Longitudinal associations of branched-chain amino acids with type 2 diabetes and markers of insulin secretion and sensitivity in Japanese Americans

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Abstract

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Background and aims: To prospectively investigate the associations of branched-chain amino acids (BCAA) with incident diabetes and estimates of insulin secretion and sensitivity.
Methods and results: We conducted a secondary analysis among participants free from diabetes in the Japanese American Community Diabetes Study (n=349). For the biomarker study, participants were assessed for diabetes at baseline and 5–6 years of follow-up and estimates of insulin sensitivity and secretion were obtained. Baseline plasma samples in adults free from diabetes were assayed for BCAA levels. We fit logistic regression models to examine associations of BCAA with incident diabetes at 5–6 years and linear regression models to examine associations with estimates of insulin secretion (oral disposition index, oDI) and insulin sensitivity (Matsuda index and HOMA2-%S) at baseline and 5–6 years. Models were adjusted for age, sex, BMI, and smoking status (Model 2); and for age, sex, BMI, smoking status, and family history of T2D (Model 3). For the oDI and Matsuda index, we fit sex-stratified models and examined whether associations depended on sex on a multiplicative scale.

Results: The mean age was 56.5. 50.7% (n=177) were men. Higher valine was associated with higher odds of incident diabetes (OR for a 1SD change 1.06, 95% CI 1.03, 1.09, Model 2). After adjustment, no BCAA was associated with oDI at baseline or follow-up. Higher isoleucine was associated with lower Matsuda index at baseline (β for 1SD change -0.02, 95%CI – (0.03, -0.01, Model 2)). Higher isoleucine and valine were associated with lower HOMA2-%S at baseline (β for 1SD change -0.43, 95%CI (-0.62, -0.23), and -0.58, 95%CI (-0.82, -0.35), Model 2, respectively). No BCAA was associated with Matsuda index or HOMA2%S at follow-up. Associations were similar in models with and without adjustment for family history of T2D. In men, no BCAA was associated with oDI or Matsuda index at baseline. In women, higher isoleucine was associated with lower Matsuda index at baseline (β for 1SD change: -0.04, 95%CI (-0.06, -0.02), Model 2, p for interaction >0.05).

Conclusion: Higher valine was associated with a higher risk of incident diabetes at 5–6 years. Higher isoleucine and valine were associated with lower insulin sensitivity (isoleucine with Matsuda index and HOMA2-%S, valine with HOMA2-%S only) at baseline but not at follow up. Results were similar with and without adjustment for family history of T2D. Associations may differ by sex. More research is needed to characterize the roles of sex and genetic variation in associations of circulating BCAA with diabetes risk and insulin secretion and sensitivity measures.

1. Background and Significance:

In 2021, type 2 diabetes (T2D) affected 11.6% of Americans[1] and is predicted to affect 783 million people worldwide by 2045[2]. Relatively deficient insulin secretion by the pancreatic β cell is necessary for the pathogenesis of T2D[3], but defects in insulin sensitivity ("insulin resistance") are also common. Risk factors for T2D are many and complex, including diet, physical activity, age, body composition, the environment, and genetics[4]. Factors that contribute to defects in insulin secretion or sensitivity could provide insight into the pathogenesis of T2D.

Branched-chain amino acids (BCAA) are essential amino acids and consist of isoleucine, leucine, and valine. These play a role in glucose metabolism and insulin action[6]. There is evidence of BCAA enhancing glucose uptake via up-regulation of glucose transporters: leucine has been noted to be involved in translocation of the glucose transporters $GLUT_1$ and GLUT4 in rat models[20]. Higher levels of BCAA activate the mTOR complex 1, resulting in insulin resistance (HOMA-IR[21]). These metabolites can also be measured in the circulation. Thus, BCAA may be circulating biomarkers that precede the development of diabetes or deficits in insulin secretion/sensitivity and assist in early diagnosis.

Mendelian randomization analyses have suggested that higher concentrations of total BCAA are associated with insulin resistance[13]. There are, however, important gaps in our understanding of associations of circulating BCAA with incident diabetes, and with measures of insulin secretion and sensitivity. Previous studies have included mainly individuals of European ancestry[14], have relied on fasting measures of insulin secretion and sensitivity, have not evaluated the role of family history of diabetes, and have not

evaluated whether associations of BCAA with diabetes risk, insulin secretion, or insulin sensitivity differ between men and women. To our knowledge, this is the first study to examine associations of BCAA levels with estimates of insulin secretion and sensitivity in Japanese Americans. Additionally, it is the first study to examine associations of BCAA with estimates of β-cell function (oral disposition index (oDI)) and insulin sensitivity (Matsuda index) that do not rely only on fasting glucose and insulin values. We also will analyze the impact of family history on these associations; and evaluate whether associations depend on sex. We hypothesize that higher levels of BCAA will be associated with a higher odds of incident diabetes, and that higher BCAA levels will be associated with lower insulin secretion and sensitivity measures. We also hypothesize that we will see the association of BCAA with insulin secretion and sensitivity measures attenuated when adjusted for family history. Finally, we hypothesize that we will see a difference in association of BCAA with insulin secretion and sensitivity measures when we stratify by sex.

2. Methods:

2.1 Study setting and population

The Japanese American Community Diabetes Study (JACDS) is a prospective, longitudinal[8], community-based cohort of second- and third-generation Japanese Americans of 100% Japanese ancestry in King County, Washington. Its methods have been described previously[8, 9]. Briefly, participants were second- (Nisei) and third generation (Sansei) Japanese Americans of 100% Japanese ancestry living in King County, Washington [8]. It was designed to investigate the risk factors for and prevalence and incidence of T2D and related conditions in Japanese Americans.

Participants were recruited between 1983 and 1991, both male and female, and between the ages of 34-76. Follow-up assessments were conducted at 5–6 and 10–11 years[10, 11]. This current analysis is part of the JACDS Biomarker Discovery Project (BDP). In the BDP, banked samples were used for biomarker analysis. No stored fasting plasma samples were available from the original (entry) visit. Thus, for this analysis, fasting samples from the JACDS 5- to 6-year visit were analyzed. This visit is termed the BDP baseline visit. The JACDS 10- to 11-year visit is termed the BDP follow-up visit. The BDP cohort comprises all participants with available fasting plasma samples at the 5–6-year study visit (n=391). We excluded 34 participants with diabetes at the BDP baseline visit. Seven participants were excluded for missing data at baseline and 1 participant was excluded for implausible data (a fasting insulin of 99 pmol/L, which may represent a code for missing data). Therefore, the final study population resulted in n = 349.

2.2 Exposures

Evaluations were performed at the General Clinical Research Center at the University of Washington, Seattle[9]. Fasting plasma samples were collected by trained phlebotomists and stored at -80° C. BCAA levels were measured via mass spectrometry: Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry analysis was used for plasma samples, using an AB Sciex quadrupole mass spectrometer 6500[9]. Sociodemographic data and family history were collected by trained interviewers[9].

2.3 Outcomes

At the BDP baseline and follow-up visits, a 75-gram OGTT was performed after a 10 h overnight fast, and samples were collected just before and at 30, 60, and 120 min after ingestion of the oral glucose load for measurement of glucose and insulin levels[10,] Plasma glucose was measured by the glucose oxidase method. Plasma insulin was measured using a modified double-antibody radioimmunoassay as described previously[16]. Diabetes diagnosis was based on fasting plasma glucose ≥126 mg/dl, 2 h plasma glucose ≥200 mg/dL[33] ,or self-report of use of glucose-lowering medications. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. The study was approved by the University of Washington Institutional Review Board and all participants provided written informed consent[7,11].

2.4 Calculations

To estimate β-cell function and insulin secretion, we calculated the oral disposition index (oDI), a composite measure of β-cell function[15], from the formula described by Utzschneider et al., (2009): $\Delta I_{0\text{-}30}/\Delta G_{0\text{-}30}$ * 1/fasting insulin[16]. To estimate insulin sensitivity, we calculated the Matsuda index and the HOMA2-%S (homeostasis model assessment of insulin sensitivity). The Matsuda index was calculated by the following formula: 1000/ sqrt([(fasting glucose * fasting insulin) * (mean glucose * mean insulin)]. Another insulin sensitivity measure, HOMA2-%S was calculated with the Web-based HOMA calculator, providing insulin sensitivity as a percentage (https://www2.dtu.ox.ac.uk/homacalculator/).

2.5 Statistical Analysis

Descriptive analyses of baseline characteristics were performed to estimate means and standard deviations. We fit logistic regression models to examine the associations of each BCAA with odds of incident diabetes at follow-up. Odds ratios with 95% CI are reported. We fit linear regression models to examine the association of BCAA with a one standard deviation increment in measures of insulin secretion and sensitivity at baseline and follow-up, and report regression coefficients with 95% CIs. For each analysis, we fit unadjusted models (Model 1); models adjusted for age, sex, BMI, and smoking status (Model 2); and models adjusted for age, sex, BMI, smoking status, and family history (Model 3). For models examining associations of BCAA with measures of insulin secretion or sensitivity at five years, we additionally adjusted for the baseline level of the corresponding BCAA measure. We fit models to examine first-order multiplicative interactions of BCAA with sex. For these models we fit an unadjusted model (Model 1) and a model adjusted for age, BMI, smoking status, and family history (Model 2). P-values <0.05 were reported as statistically significant. Data analysis was performed with R Studio Version 2023.09.0+463.

3. Results

Participants at baseline were 56.5 years old (±11.1) on average and had a mean BMI of 24.6 kg/m^2 (±3.48). 50.7% were men (Table 1). Women had a higher mean HOMA2-%S, Matsuda index and oDI. Men had higher levels of isoleucine, leucine, and valine. When stratified by incident diabetes at follow-up, 58 participants developed diabetes while 325 did not (Table 2). A higher proportion of participants who developed diabetes at follow-up had a family history of diabetes, higher mean BMI, higher mean BCAA levels, and lower mean oDI, Matsuda index and HOMA2-%S (Table 2).

In unadjusted models, valine only was associated with odds of incident diabetes at follow-up (Table 3). This association persisted after adjustment for age, sex, BMI, and smoking status (OR for a 1SD greater valine level 1.06, 95% CI (1.03, 1.09)). In the model adjusted for family history (Model 3), the magnitude of association was similar, but the association between valine and incident diabetes was no longer statistically significant at $\alpha<0.05$ (OR 1.05, 95% CI (1.02, 1.08)). In adjusted models, neither isoleucine nor leucine was associated with odds of incident diabetes at follow-up (Table 3).

In unadjusted models, higher levels of isoleucine, leucine, and valine were significantly and inversely associated with the oDI at baseline only (β for a 1SD change -0.02, 95% CI (-0.04, -0.01); -0.03, 95% CI (-0.04, -0.02); β -0.04, 95%CI (-0.06, -0.03), respectively). In adjusted models, no BCAA were associated with the oDI. (Table 4).

In unadjusted models, isoleucine, leucine, and valine were significantly and inversely associated with the Matsuda index, at both baseline and follow up (Table 5). In adjusted models, a higher isoleucine level was inversely and significantly associated with the Matsuda index at baseline only (β for a 1SD change -0.02, 95% CI (-0.03, -0.01)). (Model 2). In the model adjusted for family history (Model 3) isoleucine remained significantly associated with the Matsuda index at baseline only (Table 5).

In unadjusted models, isoleucine, leucine, and valine were significantly associated with HOMA2-%S at both baseline and follow-up (Table 6). In adjusted models (Model 2), higher plasma levels of isoleucine and valine were associated with HOMA2-%S at baseline only (β -0.43, 95% CI (-0.62, -0.23), β -0.58, 95% CI (-0.82, -0.35), respectively). In the model adjusted for family history (Model 3), both isoleucine and valine remained significantly associated with HOMA2-%S at baseline only (Table 6).

In unadjusted and adjusted models among men only, no BCAA were significantly associated with the oDI at baseline (Table 7). In the unadjusted model among men, higher levels of valine were associated with the Matsuda index at baseline (β -0.04, 95% CI (-0.05, -0.02)). In the adjusted model among men, no BCAA were significantly associated with the Matsuda index at baseline (Table 8).

In the unadjusted model for women, higher levels of leucine and valine were significantly associated with the oDI at baseline (Table 7). In the adjusted model, no BCAA were significantly associated with the oDI at baseline (Table 7). In the unadjusted model among women, higher levels of isoleucine, leucine and valine were associated with the Matsuda index at baseline (Table 8). In the adjusted model among women, higher levels of isoleucine were associated with the Matsuda index at baseline (β -0.04 (-0.06, -0.02)) (Table 8).

P-values for sex*BCAA term in relation to the oDI at baseline were not significant. (Table 7). P-values for sex*BCAA terms in relation to Matsuda index at baseline were significant for isoleucine (p for interaction= 0.022) (Table 8). The p-value for sex*leucine was also statistically significant (p=0.010); however, leucine was not significantly associated with Matsuda index in men or women (Table 8).

4. Discussion

4.1 Summary

In this prospective longitudinal analysis of Japanese Americans without diabetes at baseline, we found that higher levels of valine were associated with higher risk of diabetes at follow-up independent of age, sex, BMI, and smoking status. At baseline, higher levels of isoleucine were associated with lower Matsuda index, and higher levels of isoleucine and

valine were associated with lower HOMA2-%S. When stratified by sex, higher isoleucine was associated with lower Matsuda index at baseline in women but not men. Associations were similar with and without adjustment for family history of T2D.

To our knowledge, this study is the first to examine associations of plasma BCAA levels with insulin secretion and sensitivity using the oral disposition and Matsuda indexes among Japanese Americans. Previous studies found evidence for a positive association of all three BCAA with higher risk of diabetes[22]; however, the magnitude of the associations varied[22]. In a longitudinal study in Brazilian men and women (n=3828) investigators observed that individuals in the fourth total BCAA quartile level had a three-to-four-fold higher risk of diabetes in men and women than those in the first quartile [5]. In a prospective cohort study, women with gestational diabetes (n=172) were matched with a diabetes-free control group (n=175)[12]. Researchers reported that women with the highest total plasma BCAA concentrations (4th quartile) had four-fold greater risk of diabetes development compared to the first quartile[12]. In the PREVEND cohort study in the Netherlands (n=6244), individuals in the highest total BCAA quartile level had higher diabetes risk compared to the lowest quartile after adjustment for variables such as age, sex BMI and parental history of diabetes, resulting in a HR of 6.15[14].

We found that higher isoleucine was associated with lower Matsuda index at baseline. The Matsuda index utilizes fasting and stimulated glucose and insulin measurements to quantify whole body insulin sensitivity[24]. It measures hepatic and peripheral tissue insulin sensitivity[15], while HOMA measures hepatic insulin sensitivity, reported in other studies as an insulin resistance measure[30]. A cross-sectional study with Finnish men (n=9369) reported a strong inverse correlation with isoleucine and insulin

sensitivity measured with the Matsuda index[25]. This finding is similar to the inverse correlation we observed; however, we included both men and women in our analysis. Additionally, a cross-sectional study of participants without (n=15) and with obesity (n=33) concluded that individual BCAA were significantly associated with the Matsuda index[27]. Total summed BCAA were also reported to be significantly associated with the Matsuda index after multivariate modeling[27].

Both isoleucine and valine were associated with HOMA2-%S at baseline in adjusted models. HOMA2-%S provides another measure of insulin sensitivity[26] and is the inverse of HOMA2-IR. Higher HOMA2-IR has been linked to greater odds of T2D development[26]. In the PREVEND cohort study, total BCAA were positively associated with HOMA-IR[14]. A cross-sectional study with non-diabetic Japanese participants (n=94) observed a positive association of isoleucine, leucine, and valine, as well as total BCAA, with HOMA-IR[30]. Our analysis yielded an inverse relationship between BCAA and HOMA2-%S, demonstrating the same relationship as these previous studies.

4.2 The role of family history in associations of BCAA with diabetes, insulin secretion, and insulin sensitivity:

We also found that associations of BCAA with diabetes, and measures of insulin secretion and sensitivity were similar with and without adjustment for family history of T2D. Family history of T2D in a first-degree relative is associated with about a three-fold increase in risk of T2D[19]. Studies with monozygotic twins have a higher concordance rate (70%) than dizygotic twins (30%), also arguing for inheritance as a risk factor for T2D[18]. GWAS studies have been completed with a number of different cohorts and have

identified common variants linked to the development of T2D[18]. A recent muti-ancestry genome-wide study reported 1,289 association signals or single nucleotide variants (SNVs), mapping to over 600 loci[23]. When combining these loci and looking at polygenic risk scores (PRS) it has been observed that those with higher PRS have a higher risk for T2D[18]. In a GWAS meta-analysis of Biobank Japan participants (36,164 cases and 155,150 controls), researchers observed 88 loci[19] with genome-wide significance[34]. 77% of these variants were seen to be common in both Japanese and European cohorts[34]. Additionally, among a multi-ethnic, genome-wide case control study, it was reported that novel loci had a smaller effect on T2D[17].

The PPM1K gene has been studied with its link to circulating BCAA levels and as a susceptibility gene for T2D[13]. This gene is involved in BCAA catabolism and activation of the branched-chain-alpha-ketoacid dehydrogenase complex (BCKD)[13]. This is the first step of BCAA metabolism and is irreversible[6,13]. Expression of PPM1K has an effect on insulin secretion[36]. Studies have shown that variants with increased BCAA levels have a higher risk for the development of T2D[4] – the genetic predisposition of increased BCAA levels is linked to a higher odds ratio for the development of T2D[13]. GWAS studies on plasma BCAA identified five genomic loci that were significantly associated with BCAA[13]. The strongest signal identified was 21 kb upstream from the PPM1K gene – here, the activation of the BCKD complex is encoded[13]. In a GWAS of European participants, the common variant rs144058, located near the PPM1K gene, was found to be associated with higher plasma levels of BCAA[32]. This variant was also utilized in a Chinese prospective cohort study. The researchers reported each C-allele in this variant was associated with a 20% increase in risk of T2D[32]. Also reported was the association of

rs144058 and BMI, and changes in insulin resistance[32]. Additionally, four genomic regions involved with BCAA catabolism and insulin resistance have been investigated in European populations[31] and four variants have been seen to have an association with BCAA levels at a fasting state[7]. Further research among other populations is needed to fully understand the genetic variation of BCAA and their potential link to T2D and insulin resistance.

4.3 Sex stratified analyses

We found some evidence that associations of BCAA with the Matsuda index differ by sex. In women, higher isoleucine was associated with lower Matsuda index at baseline. In men, no BCAA was associated with Matsuda index. Two Finnish studies, one cross sectional study (n=5471) and one prospective cohort study (n=2247), analyzed insulin resistance associations with metabolites. In the combined cohort (n=7098), BCAA showed sex-dependent results: among women only, BCAA showed associations with HOMA-IR, however this association was significant if they had abdominal obesity[28]. This same association was not seen among men[28]. A cross sectional study of Japanese participants without diabetes (n=94) analyzed the relationship between BCAA and insulin resistance using HOMA-IR[30]. They reported a positive association with HOMA-IR and isoleucine, leucine, and valine in women; and with isoleucine and leucine in men[30]. A study with 106 participants with obesity and 105 without obesity in China reported significant linear relationships of BCAA with HOMA-IR in both men and women, however the relationship was stronger in men[29].

4.4 Limitations

Our study has several limitations. First, our sample size may have limited our ability to detect differences of smaller magnitude than the significant differences we observed in this analysis. Second, we used family history as we did not have access to genetic information. Family history was self-reported as having a first degree relative with T2D. Thus, second degree and further relatives are not included, thereby limiting capture of inheritance. Additionally, no genetic data was collected in this study, therefore we cannot make connections between genetic markers as seen in previous studies. Further collection of genetic information and family history is needed in future research to enable more robust conclusions about the role of genetics in BCAA and insulin secretion and sensitivity. In this analysis, sex refers to sex assigned at birth as the data collected did not provide options beyond "men" and "women." Therefore, this conclusion is not representative of all sex categories and identities.

5. **Conclusion:**

In summary, in this longitudinal cohort of Japanese Americans, higher valine was associated with a higher risk of incident diabetes at 5–6 years. Higher isoleucine and valine were associated with lower insulin sensitivity at baseline but not at follow up (isoleucine with Matsuda index and HOMA2-%S, valine with HOMA2-%S only). Associations may differ by sex. Results were similar with and without adjustment for family history of T2D. We also observed sex-dependent associations with BCAA, as women had a significant association between isoleucine and the Matsuda index at baseline.

Associations of BCAA with measures of insulin secretion and sensitivity were not meaningfully attenuated when we adjusted for family history, which is a limited proxy for

genetic information. Further research is needed to fully understand the genetic component of BCAA association with insulin resistance. Examination of genes such as PPM1K and other significant genomic regions could provide insight into determinants of circulating BCAA levels and their impact on insulin secretion and sensitivity.

These findings were consistent with some of the previous literature and provide support for the use of BCAA as a biomarker for T2D development and insulin resistance – a useful public health tool that can assist with earlier diagnosis and intervention. Further research is necessary to determine if these findings differ among other populations.

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Table 1: Baseline characteristics of participants in the Japanese American Community Diabetes Study (JACDS) Biomarker Discovery Project participants, overall and stratified by sex, excluding participants who have diabetes at baseline

Characteristics	All (n = 349)	Men (n = 177)	Women (n = 172)	p-value
Age (years)	56.5 ± 11.1	56.3 ± 10.8	56.7 ± 11.5	0.698
Body mass index (kg/m^2)	24.6 ± 3.48	25.5 ± 3.10	23.7 ± 3.62	<0.001

Abdominal circumference (cm)	87.0 ± 9.64	89.5 ± 7.92	84.5± 10.6	<0.001
Intra-abdominal fat area (cm^2)	86.3 ± 44.2	98.5 ± 44.6	73.6 ± 40.1	<0.001
Family history of T2D (%)	32.66%	27.68%	37.79%	0.058
Fasting glucose (mg/dL)	96.8 ± 8.68	97.3 ± 8.81	96.3 ± 8.53	0.276
2-h glucose (mg/dL)	138 ± 28.4	134 ± 27.8	143 ± 28.5	0.006
HOMA2 -%S	69.9 ± 29.0	65.3 ± 27.3	74.1 ± 30.1	0.005
Fasting insulin (pmol/L)	15.2 ± 8.05	16.1 ± 8.61	14.2 ± 7.32	0.022
Matsuda index	3.27 ± 1.63	3.11 ± 1.49	3.43 ±1.75	0.069
Oral disposition index (mM^{-1})	1.49 ± 1.84	1.39 ± 2.13	1.59 ± 1.50	0.296
BCAA level (uM/L):				
Isoleucine	35.2 ± 7.18	37.4 ± 7.24	33.0 ± 6.39	< 0.001
Leucine	32.8 ± 6.41	33.6 ± 6.51	31.8 ± 6.20	0.008
Valine	32.4 ± 6.19	34.1 ± 6.24	30.6 ± 5.63	<0.001

Abbreviations: BCAA (branched chain amino acids), HOMA2-%S (homeostatic model assessment for insulin sensitivity)
Data are presented as mean ± standard deviation, SD for continuous variables and n, % for categorical variables
P-values are calculated using Welch's two sample t-test for continuous variables and Pearson's chi-squared test for categorical variables

Table 2: Baseline characteristics of participants in the Japanese American Community Diabetes Study Biomarker Discovery Project stratified by development of diabetes at follow-up, excluding participants who have diabetes at baseline

Characteristics	No diabetes (n = 325)	Diabetes (n = 58)	p-value
Age (years)	56.0 ± 11.1	60.6 ± 10.8	0.018
Male (%)	50.32%	54.05%	0.798
Female (%)	49.68%	45.95%	0.798
Body mass index (kg/m^2)	24.5 ± 3.42	25.9 ± 3.76	0.035
Abdominal circumference (cm)	86.6± 9.79	90.7 ± 7.49	0.005
Intra-abdominal fat area (cm^2)	83.6 ± 43.9	111 ± 39.3	<0.001
Family history of T2D (%)	30.44%	51.35%	0.017

Fasting glucose (mg/dL)	95.9 ± 8.18	104 ± 9.16	<0.001
2-h glucose (mg/dL)	135 ± 27.3	163 ± 25.6	<0.001
HOMA2-%S	71.3 ± 29.1	55.3 ± 24.5	<0.001
Fasting insulin (pmol/L)	14.7 ± 7.81	18.8± 9.15	0.013
Matsuda index	3.36 ± 1.62	2.49 ± 1.49	0.002
Oral disposition index (mM^{-1})	1.55 ± 1.92	1.03 ± 0.93	0.008
BCAA level (uM/L):			
Isoleucine	35.0 ± 7.21	37.1 ± 6.66	0.074
Leucine	32.6 ± 6.37	34.4 ± 6.65	0.116
Valine	32.2 ± 6.04	34.1± 7.21	0.124

Abbreviations: BCAA (branched chain amino acids), HOMA2-%S (homeostatic model assessment for insulin sensitivity)
Data are presented as mean ± standard deviation, SD for continuous variables and n, % for categorical variables
P-values are calculated using Welch's two sample t-test for continuous variables and Pearson's chi-squared test for categorical variables

Table 3: Unadjusted and adjusted associations of a one-standard deviation greater concentration of individual plasma branched chain amino acid levels with odds of **incident diabetes** at follow-up among JACDS participants

	Model 1		Model 2		Model 3	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Isoleucine	1.03 (1.01, 1.05)	0.126	1.02 (1.00, 1.04)	0.301	1.01 (0.99, 1.04)	0.528
Leucine	1.03(1.01, 1.05)	0.200	1.02 (1.00, 1.05)	0.311	1.02 (0.99, 1.04)	0.534
Valine	1.04(1.02, 1.07)	0.050	1.06 (1.03, 1.09)	0.028	1.05 (1.02, 1.08)	0.070

Abbreviations:
Model 1: Unadjusted
Model 2: Adjusted for age, sex, BMI, and smoking status
Model 3: Adjusted for age, sex, BMI and smoking status, and family history of diabetes
Unadjusted and adjusted associations with incident diabetes were tested individually for each BCAA.

Table 4: Unadjusted and adjusted associations of a one standard deviation greater concentration of individual plasma branched chain amino acid levels with oral disposition index (oDI) at baseline and follow-up among JACDS participants

	Model 1		Model 2		Model 3	

	Oral disposition at baseline					
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Isoleucine	-0.02 (-0.04, -0.01)	0.048	-0.01 (-0.02, 0.00)	0.439	-0.01 (-0.02, 0.01)	0.648
Leucine	-0.03 (-0.04, -0.02)	0.033	-0.02 (-0.03, 0.00)	0.222	-0.01 (-0.03, 0.00)	0.340
Valine	-0.04 (-0.06, -0.03)	0.001	-0.03 (-0.04, -0.01)	0.091	-0.02(-0.04, -0.01)	0.161
	Oral disposition index at follow-up					
Isoleucine	-0.02 (-0.03, -0.01)	0.125	-0.01 (-0.02, 0.01)	0.583	-0.01 (-0.02, 0.01)	0.596
Leucine	-0.02(-0.03, -0.01)	0.132	-0.01(-0.02, 0.00)	0.492	-0.01 (-0.02, 0.00)	0.503
Valine	-0.02 (-0.03, -0.01)	0.132	0.00 (-0.02, 0.01)	0.759	0.00 (-0.02, 0.01)	0.774

Abbreviations:
Model 1: Unadjusted
Model 2: Adjusted for age, sex, BMI, and smoking status
Model 3: Adjusted for age, sex, BMI and smoking status, and family history of diabetes
Models examining associations of BCAA with oDI at follow-up were additionally adjusted for baseline oDI.

Table 5: Unadjusted and adjusted associations of a one-standard deviation greater concentration of individual plasma branched chain amino acid levels with Matsuda index at baseline and follow-up among JACDS participants

	Model 1		Model 2		Model 3	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
	Matsuda index at baseline					
Isoleucine	-0.05 (-0.06, -0.04)	<0.001	--0.02 (-0.03, -0.01)	0.039	-0.02 (-0.03, -0.01)	0.050
Leucine	-0.03 (-0.05, -0.02)	0.008	-0.006 (-0.02, 0.01)	0.582	-0.005 (-0.02, 0.01)	0.655
Valine	-0.05 (-0.06, -0.04)	<0.001	-0.02 (-0.04, -0.02)	0.056	-0.02 (-0.04, -0.01)	0.070

	Matsuda index at follow-up					
Isoleucine	-0.05 (-0.06, -0.03)	<0.001	-0.006 (-0.02, 0.01)	0.607	-0.006(-0.02, 0.01)	0.608
Leucine	-0.04 (-0.06, -0.03)	0.003	-0.01 (-0.03, 0.00)	0.326	-0.01 (-0.03, 0.00)	0.326
Valine	-0.06 (-0.07, -0.04)	<0.001	-0.02 (-0.04, -0.01)	0.151	-0.02 (-0.04, -0.01)	0.149

Abbreviations:
Model 1: Unadjusted
Model 2: Adjusted for age, sex, BMI, and smoking status
Model 3: Adjusted for age, sex, BMI and smoking status, and family history of diabetes
Models examining associations of BCAA with Matsuda index at follow-up were additionally adjusted for baseline Matsuda index.

Table 6: Unadjusted and adjusted associations of a one standard deviation greater concentration of individual plasma branched chain amino acid levels with HOMA2-%S at baseline and follow-up among JACDS participants

	Model 1		Model 2		Model 3	
	HOMA2-%S at baseline					
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Isoleucine	-1.02 (-1.23, -0.82)	<0.001	-0.43 (-0.62, -0.23)	0.033	-0.40 (-0.60, -0.20)	0.047
Leucine	-0.72 (-0.95, -0.48)	0.003	-0.13 (-0.34, 0.08)	0.532	-0.11 (-0.32, 0.11)	0.614
Valine	-1.25 (-1.49, -1.01)	<0.001	-0.58 (-0.82, -0.35)	0.014	-0.56 (-0.80, -0.32)	0.019
	HOMA2-%S at follow-up					
Isoleucine	-0.93 (-1.18, -0.68)	<0.001	-0.31 (-0.57, -0.05)	0.232	-0.29 (-0.55, -0.03)	0.263
Leucine	-0.89 (-1.18, -0.60)	0.002	-0.30 (-0.57, -0.03)	0.268	-0.29 (-0.56, -0.01)	0.300
Valine	-1.23 (-1.52, -0.94)	<0.001	-0.40 (-0.71, -0.10)	0.191	-0.39 (-0.70, -0.08)	0.214

Abbreviations:
Model 1: Unadjusted
Model 2: Adjusted for age, sex, BMI, and smoking status

Model 3: Adjusted for age, sex, BMI, smoking status, and family history of diabetes. Models examining associations of BCAA with HOMA2-%S at follow-up were additionally adjusted for baseline HOMA2-%S.

Table 7: Associations of a one-standard deviation greater concentration of individual plasma branched chain amino acids with oral disposition index (oDI) at baseline, stratified by sex among JACDS participants

	Model 1				Model 2				
	Men		Women		Men		Women		Interaction p-value
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	
Isoleucine	-0.02 (-0.04, 0.00)	0.371	-0.03 (-0.05, -0.01)	0.068	0.01 (-0.02, 0.03)	0.830	-0.01 (-0.02, 0.01)	0.612	0.629
Leucine	-0.02 (-0.04, 0.01)	0.462	-0.04 (-0.06, -0.03)	0.012	0.01 (-0.01, 0.04)	0.583	-0.02 (-0.04, 0.00)	0.210	0.358
Valine	-0.04 (-0.06, -0.02)	0.089	-0.04 (-0.06, -0.02)	0.039	-0.01 (-0.04, 0.02)	0.702	-0.03 (-0.05, -0.01)	0.165	0.999

Model 1: Unadjusted
Model 2: Adjusted for age, BMI, smoking status, and family history of diabetes.

Table 8: Associations of a one-standard deviation greater concentration of individual plasma branched chain amino acids with Matsuda insulin sensitivity index at baseline, stratified by sex among JACDS participants

	Model 1				Model 2				
	Men		Women		Men		Women		Interaction p-value
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	
Isoleucine	-0.02 (-0.04, -0.01)	0.088	-0.08(-0.10, -0.06)	<0.01	-0.01 (-0.02, 0.01)	0.719	-0.04(-0.06, -0.02)	0.029	0.022
Leucine	0.00 (-0.17, 0.02)	0.977	-0.07 (-0.09, -0.05)	<0.01	0.02 (0.00, 0.04)	0.205	-0.02 (-0.04, 0.00)	0.222	0.010

Valine	-0.04 (-0.05, -0.02)	0.035	-0.06 (-0.09, -0.04)	<0.01	-0.02 (-0.03, 0.00)	0.305	-0.03 (-0.05, -0.01)	0.198	0.329

Model 1: Unadjusted
Model 2: Adjusted for age, BMI, smoking status, and family history of diabetes.

Figure 1: Flow of participants across the JACDS study and participant exclusion:

Participants in biomarker dataset (n= 391)

➔ Excluded those who had diabetes at baseline (n=34)
➔ Excluded those who have missing data at baseline (n=7)
Excluded participants with insulin = Inf (n=1)

➔ Participants eligible for study at baseline (n=349)

➔ Participants eligible for study at follow-up (n=349)

Figure 2: Distribution of each branch chain amino acid among JACDS participants