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| Title    | Association between type 2 diabetes and branched chain amino acids (BCAA); a case-control study                                                                                                                                                                                                                                                  |
| Type     | Article                                                                                                                                                                                                                                                                                                                                          |
| URL      | <a href="https://clock.uclan.ac.uk/id/eprint/48077/">https://clock.uclan.ac.uk/id/eprint/48077/</a>                                                                                                                                                                                                                                              |
| DOI      | <a href="https://doi.org/10.1007/s40200-023-01247-9">https://doi.org/10.1007/s40200-023-01247-9</a>                                                                                                                                                                                                                                              |
| Date     | 2023                                                                                                                                                                                                                                                                                                                                             |
| Citation | Torki, Saheb Abbas, Bahadori, Effat, Aghakhaninejad, Zohreh, Mohseni, Golsa Khalatbari, Tajadod, Shirin, Rajabi Harsini, Asma, Azaryan, Fatemeh, Saeedirad, Zahra, Askarpour, Seyed Ali et al (2023) Association between type 2 diabetes and branched chain amino acids (BCAA); a case-control study. Journal of Diabetes & Metabolic Disorders. |
| Creators | Torki, Saheb Abbas, Bahadori, Effat, Aghakhaninejad, Zohreh, Mohseni, Golsa Khalatbari, Tajadod, Shirin, Rajabi Harsini, Asma, Azaryan, Fatemeh, Saeedirad, Zahra, Askarpour, Seyed Ali, Mahmoudi, Zahra, Khoshdooz, Sara, Bahar, Bojlul, Shafaei, Hanieh, Mosavi Jarrahi, Seyed Alireza, Doaei, Saeid, Nazemi, Samad and Gholamalizadeh, Maryam |

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<https://doi.org/10.1007/s40200-023-01247-9>

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## **Association between type 2 diabetes and branched chain amino acids (BCAA); a case-control study**

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## Abstract

**Background** Several amino acids and their derivatives have been implicated in insulin resistance (IR) and Type 2 Diabetes Mellitus (T2DM). This research sought to establish a relationship between the dietary levels of branched-chain amino acids (BCAA) and the risk of T2DM.

**Methods** This case-control study was carried out on 4200 participants consisting of 589 people with T2DM and 3611 non-diabetic aged 35 to 70 years residents in Sabzevar, Iran. Data on the economic-social, employment status, medical history, lifestyle, and sleep habits were collected via interview. The food frequency questionnaire (FFQ) was used to check the nutritional status. Participants' dietary BCAA consumption was estimated using Nutritionist IV software.

**Results** A significant negative association between the incidence of T2DM and the dietary levels of BCAAs after adjustment for age and sex (OR = 0.972, CI 95%: 0.648–0.996, P= 0.022). The negative association remained significant after additional adjustments for body mass index (BMI) and physical activity (OR = 0.967, CI 95%: 0.943–0.992, P=0.010). Interestingly, a positive association was found between T2DM and total BCAAs (OR = 1.067, CI 95%: 1.017–1.119, P= 0.008), Isoleucine (OR=1.248, CI 95%: 1.043–1.494, P=0.016), Leucine (OR=1.165, CI 95%: 1.046–1.299, P=0.006) and Valine (OR = 1.274, CI 95%: 1.088–1.492, P= 0.003) after further adjustment for calorie intake.

**Conclusions** Our results demonstrate branched-chain amino acids (BCAAs) including isoleucine, leucine, and valine are negatively associated with the incidence of type 2 diabetes (T2DM) after adjusting for age and sex, BMI, and physical activity. However, adjusting for calorie intake reversed the association between T2DM and BCAAs. These findings suggest that the association between BCAAs and T2DM may be influenced by calorie intake. Future longitudinal studies are warranted.

**Keywords** Type 2 diabetes mellitus · Branched-chain amino acids · Dietary intake

## Introduction

Type 2 Diabetes Mellitus (T2DM) is one of the most common metabolic disorders that affect a large part of the population, and based on World Health Organization (WHO), the number of the adult population living with diabetes is expected to rise to 693 million by 2045 [1]. T2DM is a multifactorial chronic disease brought on by a mix of reversible risk factors including a poor diet and inactivity as well as irreversible risk factors like genetics, age, race, and ethnicity [2, 3]. Several dietary patterns were connected to the development of type 2 diabetes, however, the ideal macronutrient profile that can reduce the risk of diabetes is still to be established [4]. The restriction of the carbohydrate content in the meal was shown as an important factor to reduce postprandial glycemia [5]. An increase in fat and protein in the diet with a moderate reduction in carbohydrates was reported to reduce postprandial glucose and improved T2DM control [6]. On the other hand, higher consumption of total protein, particularly from animal sources was associated with an increased risk of diabetes [7–10], while a diet high in plant protein was associated with a decreased risk [11]. The roles of different amino acids and their derivatives in IR and T2DM were extensively investigated [12–19]. The BCAA – Isoleucine, Leucine, and Valine – have specific structural features (non-linear aliphatic side chain) due to which they have identical catabolic pathways. BCAA are essential amino acids, which cannot be synthesized *de novo*, and dietary intake is the only source [20]. A typical diet consists of 15–25% BCAA [21] and the plasma BCAA levels increase following consumption of a meal with BCAA, which is reported to be associated with an increased risk of T2DM [20]. Hence, the BCAA metabolism and plasma concentration are reliable markers of insulin action and can be used to predict the risk of developing obesity and T2DM [22].

While the BCAA represent essential amino acids required for cellular protein synthesis, there is conflicting evidence available to support their role in the metabolic health [23–25]. For instance, a Leucine-rich diet was reportedly associated with a lower incidence of obesity and metabolic syndrome [26]. In the mice model, an increase in the dietary Leucine intake substantially decreased diet-induced obesity, hyperglycemia, and hypercholesterolemia [23]. Although the attempt to understand the role of BCAA in metabolic health dates back to 1976, their role in T2DM is still to be elucidated [24]. Therefore, this case-control study aimed to investigate the association of dietary intake of BCAA with the risk of T2DM.

## Methods

### Study population

This case-control study was carried on a total of 4200 participants of the first phase of the Persian

Sabzevar Cohort Study (PSCS), consisted of 589 patients with T2DM and 3611 non-diabetic people aged 35 to 70 years in Sabzevar, Iran. For this study, T2DM was defined as fasting blood sugar equal to or higher than 126 mg/L. Inclusion criteria were age between 35 and 70 years, no more than 3 months have passed since the diagnosis of diabetes in the cases, and written consent to participate. Exclusion criteria include people with a family history of diabetes, undergoing treatment with drugs affecting diabetes, and current use of branched-chain amino acid supplements.

## **Data collection**

Data on economic-social and employment status, lifestyle, and sleep habits were collected. Furthermore, drug history, medical history, and hospitalization status in the last year were collected during the interview. The level of physical activity was measured as the metabolic equivalent of task (MET). A MET is the ratio of the rate of energy expended during an activity to the rate of energy expended at rest. To measure weight and height, a mechanical column scale (SECA 755) and a mobile stadiometer (SECA204) were used, respectively. The BMI of the participants was calculated using the following formula:  $\text{body weight(kg)/height(m)}^2$  [1]. Furthermore, to check the nutritional and supplement status, the data was obtained from the Persian cohort food frequency questionnaire (FFQ), and its validity and reliability were confirmed. Then, the participant's dietary BCAA consumption was estimated using Nutritionist IV software.

At the time of the examination, blood samples were taken from the antecubital vein for biochemical analysis after an overnight fast of at least 10 h, and the serum was separated and maintained at a temperature of  $-70^{\circ}\text{C}$ . Diabetes was diagnosed at fasting blood sugar (FBS) of greater than or equal to 6.99 mmol/L (126 mg/dL), according to the American Diabetes Association 2020 criteria [25].

## **Statistical analysis**

The data on the variables measured were analyzed, and for the quantitative variables, means were compared between the two groups by an independent t-test or non-parametric (Mann-Whitney) test. For the qualitative variables, the two groups were compared by Chi-square test. The association between T2DM and dietary intake of BCAA was assessed using different models of logistic regression. All analyses were performed using SPSS software and  $P < 0.05$  was considered as the cut-off for the statistical probability.

## **Results**

In this study, the patient with T2DM was older (54.53 vs. 48.36 years,  $P < 0.001$ ), and had lower height (161.44 vs. 162.33 cm,  $P = 0.033$ ), higher weight (76.18 vs. 73.77 kg,  $P < 0.001$ ), higher BMI (29.24 vs. 28.02 kg/m<sup>2</sup>,  $P < 0.001$ ), and lower MET (37.25 vs. 38.86 kcal/kg/day,  $P < 0.001$ ) compared with the controls group (Table 1)(Fig. 1).

Regarding dietary intake of participants, the amount of energy (2381 vs. 2516 kcal,  $P < 0.001$ ), total fat (61.47 vs. 64.99 g/day,  $P = 0.003$ ), carbohydrate (393.16 vs. 415.9 g/day,  $P < 0.001$ ), alcohol (0.025 vs. 0.035 g/day,  $P = 0.001$ ), isoleucine (2.99 vs. 3.08 mg/day,  $P = 0.044$ ) was significantly lower in cases than in controls. No difference was found between the intake of total protein, Leucine, and Valine in the two groups (Table 2).

As shown in Table 3, there was a significant negative association between the incidence of T2DM and the dietary levels of BCAA after adjustment for age and sex (OR = 0.972, CI 95%: 0.948–0.996,  $P = 0.022$ ) (Model 1). The result remained significant after additional adjustments for BMI and physical activity (OR = 0.967, CI 95%: 0.943–0.992,  $P = 0.010$ ) (Model 2). Interestingly, a positive association was found between T2DM and BCAAs after further adjustment for the calorie intake (OR = 1.7, CI 95%: 1.017–1.119,  $P = 0.008$ ).

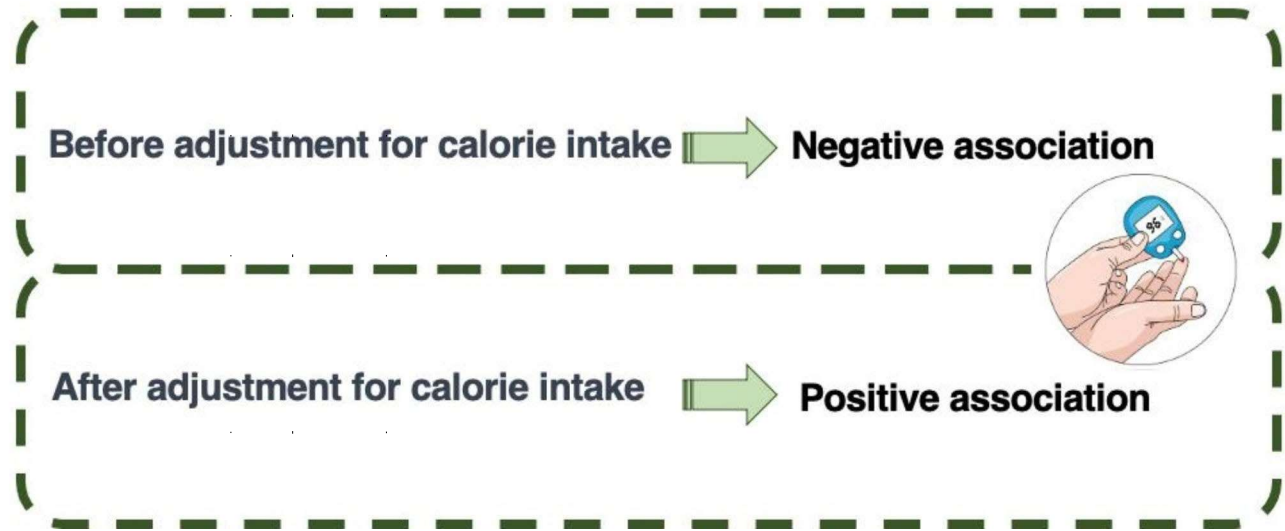
Regarding the association between T2DM and different types of BCAAs, there was a negative association between T2DM and Isoleucine (OR = 0.892, CI 95%: 0.810–0.981,  $P = 0.019$ ), Leucine (OR = 0.938, CI 95%: 0.887–0.991,  $P = 0.023$ ), Valine (OR = 0.909, CI 95%: 0.837–0.987,  $P = 0.022$ ) after adjustment for age and sex (Model 1). Also, there was a negative association between T2DM with Isoleucine (OR = 0.876, CI 95%: 0.795–0.966,  $P = 0.008$ ) and Leucine (OR = 0.929, CI 95%: 0.878–0.983,  $P = 0.011$ ) and Valine (OR = 0.937, CI 95%: 1.088–1.492,  $P = 0.094$ ) after additional adjustments for BMI and physical activity (Model 2). The result remained significantly positive between T2DM and Isoleucine (OR = 1.248, CI 95%: 1.043–1.494,  $P = 0.016$ ) Leucine (OR = 1.165, CI 95%: 1.046–1.299,  $P = 0.006$ ) and Valine (OR = 1.274, CI 95%: 1.088–1.492,  $P = 0.003$ ) after further adjustments for dietary calorie intake (Model 3).

## Discussion

This case-control study for the first time, reports on the association between type 2 diabetes and dietary intake of BCAA. The results showed a significant positive association between T2DM and BCAA after calorie adjustment despite a negative association after adjustment for age, sex, and BMI. These findings

suggest that calorie intake may be a critical component in influencing the development of type 2 diabetes and insulin resistance. Additionally, participants'

### **Type 2 Diabetes and Branched Chain Amino Acids (Isoleucine, Leucine, and Valine)**



**Fig. 1** The association between Type 2 Diabetes and Branched Chain Amino Acids (BCAA)

food consumption was substantially different between the cases and controls in terms of calories, total fat, carbohydrate, alcohol, and Isoleucine. Although there was no difference in the amounts of other dietary ingredients, including total protein, Leucine, or Valine between the two groups. This study after calorie adjustment had similar results to previous studies that type 2 diabetes can be associated with dietary intake of BCAA. A prospective cohort study [26] reported that intake of BCAA for the long-term could increase the risk of incident T2DM. These associations were independent of traditional risk factors of diabetes, like BMI. Another study showed that consumption of BCAA, Phenylalanine, and Tyrosine was associated with impaired fasting glucose and metabolic syndrome [27]. Results in a meta-analysis showed that elevation in the levels of BCAA could cause IR. In contrast, there is no association between elevated levels of Glutamine and Glycine and IR [28]. In fact, several studies, particularly in Caucasian and Asian ethnic groups, showed that high BCAA levels are associated with the development of type 2 diabetes [29–35]. In the Framingham Offspring study, elevated plasma BCAA levels had a positive correlation with fasting insulin levels that can predict the future risk of T2DM, especially in obese people [20]. Several studies also demonstrated an adverse association between BCAA and T2DM, because of BCAA's role in the development of IR [20, 36, 37]. In a Japanese study, BCAA was inversely associated with T2DM in women but were not significantly associated with T2DM in men [38]. These

differences could be in terms of the population age (35 years vs. 50–79 years) and T2DM ascertainment by self-report compared with HbA1c [7, 38]. Although another study reported that increased dietary intake of BCAA was associated with lower T2DM risk [38], ethnic differences can be responsible for such contradictory results.

The BCAA contributes to the development of T2DM via several mechanisms. BCAA can extend the secretion of glucagon and insulin secretion for a prolonged period [39]. Additionally, BCAA can play a major role in the pathophysiology of IR by promoting the production of hexosamine and gluconeogenic precursors [38]. Another hypothesis relates to the role of BCAA in activating the mechanistic target of the Rapamycin (mTORC) pathway. BCAA may activate mTORC1, with a negative effect on insulin receptors and promoting IR [40]. Amino acids can inhibit glucose transport and phosphorylation and impair glucose utilization and some studies showed that elevated levels of plasma amino acid can cause IR in skeletal muscle and stimulated endogenous glucose production in healthy men [40, 41]. Another mechanism is associated with changes in genes, especially the Protein Phosphatase,  $Mg^{2+}/Mn^{2+}$  Dependent 1 K (PPM1K), a gene encoding the mitochondrial phosphatase activating branched-chain keto acid dehydrogenase (BCKDH) complex, that has an important role in the development of T2DM [42].

This study reported an association between type 2 diabetes and dietary intake of BCAA. Using a validated FFQ to assess regular dietary intake to determine the consumption of dietary BCAA was an important strength. Thus, the current study has another important strength, including a larger sample size. Even though clinical measurements, not self-reports, were used to determine T2DM status. The primary drawback of the current investigation was the reliance on the patient's recollection of the self-reported FFQ, which was used to evaluate dietary consumption.

## Conclusion

In this study, patients with T2DM were found to have lower levels of BCAAs in their diet compared to the control group, and there was a negative association between T2DM and the dietary levels of BCAAs. However, after adjustment for calorie intake, a positive association was found between T2DM and BCAAs. Furthermore, there was a negative association between T2DM and individual BCAAs (Isoleucine, Leucine, and Valine), but this association became positive after adjustment for dietary calorie intake. Further studies need to investigate the mechanisms behind these associations and whether BCAA supplementation could potentially benefit patients with T2DM. It would also be interesting to explore the role of other dietary factors in the development and progression of T2DM.



**Acknowledgements** The research was financially supported by Sabzevar University of Medical Sciences, Sabzevar, Iran.

**Authors' contributions** SAT, EB, ZA, GKH, SHT, SR, FA, ZS, AA, ZM and SD designed the study, involved in the data collection, analysis, and drafting of the manuscript. SD, SAM, SKH, BB, HSH, SN and MGH were involved in the design of the study, analysis of the data, and critically reviewed the manuscript. All authors read and approved the final manuscript.

**Funding** The research was funded by Sabzevar University of Medical Sciences, Sabzevar, Iran (Code 99220).

**Data Availability** Datasets used and/or analyzed during the current study are available from the corresponding author on reasonable requests.

## **Declarations**

**Ethics approval and consent to participate** This study was approved by the Ethical Committee of the Research Ethics Committee of the Sabzevar University of Medical Sciences, Sabzevar, Iran. (code: IR.MEDSAB.REC.1400.040). All procedures of the studies involving human participants were by the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All participants signed informed consent forms. Informed consent was obtained from the adolescents and their parents to participate in the study.

**Consent for publication** Not applicable.

**Competing interests** The authors declare that they have no competing interests.

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**Table 1** Characteristics of the participants

|                          | Controls<br>(n = 3611) | Cases<br>(n = 589) |
|--------------------------|------------------------|--------------------|
| Age (yrs)                | 48.36 ± 8.62           | 54.53 ± 7.75       |
| Males (%)                | 1604 (44.42%)          | 267 (45.33%)       |
| Females (%)              | 2007(55.58%)           | 322(54.67%)        |
| Height (cm)              | 162.33 ± 9.18          | 161.44 ± 9.37      |
| Weight (kg)              | 73.77 ± 13.41          | 76.18 ± 13.49      |
| BMI (kg/m <sup>2</sup> ) | 28.02 ± 4.70           | 29.24 ± 4.74       |
| MET (kcal/kg/day)        | 38.86 ± 7.91           | 37.25 ± 6.86       |

Abbreviations: BMI: Body Mass Index; Met: Metabolic Equivalent Task

Quantitative and qualitative data are presented as mean ± SD and number (%), respectively

\*The analyses were performed using the independent T-test for quantitative variables and a chi

**Table 2** Average daily dietary intake of the participants

|                      | Controls       | Cases           | P*      |
|----------------------|----------------|-----------------|---------|
| Energy (Kcal)        | 2516 ± 78      | 2381 ± 80       | < 0.001 |
| Protein (g/day)      | 78.99 ± 26.06  | 76.60 ± 25.97   | 0.400   |
| Total fat (g/day)    | 64.99 ± 25.13  | 61.47 ± 26.28   | 0.003   |
| Carbohydrate (g/day) | 415.9 ± 137.51 | 393.16 ± 140.01 | < 0.001 |
| Alcohol (g/day)      | 0.035 ± 0.079  | 0.025 ± 0.061   | 0.001   |
| Isoleucine (mg/day)  | 3.08 ± 1.02    | 2.99 ± 1.04     | 0.044   |
| Leucine (mg/day)     | 5.29 ± 1.76    | 5.13 ± 1.80     | 0.053   |
| Valine (g/day)       | 3.63 ± 1.19    | 3.52 ± 1.22     | 0.051   |
| BCAAs (g/d)          | 12.01 ± 3.98   | 11.65 ± 4.08    | 0.046   |

Data are presented as mean ± SD.

\*The analyses were performed using the independent T-test