/148 | 103/129 | 123/128 | 147/128 | 140/128 |
| Simple OR (95% CI) | ref | 1.17 (0.83-1.63) | 1.32 (0.94-1.84) | 1.26 (0.87-1.83) | 0.08 |
| Multivariable OR (95% CI) | ref | 1.20 (0.84-1.71) | 1.43 (1.01-2.03) | 1.26 (0.87-1.83) | 0.17 |

**Valine**

|                | All Samples | Fasting Samples | Postmenopausal women in NHS, proximate sample collection (<10y before diagnosis, N=592 cases/controls) |
|----------------|-------------|-----------------|--------------------------------------------------------------------------------------------------|
| No. of cases/controls | 146/156 | 161/156 | 137/155 | 179/156 | 103/129 | 123/128 | 147/128 | 140/128 |
| Multivariable OR (95% CI) | ref | 1.29 (0.88-1.90) | 1.58 (1.08-2.31) | 1.31 (0.87-1.98) | 0.12 |
|                         | No. of cases/controls | Simple<sup>a</sup> OR (95% CI) | Multivariable<sup>c</sup> OR (95% CI) |
|-------------------------|-----------------------|---------------------------------|-------------------------------------|
| **No. of cases/controls** | 119/148               | 146/148                         | 158/148                             |
| Simple<sup>a</sup> OR (95% CI) | ref                   | 1.21 (0.87-1.68)                | 1.31 (0.95-1.80)                    |
| Multivariable<sup>c</sup> OR (95% CI) | ref                   | 1.23 (0.87-1.73)                | 1.33 (0.94-1.88)                    |
| **Fasting Samples**     |                       |                                 |                                     |
| No. of cases/controls   | 99/129                | 134/128                         | 111/128                             |
| Multivariable<sup>c</sup> OR (95% CI) | ref                   | 1.45 (1.00-2.10)                | 1.13 (0.76-1.67)                    |
| **Total BCAAs**         |                       |                                 |                                     |
| All Samples             |                       |                                 |                                     |
| No. of cases/controls   | 119/148               | 149/148                         | 148/148                             |
| Simple<sup>a</sup> OR (95% CI) | ref                   | 1.25 (0.90-1.74)                | 1.25 (0.90-1.74)                    |
| Multivariable<sup>c</sup> OR (95% CI) | ref                   | 1.30 (0.92-1.85)                | 1.35 (0.94-1.93)                    |
| **Fasting Samples**     |                       |                                 |                                     |
| No. of cases/controls   | 101/129               | 129/128                         | 123/128                             |
| Multivariable<sup>c</sup> OR (95% CI) | ref                   | 1.41 (0.96-2.08)                | 1.35 (0.92-1.98)                    |

<sup>a</sup> Predominantly premenopausal (see Table 1 and Figure 1 for details). CI = confidence interval; BCAA = branch chain amino acids; NHS = Nurses’ Health Study; NHSII = Nurses’ Health Study II

<sup>b</sup> Simple model: no adjustment factors were included.

<sup>c</sup> Multivariable model: BMI at age 18, weight change from age 18 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.

<sup>d</sup> Predominantly postmenopausal women (see Table 1 and Figure 1 for details)
Table 5: Odds ratios (ORs) of breast cancer according to quartiles of plasma branched-chain amino acids among premenopausal and postmenopausal women in WHS

| BCAAs | Q1          | Q2          | Q3          | Q4          | \(P_{\text{trend}}\) |
|-------|-------------|-------------|-------------|-------------|----------------------|
|       | Premenopausal women at blood collection in WHS (n=763 cases) |       |       |       |       |
| Isoleucine | No. of cases/non-cases | 191/2873 | 188/2906 | 190/2891 | 194/2980 |       |
|        | Multivariable OR (95% CI) | Ref | 0.98 (0.80, 1.20) | 0.99 (0.81, 1.21) | 0.99 (0.80, 1.20) | 0.93 |
| Leucine | No. of cases/non-cases | 183/3000 | 187/2903 | 213/2798 | 180/2949 |       |
|        | Multivariable OR (95% CI) | Ref | 1.06 (0.87, 1.31) | 1.22 (1.00, 1.49) | 1.00 (0.81, 1.24) | 0.62 |
| Valine | No. of cases/non-cases | 206/3081 | 187/2849 | 179/2836 | 191/2884 |       |
|        | Multivariable OR (95% CI) | Ref | 0.98 (0.80, 1.21) | 0.95 (0.77, 1.16) | 0.97 (0.79, 1.20) | 0.76 |
| Total BCAAs | No. of cases/non-cases | 196/3058 | 181/2850 | 193/2780 | 193/2962 |       |
|        | Multivariable OR (95% CI) | Ref | 0.98 (0.80, 1.21) | 1.07 (0.88, 1.32) | 1.01 (0.82, 1.25) | 0.76 |
|       | Postmenopausal women in WHS, distant sample collection (10-20y before diagnosis, n=515 cases) |       |       |       |       |
| Isoleucine | No. of cases/non-cases | 125/3561 | 118/3538 | 144/3525 | 128/3448 |       |
|        | Multivariable OR (95% CI) | Ref | 0.94 (0.73, 1.21) | 1.16 (0.91, 1.47) | 1.11 (0.86, 1.43) | 0.25 |
| Leucine | No. of cases/non-cases | 121/3446 | 105/3555 | 146/3593 | 143/3478 |       |
|        | Multivariable OR (95% CI) | Ref | 0.85 (0.65, 1.10) | 1.15 (0.90, 1.47) | 1.23 (0.96, 1.58) | 0.04 |
| Valine | No. of cases/non-cases | 127/3336 | 127/3587 | 129/3606 | 132/3543 |       |
|        | Multivariable OR (95% CI) | Ref | 0.94 (0.73, 1.21) | 0.95 (0.74, 1.22) | 0.99 (0.76, 1.29) | 0.95 |
| Total BCAAs | No. of cases/non-cases | 127/3369 | 121/3598 | 135/3642 | 132/3463 |       |
|        | Multivariable OR (95% CI) | Ref | 0.88 (0.69, 1.13) | 0.98 (0.76, 1.25) | 1.05 (0.81, 1.36) | 0.60 |
|       | Postmenopausal women in WHS, proximate sample collection (<10y before diagnosis, n=487 cases) |       |       |       |       |
| Isoleucine | No. of cases/non-cases | 136/3550 | 116/3540 | 120/3549 | 115/3461 |       |
|        | Multivariable OR (95% CI) | Ref | 0.87 (0.68, 1.12) | 0.93 (0.73, 1.19) | 0.97 (0.75, 1.26) | 0.85 |
| Leucine | No. of cases/non-cases | 126/3441 | 115/3545 | 123/3616 | 123/3498 |       |
|        | Multivariable OR (95% CI) | Ref | 0.91 (0.70, 1.17) | 0.97 (0.76, 1.25) | 1.05 (0.81, 1.36) | 0.68 |
| Valine | No. of cases/non-cases | 119/3344 | 133/3581 | 128/3607 | 107/3568 |       |
|        | Multivariable OR (95% CI) | Ref | 1.09 (0.85, 1.39) | 1.04 (0.81, 1.34) | 0.96 (0.73, 1.26) | 0.75 |
| Total BCAAs |           |           |           |           |           |  

30
| No. of cases/non-cases | 128/3368 | 119/3600 | 126/3651 | 114/3481 |
|------------------------|----------|----------|----------|----------|
| Multivariable OR (95% CI) | Ref | 0.89 (0.69, 1.15) | 0.97 (0.75, 1.24) | 0.98 (0.75, 1.27) | 0.98 |

*aMultivariable 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. WHS = Women’s Health Study*
Table 6: Odds ratios (ORs) of breast cancer according to 10-year change\textsuperscript{a} in plasma branched-chain amino acids in postmenopausal women in NHS

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

\textsuperscript{a} Cross-classified by median in distant/proximate sample collections

\textsuperscript{b} Multivariable model: body mass index at age 18, weight change from age 18 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. BCAAs = branched-chain amino acids; CI = confidence interval; NHS = Nurses’ Health Study
Figure Legend

Figure 1. Age and menopausal status distribution at blood collection. Panel A shows the age distribution in the three 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 three datasets: premenopausal status is shown in dark green, postmenopausal status is shown in light green and unknown status is shown in gray.
