Longitudinal Associations of Branched-chain and Aromatic Amino Acids with Decreased Insulin Secretion and Insulin Sensitivity among Japanese Americans

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Abstract

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Background: Type 2 diabetes (T2D) is characterized by diminished insulin sensitivity and insulin secretion. Identification of metabolic biomarkers such as branched-chain (BCAA) and aromatic amino acids (AAA) that could be used for early identification of increased risk for decreased insulin sensitivity and secretion among Japanese Americans could improve health outcomes and reduce health disparities for this minority population. We characterized cross-sectional and longitudinal associations of plasma BCAA and AAA levels with insulin sensitivity and secretion in Japanese Americans.

Methods: We used mass spectrometry to measure plasma BCAA and AAA levels obtained from Japanese American participants without diabetes who were in a study of diabetes risk factors (Japanese American Community Diabetes Study Biomarker Discovery Project, n=347,

51% men, 49% women). We calculated Gutt, Matsuda, and oral disposition indices (DI_O) using weight and oral glucose tolerance test results from baseline and five years later. Linear regression was used to evaluate cross-sectional and longitudinal associations between BCAA and AAA levels and insulin sensitivity and secretion indices overall and in sex-stratified models.

Results: On average, participants were 55 years old. At baseline, the mean and standard deviations were 60.9(16.5) for Gutt, 3.3(1.6) for Matsuda, and 1.1(1.2) for oral disposition indices (DI_O). In cross-sectional analyses, isoleucine was associated with Matsuda index (β -0.03 [95%CI -0.05,-0.003]). In men, tryptophan was associated with Matsuda index (β 0.05 [95%CI 0.01,0.09]) and isoleucine with DI_O (β -0.001 [95%CI -0.002,-0.00003]). In longitudinal analyses, tyrosine was associated with Gutt index (β -0.27 [95%CI -0.51,-0.02]) and phenylalanine with Matsuda index (β -0.04 [95%CI -0.08,-0.01]). In men, tyrosine was associated with Gutt index (β -0.48 [95%CI -0.86,-0.10]), and phenylalanine and tyrosine with Matsuda index (β -0.05 [95%CI -0.10,-0.01], β -0.03 [95% CI -0.06,-0.003], respectively). In women, no BCAA or AAA was associated with any index in cross-sectional or longitudinal analyses. There was no statistical evidence for effect modification by sex.

Conclusions: BCAA and AAA including tryptophan, isoleucine, tyrosine, and phenylalanine were associated with insulin sensitivity and insulin secretion; however, magnitudes of associations were small in comparison to means and standard deviations of respective indices. BCAA and AAA were inversely associated with insulin sensitivity and secretion, except tyrosine which was positively associated with Matsuda index at baseline in men. Our study highlights the need for continued metabolomics research pertaining to chronic diseases of global significance, such as T2D.

BACKGROUND & SIGNIFICANCE

Type 2 diabetes mellitus (T2D) is a complex, multifactorial disorder characterized by decreased insulin sensitivity and diminished insulin secretion.[1–3] These complexities present challenges to understanding the molecular pathways that contribute to the development of T2D.[3] The recent advent of technologies for comprehensive metabolic analysis, known as metabolomics, may facilitate progress in this area.[3] In addition to serving as potential biomarkers of disease, metabolites are hypothesized to have roles as regulatory signals with hormone-like functions that may mediate the disease process itself.[4–7]

The branched-chain amino acids (BCAA),[6,8–12] leucine,[13] isoleucine,[14,15] valine,[13,16] and the aromatic amino acids (AAA), phenylalanine,[6,8,10,12] tyrosine,[6,8,10,12,14,16] and tryptophan[17,18] have been shown to predict the development of decreased insulin sensitivity[8–12,15,17,18] and T2D,[6,8,12–14,18] with stronger associations for men[8,9,11,14] – however, the majority of studies are only cross-sectional[6,9,13,16] and none are in an exclusively Japanese American ethnic group. These studies primarily assessed insulin sensitivity using homeostasis model assessment of insulin resistance[6,8,11,12,16–18] hyperinsulinemic-euglycemic clamp method,[15] Matsuda index,[17] or Bergman's minimal model[9], and T2D diagnosis based on hemoglobin A1C[13] or using the oral glucose tolerance test (OGTT).[18] These findings raise the possibility that alterations in plasma metabolite levels could presage the onset of overt T2D and therefore aid in the identification of 'at risk' individuals by adding information not available in standard clinical markers.[6] Most prior studies using mass spectrometry as a tool for biomarker discovery[9] have been cross-sectional, providing limited information regarding the use of metabolomic biomarkers in the prediction of decreased insulin sensitivity or T2D.[6] To our knowledge no longitudinal studies have been conducted evaluating the association of plasma BCAA and AAA with insulin sensitivity using the Gutt or Matsuda indices in an exclusively Japanese American population. There is a lack of prior studies evaluating insulin secretion, but one such study showed an inverse association

between plasma BCAA and AAA with insulin secretion estimated by disposition index among Finnish participants.[14] To our knowledge no studies have been done evaluating the association of plasma BCAA and AAA with insulin secretion using the oral disposition index in an exclusively Japanese American population.

Elevated plasma levels of all BCAA,[9,19,20] phenylalanine, and tyrosine have been associated with obesity.[7,10,21] Excess body fat,[22] and in particular visceral fat area (VFA),[5,23–25] has been associated with decreased insulin sensitivity[5,25,26] and T2D[23,24]. VFA has also been associated with BCAA.[5,27,28] These findings suggest a possible complex role of adipose tissue in BCAA homeostasis and insulin sensitivity.[5,10,20,29] Several previous studies adjusted for body mass index (BMI) as a surrogate for adiposity;[6,21] however, this cannot distinguish between lean muscle and fat body mass and certainly does not differentiate between subcutaneous and visceral fat compartments.[30] BMI is an imperfect measure of adiposity, especially among Japanese,[31] and is not a sensitive measure of visceral adipose tissue,[30] which is the fat type most strongly associated with insulin sensitivity[5,25,26] and T2D.[23,24] Whether VFA specifically may confound associations of BCAA and AAA plasma levels with insulin sensitivity or secretion has not been well evaluated.

Additionally, there is a paucity of research in this area in the Japanese American population. When compared to Caucasian populations, Asians tend to have more visceral fat and a higher body fat percentage for a given body mass index.[31,32] Adiposity-related risk factors derived in non-Asian cohorts might therefore perform poorly when applied to Japanese American populations,[28,31] highlighting the importance of metabolomic profiling studies in this population. It is not well established if elevated BCAA or AAA plasma levels are associated with alterations in insulin sensitivity or secretion in Japanese Americans.

To address these gaps, we evaluated cross-sectional and longitudinal associations of plasma BCAA and AAA with Gutt, Matsuda, and oral disposition indices among Japanese Americans with and without adjustment for VFA measured from abdominal computed tomography (CT) scans. To our knowledge the characterization of the longitudinal associations of BCAA and AAA plasma levels with insulin sensitivity and insulin secretion in Japanese Americans with adjustment for VFA is unique and will help to expand the roles of these metabolites in glucose metabolism.

METHODS

Study Design and Population

Participants for this cohort, termed the Japanese American Community Diabetes Study (JACDS) Biomarker Discovery Project (BDP), were taken from the parent JACDS study, a prospective community-based longitudinal cohort study that was conducted in King County, WA, between 1983 and 2002.[33] The cohort is described in more detail in previous reports.[25] Briefly, JACDS participants (n=761) were male and female Japanese American residents between the ages of 34 and 76 years at the enrollment visit[25,34,35] and were American-born residents of King County, WA, whose parents or grandparents immigrated from Japan.[36] Participants were required to be of 100% Japanese ancestry.[25,36] A comprehensive mailing list and telephone directory was used for participant recruitment that included almost 95% of the Japanese American population of King County, WA, at the time.[36] The Human Subjects Review Committee at the University of WA approved the study protocol. Signed informed consent was obtained from all participants.[23]

Among the 761 JACDS participants, there was an approximately 20% attrition rate over the course of the entire JACDS study. Only those who remained in the JACDS study and had OGTT results available at year 5-6 and year 10-11 follow-up, and who were without diabetes at

year 5-6 follow-up visit with available fasting plasma samples from the year 5-6 follow-up visit were included in the JACDS BDP (n=351). The exclusion criterion of diabetes was defined as having either a fasting plasma glucose ≥126 mg/dl or a plasma glucose level ≥200 mg/dl measured two hours after oral administration of a 75-gram glucose load.[37] Four participants with aberrant or missing data for exposures or outcomes were additionally excluded, leaving a final analytic sample of n=347.

Data collection

Evaluations were completed at the General Clinical Research Center at the University of WA, Seattle. JACDS study visits were performed at enrollment, year 5–6, and year 10–11 follow-up visits. For the purposes of the current analysis, the original JACDS five-year visit is termed the BDP baseline visit, and the JACDS 10-year visit is termed the BDP follow-up visit.

Branched-chain and Aromatic Amino Acids

Plasma levels of BCAA (leucine, isoleucine, valine) and AAA (phenylalanine, tyrosine, and tryptophan) were measured using previously frozen plasma samples from venous blood collected at the BDP baseline visit. BCAA and AAA were measured using targeted metabolomics protocols with GC-TOF-HRMS, hydrophilic (HILIC)-LC-HRMS, and HILIC-LC-MS/MS instruments as previously reported[38] and were quantified in micromoles per liter (μmol/L).

Measures of insulin sensitivity: Gutt and Matsuda indices

Measures of insulin sensitivity were calculated from three-hour oral glucose tolerance tests performed at BDP baseline and 5-year follow-up. The Gutt index was chosen since studies show it is a good tool to predict onset of T2D[39,40] and correlates well with the insulin sensitivity index obtained from the hyperinsulinemic-euglycemic clamp, the costly and time intensive “gold standard” for direct measurement of insulin sensitivity.[39,41] [35]A Gutt index score < 45 predicts

decreased insulin sensitivity,[40] a condition where greater than normal amounts of insulin are required to obtain a quantitatively normal response to postprandial glucose levels in the body.[39,40] The Gutt index uses weight (w) measured in kilograms in conjunction with an OGTT, where insulin (I) and glucose (G) are measured at 0 and 120 minutes post 75g oral glucose load,[41] and is calculated as follows[40–42]:

$$\text{Gutt index} = 75{,}000 + (G_0 - G_{120}\ \text{mg/dL})(0.19w) / (120)(G_{mean(0,120)}\ \text{mmol/L})(\text{Log}[I_{mean(0,120)}\ \text{mU/L}])$$

The Matsuda index was chosen as a second measure of insulin sensitivity as it provides a reasonable approximation of whole-body insulin sensitivity— representing both hepatic and peripheral tissue sensitivity to insulin.[39,40] In general, higher Matsuda index values indicate greater insulin sensitivity, whereas reduced values indicate decreased insulin sensitivity.[43,44] Matsuda index calculation uses glucose (G) and insulin (I) measurements at 0, 30, 60, and 120 minute timepoints during the OGTT to calculate G_{mean} and I_{mean}, and is calculated as follows[45]:

$$\text{Matsuda index} = 10{,}000 / \sqrt{(G_0 \times I_0 \times G_{mean} \times I_{mean})}$$

Oral Disposition Index

The oral disposition index (DI_O) was chosen as a surrogate measure for insulin secretion since it provides a composite measure of beta-cell function adjusted for insulin sensitivity. Lower baseline values have been shown to be predictive of development of diabetes over 10 years.[46] The DI_O decreases as an individual progresses from normal glucose tolerance to impaired glucose metabolism to diabetes.[46,47] DI_O calculation uses glucose (G) and insulin (I) concentration measurements at 0 and 30 minutes during the OGTT, and is calculated as follows:

$$DI_O = (I_0 - I_{30}) / (G_0 - G_{30}) \times (1 / \text{fasting insulin})$$

Covariates

Age in years,[48] sex defined as female or male at birth,[49,50] and smoker status (currently smoking vs. never or former smoker)[51] were obtained by interview. Weight was measured using a digital scale after shoes and outer clothing were removed. VFA was quantified using cross-sectional measurement of the intra-abdominal fat area (within the confines of the tranversalis fascia) in centimeters squared (cm^2) as determined using a single 1-cm slice CT scan obtained at the umbilicus (L4-L5) level,[25,52,53] where areas corresponding to a density of -250 to -50 Hounsfield units were classified as adipose tissue.[54]

Data Analyses

Descriptive analyses of demographic, clinical, radiological, and laboratory characteristics were performed, including percentages, means, standard deviations, medians, and ranges. Multiple linear regression was used for inferential statistical analyses. Continuous dependent and independent variables were used. For the primary aim, statistical analyses were performed to characterize the association of each BCAA with each outcome (insulin secretion or insulin sensitivity). For the secondary aim, this process was repeated with the AAA. All analyses were conducted with adjustment for the following covariates thought to be confounders: age, sex, smoking status, and VFA at study baseline— except for the partially adjusted primary analyses presented in table 3 which do not adjust for VFA, which were done for comparison with the fully adjusted primary analyses presented in table 4. Longitudinal analyses additionally adjusted for the baseline insulin sensitivity or secretion index being evaluated. Multiple linear regression analyses were also performed to assess for effect modification by sex (tables 5-7). Data analyses were performed using R statistical analysis software version 4.0.3 GUI 1.73 Catalina build (7892) in combination with R Studio Version 1.4.1103. An alpha = 0.05 level of significance was used when interpreting p-values from primary results.

RESULTS

Descriptive Statistics

The BDP study population consisted of 177 men and 170 women. The mean age of participants was 55 years old (range 40-80 years). Participant BMI ranged from 17.0 to 37.5 kilogram/meter squared (kg/m^2), averaging 24.6 kg/m^2 with a standard deviation of 3.5 kg/m^2. Forty-eight percent of participants reported they had never smoked, 41% reported being former smokers, and 11% reported currently smoking. Baseline characteristics of participants are presented in further detail in table 1. The distribution of baseline plasma levels of BCAA and AAA of participants are visually displayed using box plots in figure 1. The means, standard deviations, 1^{st} quartile, median, and 3^{rd} quartile of BDP baseline plasma levels of BCAA and AAA of participants are also presented in table format in the appendix. The distribution of BDP baseline Gutt, Matsuda, and oral disposition indices of participants are presented in table 2.

Inferential Statistics

Primary Analyses

Partially adjusted cross-sectional and longitudinal analyses (not adjusted for VFA) are presented in table 3. In partially adjusted cross-sectional analyses, isoleucine and valine were inversely associated with the Gutt index (β -0.30 [95% CI -0.55, -0.05], β -0.49 [95% CI -0.78, -0.20], respectively). Leucine, isoleucine, and valine were inversely associated with the Matsuda index (β -0.03 [95% CI -0.05, -0.001], β -0.04 [95% CI -0.07, -0.02], β -0.05 [95% CI -0.08, -0.03], respectively). Valine was inversely associated with the DI_O (β -0.002 [95% CI -0.004, -0.001]). In partially adjusted longitudinal analyses, valine and tyrosine were inversely associated the Gutt index (β -0.34 [95% CI -0.64, -0.04], β -0.37 [95% CI -0.61, -0.13], respectively). Valine and phenylalanine were inversely associated with the Matsuda index (β -0.04 [95% CI -0.07, -0.01], β -0.05 [95% CI -0.08, -0.02], respectively). No statistically significant longitudinal associations were observed between BCAA or AAA with the DI_O.

Cross-sectional and longitudinal analyses additionally adjusted for VFA are presented in table 4. In cross-sectional analyses additionally adjusted for VFA, no BCAA or AAA was significantly associated with the Gutt index. Isoleucine was inversely associated with the Matsuda index (β -0.03 [95% CI -0.05, -0.003]). In longitudinal analyses additionally adjusted for VFA, tyrosine was inversely associated with the Gutt index (β -0.27 [95% CI -0.51, -0.02]). Phenylalanine was inversely associated with the Matsuda index (β -0.04 [95% CI -0.08, -0.01]). No statistically significant cross-sectional or longitudinal associations existed between BCAA or AAA with the DI_O.

Sex-stratified analyses

Sex-stratified analyses are presented in tables 5-7. In cross-sectional analyses among men, no BCAA or AAA was significantly associated with the Gutt index or DI_O. Tryptophan was positively associated with the Matsuda index (β 0.05 [95% CI 0.01, 0.09]). In cross-sectional analyses among women, no BCAA or AAA was significantly associated with any measure of insulin sensitivity or secretion. In longitudinal analyses among men, tyrosine was inversely associated with the Gutt index (β -0.48 [95% CI -0.86, -0.10]). Phenylalanine and tyrosine were inversely associated with the Matsuda index (β -0.05 [95% CI -0.10, -0.01], β -0.03 [95% CI -0.06, -0.003], respectively). Isoleucine was inversely associated with DI_O (β -0.001 [95% CI -0.002, -0.00003]). In longitudinal analyses among women, no BCAA or AAA was significantly associated with any measure of insulin sensitivity or secretion. There was no statistically significant evidence of effect modification by sex for cross-sectional or longitudinal associations between BCAA or AAA with insulin sensitivity or secretion indices.

DISCUSSION

Summary

In summary, we evaluated cross-sectional and longitudinal associations of BCAA and AAA with insulin sensitivity and insulin secretion in a prospective community-based study of Japanese Americans (n=347). In models that did not adjust for VFA, we found cross-sectional associations between isoleucine and valine with Gutt index, between leucine, isoleucine, and valine with Matsuda index, and between valine with DI_O. In models that did not adjust for VFA, we found longitudinal associations between valine and tyrosine with Gutt index and between valine and phenylalanine with Matsuda index. After adjustment for VFA, however, the number of statistically significant findings was reduced from ten to three (table 3 compared to table 4). Isoleucine was inversely associated with Matsuda index at baseline, phenylalanine inversely associated with Matsuda index 5 years later, and tyrosine inversely associated with Gutt index 5 years later. Tryptophan was positively associated with Matsuda index at baseline in men (β 0.05 [95% CI 0.01, 0.09]). No statistically significant associations were found between BCAA or AAA and insulin secretion, except for baseline isoleucine plasma levels, which were inversely associated with DI_O at 5 year follow-up in men (β -0.001 [95% CI -0.002, -0.00003]). Associations did not differ by sex. The magnitude of associations for all statistically significant findings were small in comparison to BDP baseline means and standard deviations for Gutt, Matsuda, and oral disposition indices, and likely not of practical clinical relevance. After Bonferroni correction for multiple hypotheses tested, no statistically significant associations remained. To our knowledge, this is the first longitudinal study to evaluate the association of BCAA and AAA plasma levels with both insulin sensitivity and secretion in a homogenously Japanese American sample while adjusting for VFA.

Comparison of results to existing literature

The branched-chain amino acids (BCAA),[6,8–12] leucine,[13] isoleucine,[14,15] valine,[13,16] and the aromatic amino acids (AAA), phenylalanine,[6,8,10,12] tyrosine,[6,8,10,12,14,16] and tryptophan[17,18] have been shown to predict the development of decreased insulin sensitivity[8–12,15,17,18] and T2D,[6,8,12–14,18] with stronger associations for men.[8,9,11,14] There is a lack of prior studies evaluating insulin

secretion, but one such study showed an inverse association between plasma BCAA and AAA with insulin secretion estimated by disposition index among Finnish participants.[14] There are several plausible reasons for the lack of statistically significant findings for associations between plasma BCAA and AAA and insulin sensitivity and secretion in our study compared to prior studies. Our study was able to adjust for confounding by VFA as measured by CT scan, a more accurate estimate of the most deleterious adipose depot compared to BMI. This distinction is especially true for our population, since Asians generally have more visceral fat and higher body fat percentage for a given body mass index compared to Caucasians.[31] The number of statistically significant findings for primary analyses were reduced from ten to three after adjusting for VFA (table 3 compared to table 4). Our findings highlight the importance of adjusting for VFA as a confounder and may account for the differences in our results compared to other studies that adjusted for BMI instead. The strength of confounding by VFA is not surprising since VFA is a hormonally active component of total body fat, possessing unique biochemical characteristics influencing several normal and pathological processes in the human body,[30] and which has been associated with both BCAA[5,27,28] and decreased insulin sensitivity[5,25,26] and T2D.[23,24] Furthermore, our findings may differ from others as most other studies were performed using an ethnically heterogenous sample or a completely different ethnic group than the one we examined. The importance of this difference can be appreciated when one considers possible ethnic differences in physiology. For example, it has been recognized that compensatory function of the pancreatic β-cells in Japanese is lower than that observed in Caucasian populations.[55] For example, Wang et al performed a nested case-control study from the Framingham Offspring Cohort Study, showing that leucine, isoleucine, valine, phenylalanine, and tyrosine were associated with future onset of T2D; however, their research used an ethnically heterogeneous sample and did not adjust for VFA,[6] which could account for differences between their findings and ours. Though we observed a statistically significant positive association between tryptophan plasma levels and the Matsuda index at baseline for men, previous literature has shown an inverse association exists between tryptophan and

insulin sensitivity as measured by homeostasis model assessment of insulin resistance[17] and Matsuda index[17] or no association at all.[6,10,13] Research by Yu et al, suggests that tryptophan levels may initially increase and then deplete as diabetes progresses in severity.[18] Future studies may help to elucidate whether or not our finding represents a novel association in this ethnic group.

Lastly, our study used plasma samples which were frozen for approximately 30 years. While specific standard operating procedures should be used for sample collection, processing, and transport— changes in metabolite concentrations during storage are challenging to control and cannot be completely avoided,[56] and the impact of long-term storage on plasma metabolites is not yet fully understood.[56,57] Research by Hustad et al using archival samples stored up to 29 years found that metabolites were most stable in EDTA plasma,[58] and of note, tryptophan remained stable under multiple different temperature-controlled conditions.[58] Generally though, storage in freezers at -80 °C or below is recommended to maintain long-term integrity of biomarkers.[59] Abuja et al found that storage temperature affected metabolite concentrations minimally, and rather a linear dependence on number of temperature change cycles, with elevated sample temperature during prolonged retrieval time led to a distinctly different signature of metabolite changes that were induced by repeated temperature cycling.[60] A typical concern is that biomarker concentrations may decay over time,[61] for example predominantly due to hydrolysis of peptides and lipids,[60] and concentrations of some biomarkers may even increase during storage.[61] Ultimately, instability of biomarkers may increase preanalytical variability, which could result in failure to detect associations between biomarkers and disease risk.[62] In order to allow for unbiased comparison of samples collected at study baseline and 5 years later, we used specific standard operating procedures for sample collection, processing, transport, and frozen storage of plasma samples with similar numbers of freeze-thaw cycles, as well as using standardized handling and testing methods.[48,63] However, despite using standard

operating procedures, we expect there may be random changes in levels of BCAA and AAA, which could result in attenuated associations.[56,57,62]

Sex Stratification

There was no statistical evidence of effect modification by sex for the association between BCAA or AAA with insulin sensitivity or secretion. We were likely underpowered to detect sex-specific associations of small effect size. Our findings suggest that for men, increased isoleucine plasma levels may predict decreased insulin secretion 5-6 years later. Furthermore, our study found that baseline phenylalanine plasma levels may predict decreased Matsuda insulin sensitivity index levels 5-6 years later. It is plausible that multiple associations were statistically significant for men only but not for women only (tables 5-7), since amino acids are known to exhibit sex-specific associations with insulin resistance.[8] Sex differences have been found for metabolic predictors of insulin sensitivity and the disposition index in previous research, to include a much stronger inverse association of large neutral amino acids,[9] and specifically, BCAA[8], phenylalanine[8], and tyrosine[8] with diminished insulin sensitivity for men than for women.[8,9] Previous investigations have found that men exhibit stronger relationships between the insulin-like growth factor axis and insulin sensitivity,[64] supporting the hypothesis that in men, gestational programming, which is designed to meet greater anabolic demands and that operates under abundant amino acid exposure later in life, could help to explain the findings of a stronger relationship between amino acid concentrations and decreased insulin sensitivity in men compared to women.[9] Sex differences, in fact, play a key role in the onset as well as in the progression of T2D.[50]

Hypothesized mechanisms: BCAA/AAA and insulin sensitivity

Experimental and clinical data suggest that certain amino acids may be both markers and effectors of insulin resistance.[6,7] Research suggests a possible pathway exists by which dysregulated BCAA metabolism makes an independent contribution to development of

decreased insulin sensitivity and glucose intolerance, ultimately leading to T2D.[7] Specifically, BCAA have been reported to interfere with insulin signaling via stimulation of the nutrient-sensitive kinase, mammalian target of rapamycin (mTOR), and its downstream target, ribosomal protein S6 kinase 1 (S6K1), causing inhibitory phosphorylation of insulin receptor substrate-1 (IRS-1) serine residues (i.e. Ser-1101) – resulting in amino-acid induced diminished insulin sensitivity in skeletal muscle.[7,65–68] Recent studies have revealed that S6K1, an effector of mTOR, is sensitive to both insulin and amino acids.[65] Moreover, rather than signaling through the class 1 PI3K pathway, amino acids appear to mediate mTOR activation through class 3 PI3K, or hVps34.[65] Consistent with this, amino acids lead to S6K1 activation, inhibition of insulin induced class 1 PI3K activation, and decreased insulin sensitivity.[65] Another metabolic pathway to consider is that elevated BCAA lead to a high rate of flux through BCAA catabolic pathways, leading to accumulation of glutamate, which increases transamination of pyruvate to alanine – a highly gluconeogenic amino acid, which in turn, may further contribute to development of glucose intolerance.[7]

Limitations

Although we adjusted for measured covariates known to be associated with BCAA or AAA and insulin sensitivity, potential for confounding by unmeasured factors still exists given the observational study design. Furthermore, due to the observational nature of the study, we are unable to infer a causal relationship. It is also possible that slight variability in measurement of study participant weight or performance of laboratory tests may have introduced error. However, this would most likely introduce random error, and any resulting bias would be expected to be towards the null, underestimating true associations. In particular, variability in participant weight measurement methods could affect the calculation of the Gutt index, one of our primary outcomes. However, this limitation was mitigated by the fact that weight was measured by trained examiners at each follow-up visit and not self-reported. Calculation of the Matsuda index includes G_{mean} and I_{mean}, which are average glucose and insulin concentrations, respectively,

over different timepoints during the OGTT.[45] Generally, to calculate the Matsuda index it is ideal to have glucose and insulin measurements at 0, 30, 60, 90, and 120 minutes post 75g oral glucose load during the OGTT. For our study, glucose and insulin measurements were not available at 90 minutes post 75g oral glucose load. However, research by DeFronzo and Matsuda demonstrated the Matsuda index could be calculated with fewer time points, even as few as 0, 60, and 120 minutes or only 0 and 120 minutes, obtained during the OGTT.[69] Although the Matsuda index should not be compared between studies using different timepoints for its calculation, within any given study the index provides a reasonable index of whole-body insulin sensitivity.[69] Another limitation is that multiple comparisons increase the chance of false positive results. In order to address this, a conservative approach was taken, using Bonferroni correction, after which no results remained statistically significant.

Strengths

The strengths of this study include having a large sample size with power to detect a relatively small effect size, ethnically homogenous study population, longitudinal study design, ability to adjust for VFA as a confounder, and availability of laboratory measurements to calculate both insulin sensitivity and secretion in the same study. The homogeneity of our study population, which comprised only 2nd and 3rd generation Japanese Americans with 100% pure Japanese ancestry, allowed for specific evaluation of the association between plasma BCAA and AAA with insulin sensitivity and insulin secretion indices in this ethnic minority group. This value is highlighted by the fact that there are important physical[31] and metabolic[55] differences observed among different ethnicities. Having an ethnically homogenous study population enhances the external validity of our findings to other Japanese Americans outside this study.

Conclusion

Given the importance of effective interventions for delaying or preventing the onset of T2D[70] and the increasing burden of the condition worldwide,[71] earlier identification of individuals at risk is

particularly important.[6] With ongoing intensified research, metabolites such as amino acids are gaining attention as surrogate biomarkers in assessment of insulin sensitivity.[40] Metabolic profiling will likely continue to develop as a tool to identify individuals who have increased metabolic susceptibility to T2D and to direct therapies that appropriately address the identified metabolic abnormalities.[36] Identifying specific metabolic biomarkers, such as BCAA and AAA, which could be used for early identification of decreased insulin secretion or sensitivity among the Japanese American population could improve health outcomes and decrease health disparities for this minority population. While our study observed several statistically significant associations between plasma BCAA and AAA with insulin sensitivity and secretion indices, the magnitude of associations appeared to be small in comparison to the means and standard deviations of these indices. However, all these associations were in the direction predicted by our hypotheses, with plasma BCAA and AAA inversely associated with insulin sensitivity and insulin secretion, with one exception. The relationship between tyrosine and Matsuda index at baseline in men appeared to have an unexpected positive association. Future research could evaluate whether this finding is replicable, and if so, explore possible underlying mechanisms. It is unknown if our study findings may be generalizable to other Asian Americans – which could be explored in future studies. Our study contributes to the growing body of knowledge regarding metabolites which could serve as predictors for future decreased insulin sensitivity and secretion, but also highlights the need for continued studies in the field of metabolomics as it pertains to chronic diseases of global significance, such as T2D.

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TABLES

Table 1. Baseline characteristics of participants in the Japanese American Community Diabetes Study Biomarker Discovery Project, total sample size n = 347, frequency (n), percent (%)[a]

Characteristic	Baseline n (%)
Sex[b]	
Male	177 (51)
Female	170 (49)
Age (years)	
40-49	136 (39)
50-59	55 (16)
60-69	112 (32)
≥ 70	44 (13)
Body Mass Index (kilogram/meter^2)	
≤ 25	208 (60)
25–29.9	111 (32)
≥ 30	28 (8)
Visceral Fat Area[c] (centimeter^2)	
< 50	76 (22)
50-99	133 (39)
100-149	98 (29)
≥ 150	31 (9)
Smoker	
Never	168 (48)
Former	141 (41)
Current	38 (11)
Fasting Glucose (milligram/deciliter)	
< 100 (normal fasting glucose)	220 (63)
100-125 (impaired fasting glucose)	127 (37)
Fasting Insulin (microunits/milliliter)	
2-30 (normal)	328 (95)
> 30 (high)	19 (5)
Hypertension[d]	
Yes	102 (29)
No	245 (71)
Total cholesterol (milligram/deciliter)	
< 200 (normal)	132 (38)
200-239 (borderline high)	136 (39)
≥ 240 (high)	79 (23)
Physical Activity level[e]	

Sedentary	39 (11)
Light	219 (63)
Moderate	82 (24)
Heavy	7 (2)

[a]Percentages may not sum to 100 due to rounding. When there is missing data, percentages are calculated using the non-missing data. [b]Sex is defined as male or female at birth. [c]Visceral fat area is calculated using computed tomography single 1-centimeter cross-sectional slice at level of the umbilicus and measured in centimeters^2 and has baseline missing data = 9. [d]Hypertension is defined as systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 mm Hg at time of visit and/or on antihypertensive medication. [e]Physical activity level was self-reported by participants on a numeral scale where 0 = sedentary, 1 = light, 2 = moderate, and 3 = heavy activity level. If participant scores were between whole numbers, the score was rounded to the nearest representative category.

Figure 1. Baseline plasma levels of branched-chain and aromatic amino acids of participants in the Japanese American Community Diabetes Study Biomarker Discovery Project, n = 347, with outliers indicated by data points outside of box plot whiskers and defined as any data points outside 1.5 times the interquartile range above the upper quartile or below the lower quartile

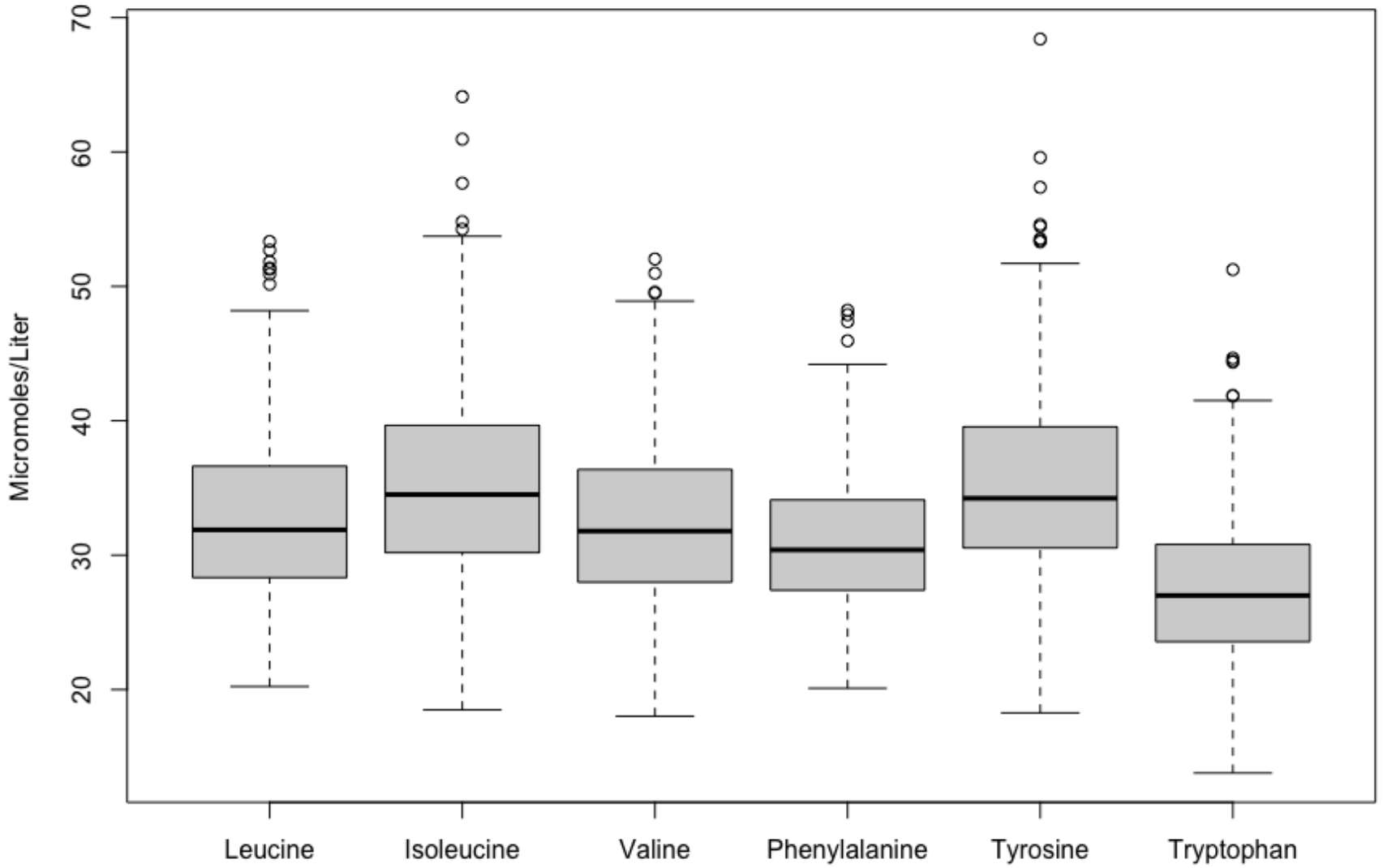

Table 2. Gutt insulin sensitivity index, Matsuda insulin sensitivity index, and oral disposition index baseline summary statistics for Japanese American Community Diabetes Study Biomarker Discovery Project, n = 347

Amino Acid	Median (1ST Quartile, 3rd Quartile)	Mean (Standard Deviation)
Gutt Insulin Sensitivity Index	58.0 (49.4, 69.1)	60.9 (16.5)
Matsuda Insulin Sensitivity Index	3.0 (2.2, 4.0)	3.3 (1.6)
Oral Disposition Index	0.8 (0.6, 1.3)	1.1 (1.2)

Table 3. Partially adjusted models for association of baseline plasma levels of branched-chain and aromatic amino acids (micromoles/liter) with Gutt insulin sensitivity index, Matsuda insulin sensitivity index, and oral disposition index at baseline and five years among participants free from diabetes at baseline in the Japanese American Community Diabetes Study Biomarker Discovery Project, n = 347

	Gutt Index		**Matsuda Index**		**Oral Disposition Index**	
	Baseline	**5-year Follow-up**	**Baseline**	**5-year Follow-up**	**Baseline**	**5-year Follow-up**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Branched-chain Amino Acid						
Leucine	-0.21(-0.48, 0.06)	-0.06(-0.33, 0.21)	**-0.03(-0.05, -0.001)**	-0.02(-0.05, 0.01)	-0.002 (-0.003, 0.0001)	-0.001 (-0.002, 0.001)
Isoleucine	**-0.30(-0.55, -0.05)**	-0.17(-0.42, 0.09)	**-0.04(-0.07, -0.02)**	-0.02(-0.04, 0.01)	-0.001 (-0.003, 0.0004)	-0.001 (-0.002, 0.001)
Valine	**-0.49(-0.78, -0.20)**	**-0.34(-0.64, -0.04)**	**-0.05(-0.08, -0.03)**	**-0.04(-0.07, -0.01)**	**-0.002 (-0.004, -0.001)**	-0.001 (-0.002, 0.001)
Aromatic Amino Acid						
Phenylalanine	-0.25(-0.60, 0.09)	-0.33(-0.67, 0.01)	-0.01(-0.04, 0.03)	**-0.05(-0.08, -0.02)**	-0.001 (-0.003, 0.001)	-0.0005(-0.002, 0.001)
Tyrosine	-0.11(-0.35, 0.13)	**-0.37(-0.61, -0.13)**	-0.01(-0.04, 0.01)	-0.02(-0.05, 0.001)	-0.0004(-0.002, 0.001)	-0.0001(-0.001, 0.001)
Tryptophan	-0.08(-0.42, 0.26)	-0.35(-0.70, 0.004)	0.02(-0.01, 0.06)	-0.02(-0.06, 0.01)	-0.0001(-0.002, 0.002)	0.001 (-0.001, 0.003)

Coefficients from the linear regression models show magnitude and direction of change in the specified index associated with each one micromole/liter increase in baseline plasma amino acid level of interest.
All models are adjusted for baseline age, sex, and smoker status. Five-year follow-up models are additionally adjusted for their respective index at baseline.

Table 4. Fully adjusted models for association of baseline plasma levels of branched-chain and aromatic amino acids (micromoles/liter) with Gutt insulin sensitivity index, Matsuda insulin sensitivity index, and oral disposition index at baseline and five years among participants free from diabetes at baseline in the Japanese American Community Diabetes Study Biomarker Discovery Project, n = 347

	Gutt Index		**Matsuda Index**		**Oral Disposition Index**	
	Baseline	**5-year Follow-up**	**Baseline**	**5-year Follow-up**	**Baseline**	**5-year Follow-up**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Branched-chain Amino Acid						
Leucine	-0.08(-0.33, 0.18)	0.05(-0.22, 0.32)	-0.01 (-0.03, 0.01)	-0.01 (-0.04, 0.01)	-0.001(-0.003, 0.0003)	-0.001 (-0.002, 0.001)
Isoleucine	-0.15(-0.39, 0.09)	-0.04(-0.29, 0.22)	**-0.03 (-0.05, -0.003)**	-0.01 (-0.03, 0.02)	-0.001(-0.003, 0.001)	-0.001 (-0.002, 0.001)
Valine	-0.23(-0.51, 0.06)	-0.16(-0.47, 0.14)	-0.03 (-0.05, 0.001)	-0.02 (-0.05, 0.01)	-0.002(-0.004, 0.0001)	-0.0004 (-0.002, 0.001)
Aromatic Amino Acid						
Phenylalanine	-0.23(-0.55, 0.10)	-0.27(-0.62, 0.07)	-0.00005(-0.03, 0.03)	**-0.04(-0.08, -0.01)**	-0.001 (-0.003, 0.001)	-0.0005(-0.002, 0.001)
Tyrosine	0.01(-0.22, 0.24)	**-0.27(-0.51, -0.02)**	0.001 (-0.02, 0.02)	-0.01(-0.04, 0.01)	-0.0001(-0.002, 0.001)	0.0001(-0.001, 0.002)
Tryptophan	-0.06(-0.38, 0.27)	-0.25(-0.60, 0.10)	0.03 (-0.0005, 0.06)	-0.01(-0.05, 0.02)	0.0001(-0.002, 0.002)	0.001 (-0.001, 0.003)

Coefficients from the linear regression models show magnitude and direction of change in the specified index associated with each one micromole/liter increase in baseline plasma amino acid level of interest.
All models are adjusted for baseline age, sex, smoker status, and visceral fat area. Five-year follow-up models are additionally adjusted for their respective index at baseline.

Table 5. Sex-stratified associations of baseline plasma levels of branched-chain and aromatic amino acids (micromoles/liter) with the Gutt insulin sensitivity index at baseline and five years among participants free from diabetes at baseline in the Japanese American Community Diabetes Study Biomarker Discovery Project

	Gutt Index Baseline			**Gutt Index Five-year Follow-up**		
	Men (n = 177)	**Women (n = 170)**		**Men (n = 177)**	**Women (n = 170)**	
	β (95% CI)	**β (95% CI)**	**p for interaction**	**β (95% CI)**	**β (95% CI)**	**p for interaction**
Branched-chain Amino Acid						
Leucine	0.06(-0.35, 0.47)	-0.14(-0.49, 0.20)	0.51	0.13(-0.31, 0.57)	-0.08(-0.45, 0.28)	0.51
Isoleucine	-0.14(-0.50, 0.22)	-0.12(-0.46, 0.22)	0.90	-0.05(-0.44, 0.34)	-0.07(-0.42, 0.29)	0.97
Valine	-0.21(-0.65, 0.22)	-0.21(-0.59, 0.17)	0.87	-0.29(-0.76, 0.18)	-0.06(-0.46, 0.34)	0.45
Aromatic Amino Acid						
Phenylalanine	-0.42(-0.93, 0.09)	-0.003(-0.42, 0.41)	0.17	-0.50(-1.05, 0.06)	-0.10(-0.54, 0.33)	0.31
Tyrosine	-0.09(-0.44, 0.27)	0.12(-0.18, 0.41)	0.32	**-0.48(-0.86, -0.10)**	-0.02(-0.34, 0.29)	0.08
Tryptophan	-0.18(-0.64, 0.28)	0.15(-0.31, 0.60)	0.19	-0.47(-0.99, 0.06)	0.05(-0.43, 0.53)	0.28

Coefficients and 95% confidence intervals are stratified by sex for the linear regression models and show the magnitude and direction of change in the Gutt index associated with each one micromole/liter increase in baseline plasma amino acid level of interest.
The p-value refers to the sex interaction term coefficient.
All models are adjusted for baseline age, sex, smoker status, and visceral fat area. Five-year models are adjusted for Gutt index at baseline.

Table 6. Sex-stratified associations of baseline plasma levels of branched-chain and aromatic amino acids (micromoles/liter) with the Matsuda insulin sensitivity index at baseline and five years among participants free from diabetes at baseline in the Japanese American Community Diabetes Study Biomarker Discovery Project

	Matsuda Index Baseline			**Matsuda Index 5-year Follow-up**		
	Men (n = 177)	**Women (n = 170)**		**Men (n = 177)**	**Women (n = 170)**	
	β (95% CI)	**β (95% CI)**	**p for interaction**	**β (95% CI)**	**β (95% CI)**	**p for interaction**
Branched-chain Amino Acid						
Leucine	0.02(-0.02, 0.05)	-0.03(-0.06, 0.01)	0.08	0.0001(-0.03, 0.03)	-0.03(-0.07, 0.02)	0.29
Isoleucine	-0.01(-0.04, 0.02)	-0.04(-0.07, 0.001)	0.17	-0.002 (-0.03, 0.03)	-0.01(-0.06, 0.03)	0.69
Valine	-0.01(-0.05, 0.02)	-0.03(-0.08, 0.01)	0.43	-0.03 (-0.06, 0.01)	-0.02(-0.07, 0.03)	0.68
Aromatic Amino Acid						
Phenylalanine	0.02(-0.02, 0.06)	-0.01 (-0.05, 0.04)	0.52	**-0.05(-0.10, -0.01)**	-0.03 (-0.08, 0.02)	0.62
Tyrosine	0.02(-0.01, 0.05)	-0.02 (-0.05, 0.01)	0.05	**-0.03(-0.06, -0.003)**	0.01 (-0.03, 0.04)	0.20
Tryptophan	**0.05(0.01, 0.09)**	0.002(-0.05, 0.05)	0.38	-0.03(-0.07, 0.01)	0.02 (-0.04, 0.07)	0.29

Coefficients and 95% confidence intervals are stratified by sex for the linear regression models and show the magnitude and direction of change in the Matsuda index associated with each one micromole/liter increase in baseline plasma amino acid level of interest.
The p-value refers to the sex interaction term coefficient.
All models are adjusted for baseline age, sex, smoker status, and visceral fat area. Five-year models are adjusted for Matsuda index at baseline.

Table 7. Sex-stratified associations of baseline plasma levels of branched-chain and aromatic amino acids (micromoles/liter) with the oral disposition index at baseline and five years among participants free from diabetes at baseline in the Japanese American Community Diabetes Study Biomarker Discovery Project

	Oral Disposition Index Baseline			**Oral Disposition Index 5-year Follow-up**		
	Men (n = 177)	**Women (n = 170)**		**Men (n = 177)**	**Women (n = 170)**	
	β (95% CI)	**β (95% CI)**	**p for interaction**	**β (95% CI)**	**β (95% CI)**	**p for interaction**
Branched-chain Amino Acid						
Leucine	0.0005(-0.002, 0.003)	-0.002(-0.004, 0.0002)	0.26	-0.001(-0.002, 0.0002)	0.0001 (-0.003, 0.003)	0.73
Isoleucine	0.0001(-0.003, 0.003)	-0.001(-0.003, 0.001)	0.58	**-0.001(-0.002, -0.00003)**	0.0002 (-0.003, 0.003)	0.60
Valine	-0.001 (-0.004, 0.002)	-0.002(-0.004, 0.0002)	0.91	-0.001(-0.003, 0.00002)	0.001 (-0.003, 0.004)	0.48
Aromatic Amino Acid						
Phenylalanine	-0.001 (-0.005, 0.003)	0.0001(-0.003, 0.003)	0.46	-0.001 (-0.002, 0.001)	-0.0003 (-0.004, 0.003)	0.93
Tyrosine	-0.0005(-0.003, 0.002)	0.0003(-0.002, 0.002)	0.44	0.001 (-0.001, 0.001)	0.0002 (-0.003, 0.003)	0.82
Tryptophan	-0.0001(-0.003, 0.003)	0.001 (-0.002, 0.004)	0.15	-0.001 (-0.002, 0.001)	0.003 (-0.001, 0.01)	0.05

Coefficients and 95% confidence intervals are stratified by sex for the linear regression models and show the magnitude and direction of change in the oral disposition index associated with each one micromole/liter increase in baseline plasma amino acid level of interest. The p-value refers to the sex interaction term coefficient.
All models are adjusted for baseline age, sex, smoker status, and visceral fat area. Five-year models are adjusted for oral disposition index at baseline.

APPENDIX

Plasma levels of branched-chain and aromatic amino acids summary statistics for Japanese American Community Diabetes Study Biomarker Discovery Project, n = 347, measured in micromoles/liter

Amino Acid	Median (1ST Quartile, 3rd Quartile)	Mean (Standard Deviation)
Branched-chain Amino Acids		
Leucine	31.9 (28.3, 36.6)	32.8 (6.4)
Isoleucine	34.5 (30.2, 39.7)	35.2 (7.2)
Valine	31.8 (28.0, 36.4)	32.4 (6.2)
Aromatic Amino Acids		
Phenylalanine	30.4 (27.4, 34.1)	30.9 (5.1)
Tyrosine	34.2 (30.5, 39.6)	35.4 (7.3)
Tryptophan	27.0 (23.6, 30.8)	27.5 (5.4)