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Dietary Intakes and Circulating Concentrations of Branched-Chain Amino Acids in Relation to Incident Type 2 Diabetes Risk Among High-Risk Women with a History of Gestational Diabetes Mellitus

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BACKGROUND: Circulating branched-chain amino acids (BCAAs; isoleucine, leucine, valine) are consistently associated with increased type 2 diabetes (T2D) risk, but the relationship with dietary intake of BCAAs is less clear.

METHODS: The longitudinal Nurses' Health Study II cohort conducted a blood collection from 1996 to 1999. We profiled plasma metabolites among 172 incident T2D cases and 175 age-matched controls from women reporting a history of gestational diabetes before blood draw. We estimated dietary energy-adjusted BCAAs from food frequency questionnaires. We used conditional logistic regression models to estimate odds ratios (OR) and 95% CI of T2D risk across quartiles (Q1–Q4) of BCAAs, adjusting for age, body mass index (BMI), physical activity, family history, and other established risk factors. We also assessed joint exposure to below/above medians of diet and plasma concentrations, with lower diet/lower plasma as reference.

RESULTS: Dietary and plasma BCAA concentrations were positively associated with incident T2D (diet Q4 vs Q1 OR = 4.6, CI = 1.6, 13.4; plasma Q4 vs Q1 OR = 4.4, CI = 1.4, 13.4). Modeling the joint association indicated that higher diet BCAAs were associated with T2D when plasma concentrations were also higher (OR = 6.0, CI = 2.1, 17.2) but not when concentrations were lower (OR = 1.6, CI = 0.61, 4.1). Conversely, higher plasma BCAAs were associated with increased T2D for either lower or higher diet.

CONCLUSIONS: Independent of BMI and other risk factors, higher diet and plasma BCAA concentrations were associated with an increased incident T2D risk among high-risk women with a history of gestational diabetes, supporting impaired BCAA metabolism as conferring T2D risk.

Type 2 diabetes (T2D)[10] has reached epidemic proportions in the US and globally, bringing with it debilitating and costly consequences, including cardiovascular disease, renal dysfunction, and vision loss. Similarly, trends in the prevalence of pregnancies complicated by gestational diabetes mellitus (GDM; glucose intolerance with onset during pregnancy) have increased *(1)*. Compared with women with a previous normoglycemic pregnancy, those with a history of GDM have a >7-fold risk of developing T2D *(2)*. Targeting high-risk groups for prevention, such as women with a history of GDM, represents a strategy for reducing T2D incidence.

T2D is a complex chronic disease influenced by lifestyle, environmental, and genetic factors, as well as the complex interactions between them. Novel approaches to identifying relevant biomarkers of underlying pathways have recently included metabolomics, which has allowed for the identification of circulating small molecules associated with T2D risk. Among the most consistent metabolites associated with T2D risk in prospective cohort studies include the branched-chain amino acids (BCAAs) isoleucine, leucine, and valine, each associated

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[10] Nonstandard abbreviations: T2D, type 2 diabetes; GDM, gestational diabetes mellitus; BCAA, branched-chain amino acids; NHS II, Nurses' Health Study II; FFQ, food frequency questionnaire; BMI, body mass index; AHEI-2010, Alterative Healthy Eating Index 2010; MET, metabolic equivalent tasks; OR, odds ratio.

with approximately 35% greater T2D risk (per 1 SD in prediagnostic concentrations) in a recent metaanalysis *(3)*. BCAAs are exogenous amino acids derived from a variety of food sources, including both animal and vegetable proteins. Long-term dietary intakes of isoleucine, leucine, and valine have also been associated with incident T2D risk in a pooled cohort analysis of US adults *(4)*. It remains unknown, however, whether higher dietary intakes of BCAAs per se, increased circulating concentrations sustained by impaired catabolism, or both, confer T2D risk.

Thus, we sought to prospectively evaluate both dietary intake and circulating concentrations of BCAAs with incident T2D in a prospective nested case–control study among high-risk women with a history of GDM in the Nurses' Health Study II (NHS II) cohort. We further evaluated the joint relationship between diet and circulating concentrations to assess their relative contributions to T2D risk.

Materials and Methods

We conducted a prospective nested case–control study among women with a history of GDM in the NHS II (see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol64/issue8). Briefly, the NHS II longitudinal cohort was established in 1989, enrolling 116 430 female nurses aged 24 to 44 years at baseline. Biennial questionnaires update information on numerous reproductive, lifestyle, and other health-related characteristics and outcomes. Beginning in 1991 and every 4 years thereafter, participants also completed a food frequency questionnaire (FFQ) to capture usual dietary intake. A biospecimen collection began in 1996, with 29 611 women free of cancer and responding to the 1995 questionnaire consenting and providing blood samples. This study has been approved by the Institutional Review Board of the Partners Health Care System (Boston, MA), with participants' consent implied by the return of the questionnaires and blood samples.

ANALYTICAL POPULATION

A random subset of 179 incident-confirmed T2D cases (date of diagnosis >1 years from date of blood draw) was selected and matched to 179 non-T2D controls on age at blood draw in 5-year strata for metabolomic profiling among NHS II women reporting a history of GDM (see Fig. 1 in the online Data Supplement). Eligibility criteria included a history of GDM reported on the baseline (1989) or follow-up questionnaires *(5)* and having an available plasma sample from the biospecimen collection. We excluded 5 participants from this analysis with GDM occurring after blood draw, 4 with missing dietary data, and 2 with missing metabolite data, leaving 172 T2D cases and 175 controls for the current analysis. The mean (SD) age of T2D cases and controls at baseline blood draw was 43.0 (4.4) years, with a mean of 12.7 (6.6) years since first GDM pregnancy. The mean (SD) time to T2D diagnosis from blood draw for incident cases was 7.4 (3.6) years.

SAMPLE COLLECTION AND METABOLOMIC PROFILING

A blood collection kit was mailed to NHS II volunteers between 1996 and 1999, and samples were returned on ice via overnight courier to our laboratory and subsequently processed and stored in liquid nitrogen freezers *(6)*. A questionnaire with the blood collection kit captured information including the date and time of blood draw, number of hours since eating before blood draw, current medication use, and current body weight. Case–control pairs were randomly selected among eligible samples, and aliquots were shipped to the Broad Institute of MIT and Harvard (Cambridge, MA) on dry ice for metabolomic profiling. High-throughput LC-MS techniques were used to profile the plasma levels of untargeted polar metabolites *(7, 8)*. The interassay reproducibility among stored NHS plasma samples was previously estimated with excellent CVs for isoleucine (1.4%), leucine (3.6%), and valine (2.2%) *(9)*. Data processing was conducted with MultiQuint Software (AB SIEX) to integrate chromatographic peaks.

DIETARY ASSESSMENT

Diet was assessed every 4 years via a 131-item semiquantitative FFQ, asking about usual intake over the past year. The nutrient content of foods and beverages was derived according to the US Department of Agriculture database, food manufacturer data, and other published resources. A previous validation study for the derived dietary intake of protein observed a correlation coefficient of 0.4 for FFQ compared with diet records *(10)*.

T2D RISK FACTOR ASSESSMENT

Height reported at baseline and body weight at blood draw were used to derive body mass index (BMI) (kg/m^2). Current use of menopausal hormone therapy was ascertained at blood collection. Fasting status at blood draw was defined as at least 8 h since eating (76% were fasting). Race/ethnicity was self-reported at baseline in 1989. Time-varying characteristics, including family history of diabetes, alcohol and dietary intake, usual physical activity, smoking status, and reproductive and health-related characteristics were assessed every 2 to 3 years and derived from the most recent biennial questionnaire preceding blood draw. We calculated individuals' score of adherence to the 2010 Alterative Healthy Eating Index 2010 dietary pattern (AHEI-2010), based on intakes of healthful and unhealthful factors, with possible scores ranging from 2.5 to 87.5 *(11)*. We previously observed

this dietary pattern was associated with lower risk of progression from GDM to T2D in NHS II *(11)*. Total physical activity was captured as the frequency in engaging in common recreational activities, and converted into total metabolic equivalent tasks (MET-hours) per week *(12)*.

STATISTICAL ANALYSIS

Intakes of the BCAAs isoleucine, leucine, and valine were energy-adjusted using the residual method *(13)*. We averaged the dietary intakes reported on the 1995 and 1999 FFQs to estimate long-term usual diet over the period in which blood samples were collected. Plasma concentrations were natural log-transformed to improve normal distribution and standardized. Pearson correlation coefficients were derived to compare dietary intakes vs circulating plasma BCAA concentrations. We compared characteristics at time of blood draw for incident T2D cases vs controls with 2-sided *t*-tests and χ^2 tests.

Age- and multivariable-adjusted conditional logistic regression models estimated the odds ratios (ORs) and 95% CIs for dietary and plasma concentrations of BCAAs categorically across quartiles (Q1–Q4), with the first quartile as the reference group. We determined the quartile cutpoints on the overall study population combined, given the high incidence of T2D among women with GDM. We also evaluated continuous exposures per 1 SD. The multivariable models adjusted for traditional T2D risk factors captured at the time of blood draw, including age, BMI (kg/m^2), family history of T2D, total physical activity (MET-hour/week), current smoking status, white race/ethnicity, AHEI-2010 diet quality score, and alcohol intake (g/day), as well as fasting status at blood draw, total calorie intake, and current menopausal hormone therapy use. Stratified analyses were conducted to evaluate potential effect modification by family history of diabetes (yes vs no), BMI (nonobese BMI $<$25 kg/m^2 vs overweight/obese BMI $\geq$25 kg/m^2), and physical activity level (above vs below median MET-hour/week). The continuous multivariable models of dietary intake and plasma levels were further mutually adjusted for one another in analytical sensitivity analyses to determine the extent to which the relationships with T2D were independent of the other.

We further conducted joint classification of participants according to being above/below the medians for diet and plasma BCAA concentrations to evaluate discordance in relation to T2D risk. Participants with low intake/low plasma concentrations served as the reference group. Statistical tests for interaction were performed with likelihood ratio tests comparing the multivariable models with and without the multiplicative interaction term.

We performed analytical sensitivity analyses to assess the robustness of our findings by repeating analyses restricting to fasting samples only and by excluding extreme 1% of outliers for both diet and plasma concentrations. Finally, we further adjusted our multivariable models controlling for nutrients that often coexist in foods rich in BCAAs and related to T2D risk, including intakes of total animal fat, trans fat, heme iron, and cereal fiber (g/day). All analyses were conducted using SAS® (version 9.3 for UNIX, SAS Institute), using 2-sided statistical tests with level of significance at $P < 0.05$.

ROLE OF THE FUNDING SOURCE

The funding sources had no role in the design, data collection, analysis, or interpretation of the data.

Results

In our nested case–control study population of women with a history of GDM, BMI at blood draw was higher among women who developed T2D during the follow-up period compared with controls (31.6 vs 25.5 kg/m^2; $P < 0.0001$) with a 2-fold higher prevalence of overweight/obesity (86% vs 42%; $P < 0.0001$) (Table 1). Cases were also more likely to report a family history of T2D (50% vs 39%; $P = 0.048$) and less likely to be fasting at blood draw (71% vs 81%; $P = 0.036$). Other characteristics were similar between cases and controls, including overall AHEI-2010 diet quality score, total physical activity, menopausal status, parity, and breastfeeding history.

Dietary intakes of individual BCAAs modestly correlated positively with circulating plasma levels: isoleucine, $r = 0.15$ ($P = 0.004$); leucine, $r = 0.19$ ($P = 0.0003$); and valine, $r = 0.19$ ($P < 0.0001$) (see Table 1 of the online Data Supplement). Greater dietary intakes (Table 2) and plasma BCAA concentrations (Table 3) were significantly associated with an increased risk of T2D in the age-adjusted model, with total dietary (energy-adjusted g/day) and plasma BCAAs associated with 4-fold greater odds of developing T2D comparing Q4 vs Q1 (diet: OR = 3.3, CI = 1.7, 6.4, *P* trend = 0 $<$ 0.001; plasma: OR = 7.3, CI = 3.3, 16.2, *P* trend $<$ 0.001). Adjusting for traditional T2D risk factors, including overall diet quality and BMI, attenuated the association between plasma BCAAs with T2D, although both diet and plasma concentrations remain significantly associated with increased odds of T2D when comparing extreme quartiles (diet: OR = 4.6, CI = 1.6, 13.4, *P* trend = 0.01; plasma: OR = 4.4, CI = 1.4, 13.4, *P* trend = 0.002). The magnitudes of association for individual BCAAs with T2D were similar in magnitude to total BCAAs, and all *P* values for tests of linear trend indicated significant positive associations between diet and plasma BCAAs with odds of progression from GDM to T2D. Results from modeling the exposures as continuous variables demonstrated similar findings (see Fig. 2 in the online Data Supplement). Further adjusting diet and

Table 1. Characteristics of incident T2D cases and controls at baseline blood draw among women with a history of GDM in the NHS II cohort.

Characteristics at baseline blood draw	Incident T2D status	
	Control (n = 175)	T2D case (n = 172)
Age, years	43.0 (4.4)[a]	43.0 (4.4)
BMI, kg/m^2	25.5 (5.4)	31.6 (6.0)[b]
BMI < 25 kg/m^2, %	58.3	14.0[b]
BMI ≥ 25 kg/m^2, %	41.7	86.1
Family history diabetes, %	39.4	50.0[c]
Years since first GDM pregnancy	12.9 (7.0)	12.3 (6.1)
Age at first birth, years	26.9 (5.1)	26.8 (4.9)
Parity (pregnancies ≥6 months)	2.5 (1.0)	2.6 (1.0)
Total breastfeeding, months	17.3 (15.4)	15.7 (13.2)
Menopausal status, %		
Premenopausal	77.7	80.2
Postmenopausal, no current hormone therapy	9.7	8.1
Postmenopausal, current hormone therapy	12.6	11.6
Smoking status, %		
Never smoker	68.0	70.4
Past or current smoker	32.0	29.7
White race/ethnicity, %	94.3	90.7
AHEI-2010 diet quality score	49.0 (9.2)	47.6 (10.0)
Total physical activity, MET-hour/week	15.6 (15.2)	13.9 (14.7)
Plasma BCAAs[d]		
Fasting status (>8 h from last eating), %	80.6	70.9[c]
Total BCAAs	−0.98 (2.5)	1.0 (3.0)[b]
Isoleucine	−0.32 (0.9)	0.35 (1.0)[b]
Leucine	−0.31 (0.9)	0.32 (1.0)[b]
Valine	−0.35 (0.8)	0.37 (1.0)[b]
Dietary intake, g/day[e]		
Total BCAAs	14.9 (2.3)	15.9 (2.4)[b]
Isoleucine	3.9 (0.6)	4.2 (0.7)[b]
Leucine	6.6 (1.0)	7.0 (1.0)[b]
Valine	4.4 (0.7)	4.6 (0.7)[b]

[a] Values are mean (SD) unless otherwise noted.
[b] $P < 0.01$ for controls vs T2D cases.
[c] $P < 0.05$ for controls vs T2D cases.
[d] Log-transformed and standardized.
[e] Adjusted for total energy intake.

plasma concentrations for each other did not modify the estimates.

There was a nonsignificant trend for an interaction between dietary intakes and circulating BCAA concentrations (Fig. 1), with the highest odds of T2D observed among women with both high dietary intake and high plasma concentrations, compared with low intake/low plasma (*P* values for diet–plasma interaction, total BCAAs = 0.22, isoleucine = 0.20, leucine = 0.13, valine = 0.084). High total dietary BCAA intake was associated with T2D risk only among women with high plasma concentrations (vs low diet/low plasma OR = 6.0, CI = 2.1, 17.3) but not among women with low plasma (OR = 1.6, CI = 0.6, 4.1). Conversely, women with high total BCAA plasma concentrations had an increased T2D risk, even for women reporting low dietary

Table 2. Quartiles of dietary intake of BCAAs in relation to T2D among women with previous GDM.[a]

Dietary intake (energy-adjusted), g/day	Q1 OR (95% CI)	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)	*P* for trend
Isoleucine					
Age-adjusted model	[ref]	1.12 (0.59, 2.13)	1.72 (0.95, 3.12)	3.48 (1.76, 6.88)	0.0002
Multivariable-adjusted model	[ref]	2.20 (0.81, 5.93)	1.77 (0.74, 4.20)	5.78 (1.86, 17.95)	0.004
Leucine					
Age-adjusted model	[ref]	1.19 (0.64, 2.21)	1.65 (0.92, 2.94)	2.93 (1.56, 5.53)	0.0007
Multivariable-adjusted model	[ref]	2.41 (0.87, 6.68)	1.35 (0.58, 3.16)	4.66 (1.60, 13.53)	0.01
Valine					
Age-adjusted model	[ref]	1.45 (0.77, 2.74)	1.47 (0.82, 2.65)	3.04 (1.58, 5.82)	0.001
Multivariable-adjusted model	[ref]	3.01 (1.06, 8.51)	1.35 (0.58, 3.16)	5.71 (1.85, 17.60)	0.009
Total BCAAs					
Age-adjusted model	[ref]	1.35 (0.72, 2.52)	1.55 (0.86, 2.82)	3.33 (1.73, 6.39)	0.0003
Multivariable-adjusted model	[ref]	2.29 (0.85, 6.15)	1.49 (0.63, 3.52)	4.63 (1.61, 13.36)	0.01

[a] Conditional logistic regression multivariable model adjusts for age (continuous), total calorie intake (continuous), fasting status ≥8 h (yes/no), alcohol intake g/day (continuous), family history of diabetes (yes/no), menopausal status and current menopausal hormone therapy use (premenopausal, postmenopausal use, yes/no), total physical activity MET-hour/week (continuous), smoking status (ever/never), white race/ethnicity (yes/no), BMI kg/m^2 (continuous), AHEI-2010 adherence dietary quality score (continuous). ref, reference category.

intake (OR = 3.2, CI = 1.2, 9.1). Trends were similar for individual BCAAs.

There was no statistically significant effect modification for associations of diet or plasma concentrations with T2D by BMI status (*P* values for interaction: diet = 0.88, plasma = 0.67), physical activity level (*P* values for interaction: diet = 0.95, plasma = 0.61), or family history of T2D (*P* values for interaction: diet = 0.05, plasma = 0.59) (see Fig. 3 in the online Data Supplement). Excluding nonfasting samples (n = 84) and data at the extremes (1 and 99 percentiles) of the dietary and plasma distributions did not appreciably affect the results

Table 3. Quartiles of circulating plasma BCAAs in relation to T2D among women with previous GDM.[a]

Plasma levels	Q1 OR (95% CI)	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)	*P* for trend
Isoleucine					
Age-adjusted model	[ref]	2.37 (1.23,4.57)	3.38 (1.65, 6.92)	8.30 (3.80,18.12)	<0.0001
Multivariable-adjusted model	[ref]	1.50 (0.61,3.71)	2.94 (1.06, 8.20)	4.54 (1.56,13.17)	0.003
Leucine					
Age-adjusted model	[ref]	2.43 (1.19,4.96)	3.22 (1.58, 6.58)	7.75 (3.50,17.17)	<0.0001
Multivariable-adjusted model	[ref]	1.26 (0.45,3.56)	2.29 (0.77, 6.79)	5.83 (1.83,18.62)	0.001
Valine					
Age-adjusted model	[ref]	1.87 (0.92,3.81)	5.15 (2.41,11.02)	8.44 (3.76,18.94)	<0.0001
Multivariable-adjusted model	[ref]	1.54 (0.58,4.10)	4.05 (1.35,12.11)	5.46 (1.76,16.96)	0.0008
Total BCAAs					
Age-adjusted model	[ref]	2.29 (1.15,4.55)	4.87 (2.24,10.59)	7.32 (3.30,16.24)	<0.0001
Multivariable-adjusted model	[ref]	1.25 (0.48,3.29)	3.81 (1.22,11.95)	4.38 (1.43,13.39)	0.002

[a] Conditional logistic regression multivariable model adjusts for age (continuous), total calorie intake (continuous), fasting status ≥8 h (yes/no), alcohol intake g/day (continuous), family history of diabetes (yes/no), menopausal status and current menopausal hormone therapy use (premenopausal, postmenopausal use, yes/no), total physical activity MET-hour/week (continuous), smoking status (ever/never), white race/ethnicity (yes/no), BMI kg/m^2 (continuous), AHEI-2010 adherence dietary quality score (continuous). ref, reference category.

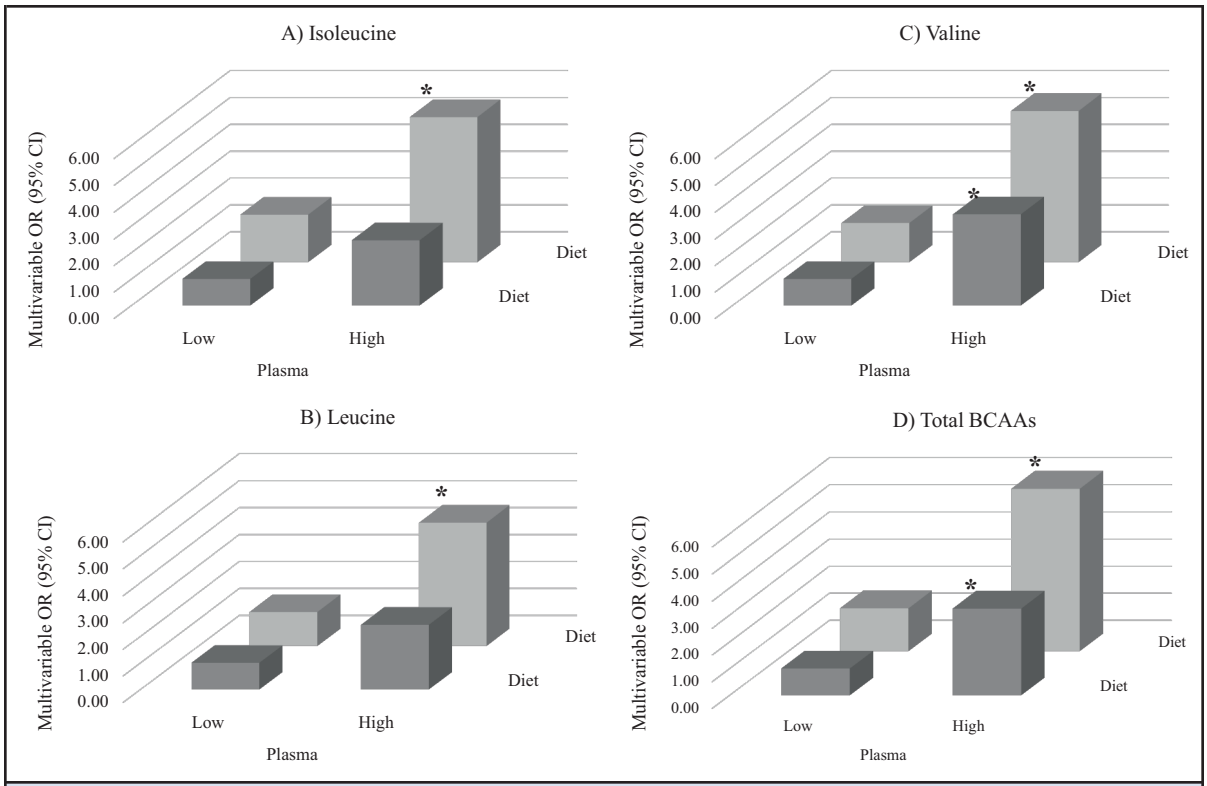

Fig. 1. Joint relationship between low (below median) vs high (above median) dietary intakes and circulating metabolite levels of (A) isoleucine, (B) leucine, (C) valine, and (D) total BCAAs with T2D risk, among women with a history of GDM.

Dietary BCAA intakes are energy-adjusted. Bars represent ORs. Asterisk indicates statistical significance at $P < 0.05$ vs the reference category of low diet/low diet. Conditional logistic regression multivariable model adjusts for age (continuous), total calorie intake (continuous), fasting status ≥8 h (yes/no), alcohol intake g/day (continuous), family history of diabetes (yes/no), menopausal status and current menopausal hormone therapy use (premenopausal, postmenopausal use yes/no), total physical activity MET-hour/week (continuous), smoking status (ever/never), white race/ethnicity (yes/no), BMI kg/m^2 (continuous), and AHEI-2010 adherence dietary quality score (continuous).

(data not shown). In addition, further adjusting for heme iron, trans and animal fats, and cereal fiber intakes did not change the multivariable model estimates (see Table 5 in the online Data Supplement).

Discussion

The present study was a prospective study to evaluate both dietary intakes and circulating concentrations of BCAAs simultaneously. Overall, we observed positive relationships between dietary intakes and circulating plasma concentrations of isoleucine, leucine, and valine with incident T2D among women with a history of GDM, indicating a striking 4- to 6-fold increased risk for those at the highest vs lowest quartiles. Analyses classifying women according to being above/below the medians for both diet and plasma concentrations supported that an increased T2D risk was limited to women with high plasma concentrations, with the highest risk observed for women with both high dietary intakes and high circulating plasma concentrations. Further, high dietary BCAA intake was not associated with T2D risk among women with low circulating plasma concentrations.

Previous studies for the relationship between dietary BCAA intake and T2D risk are limited, with conflicting results and all among the general population. In an analysis combining data across multiple US cohorts, including participants from the NHS II (not specifically with a history of GDM), long-term dietary intake of total BCAAs was associated with a modest 8% increased T2D risk, comparing the Q5 vs Q1 of energy-adjusted g/day (P for trend = 0.002) *(14)*. Conversely, a population-based Japanese cohort observed an inverse association between baseline dietary BCAAs (as a percent of calories from protein) with T2D risk in women over 10 years of

follow-up *(15)*. Notable differences in major dietary protein sources of BCAAs between the study populations were discussed, with meat being the top contributor in the US cohorts, whereas cereals, potatoes, and starches were among the top contributors in the Japanese cohort. Thus, constituents of the sources of BCAAs, preparation methods, or correlated dietary factors, rather than BCAAs themselves, may lead to increased T2D risk. In our population, we previously observed positive associations between dietary iron, animal fat, and animal protein intakes and risk of progression from GDM to T2D in the NHS II study population *(16, 17)*. Vegetable sources of protein were not associated with T2D risk in GDM women *(17)*. However, further adjusting for heme iron and trans and animal fats in the present analysis did not impact effect estimates for the associations between dietary BCAAs and T2D.

We are unaware of previous studies on circulating BCAA concentrations and T2D risk among high-risk women with a history of GDM. The significant and positive association we observed is consistent with the previously published prospective studies of non-GDM populations but 2-fold greater in the magnitude of the association. For instance, in a recent metaanalysis, isoleucine, leucine, and valine were associated with 36%, 36%, and 35% greater odds of T2D, respectively, per 1 SD in plasma concentrations *(3)*. The corresponding estimates per 1 SD in the present study were 87%, 77%, and 71%, respectively. Accumulating evidence supports the hypothesis that increased circulating concentrations of BCAAs directly affect the development of insulin resistance and T2D, possibly by increasing the presence of toxic BCAA intermediate metabolites, which in turn interfere with β-cell mitochondrial function *(18)*. Further, obesity may lead to decreased expression of BCAA metabolism genes in adipose tissue; thus, increased BCAAs may be a component of the causal pathway between obesity and T2D *(18)*.

Women with a history of GDM represent a subgroup at high risk of developing T2D later in life, and as such may have underlying impaired metabolism several years before T2D onset and diagnosis. We observed that relatively high BCAA concentrations, despite being below the median for dietary intake, conferred increased T2D risk and, thus, may signal this developing pathology. A recent Mendelian randomization study supports the hypothesis that the underlying rate of BCAA metabolism contributes to T2D development, indicating that specific genomic predictors of circulating BCAA pathway metabolites were associated with an increased T2D risk *(19)*. However, <8% of the heritability for isoleucine, leucine, and valine could be explained by the genetic variants identified, suggesting additional and potentially modifiable factors may have a greater influence on BCAA concentrations. Further research is needed to identify determinants of BCAA metabolite concentrations.

We did not observe a T2D risk for the subgroup of women with GDM who had above median dietary BCAA intake but low relative metabolite concentrations. It is possible that preventive strategies aimed at improving BCAA catabolism and clearance may help to mitigate risk. Two randomized weight-loss intervention trials demonstrated an effect of weight loss on lowering circulating BCAA metabolites *(20)*. Additionally, an aerobic exercise training intervention induced greater plasma BCAA turnover and increased insulin sensitivity among overweight trained participants vs overweight untrained individuals over 6 months *(21)*. A nonrandomized intervention study also observed lowered plasma BCAAs when healthy participants abstained from animal products and followed a modified vegan diet allowing fish intake *(22)*. Interestingly, BCAA metabolite concentrations of obese women receiving whey protein supplements, enriched in BCAAs, did not differ from a group receiving protein-matched gelatin supplements after 8 weeks. Plasma BCAA metabolite concentrations, therefore, constitute biomarkers beyond dietary intake, capturing a constellation of T2D-related mechanistic pathways even several years before diagnosis and, importantly, may be amenable to lifestyle interventions.

Strengths of this study include its prospective nested case–control design, allowing for the ascertainment of exposures before T2D diagnosis, mitigating the influence of the outcome and related treatments on participants' metabolomic profiles. Limitations of this analysis include our measurement of plasma BCAA at a single time point, which may be less representative of individuals' long-term metabolome status, and the mail-based blood collection susceptible to processing delays. However, a pilot study in the similar NHS cohort observed good within-person reproducibility for samples collected 1 year apart, with intraclass correlations of 0.56, 0.44, and 0.58 for isoleucine, leucine, and valine, respectively *(9)*. There was also little impact of sample processing delays for isoleucine, leucine, and valine metabolites, all with interclass correlation coefficients >0.86 comparing immediate vs delayed (>24 h) processing times. Assessment of long-term usual dietary intake via FFQ may be prone to measurement error, although the performance of the FFQ to estimate nutrient intake has been extensively validated against multiple-week diet records. Additionally, we derived dietary BCAAs from the cumulative mean of 2 FFQs to minimize measurement error. Random measurement error in the diet and plasma BCAA measurements may lead to our underestimation of their relationship with T2D. Residual and unmeasured confounding by other dietary components or T2D risk factors may be possible. The relatively racially/ethnically homogenous study population and small sample size preclude subgroup analyses to investigate potential effect modifica-

tion. Replication of these findings in a separate cohort would enhance the validity of our results.

Summary

Our prospective nested case–control study indicates that higher dietary and circulating concentrations of isoleucine, leucine, valine, and total BCAAs are related to a greater risk of progression from GDM to T2D later in life. Uncovering dietary and/or plasma BCAA's role in T2D pathophysiology may help to develop targeted therapies and identify high-risk individuals. Follow-up studies are warranted to determine whether interventions, including lifestyle modifications, lead to reductions in isoleucine, leucine, or valine metabolite levels and subsequently reduced T2D risk.

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