Brief Report

Branched-Chain Amino Acids Associate Negatively With Postprandial Insulin Secretion in Recent-Onset Diabetes

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Abbreviations: AA, amino acid; Adipo-IR, adipose tissue insulin resistance index; AUC, area under the curve; BCAA, branched-chain amino acid; BMI, body mass index; CON-T1D, control group for type 1 diabetes group; CON-T2D, control group for type 2 diabetes group; FFA, free fatty acids; iAUC, incremental area under the concentration time curve; ILE, isoleucine; LEU, leucine; MMTT, mixed meal tolerance test; mTOR, mechanistic target of rapamycin; PREDIM, PREDicted M-value index; TG, triglyceride; VAL, valine.

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Abstract

Context: In addition to unfavorable effects on insulin sensitivity, elevated plasma branched-chain amino acids (BCAA) stimulate insulin secretion, which, over the long-term, could impair pancreatic β-cell function.

Objective: To investigate cross-sectional and prospective associations between circulating BCAA and postprandial β-cell function in recently diagnosed type 1 and type 2 diabetes.

Methods: The study included individuals with well-controlled type 1 and type 2 diabetes (known diabetes duration <12 months) and glucose-tolerant participants (controls) of similar age, sex, and body mass index (n = 10/group) who underwent mixed meal tolerance tests. Plasma BCAA levels were quantified by gas chromatography–mass spectrometry, postprandial β-cell function was assessed from serum C-peptide levels, and insulin sensitivity was determined from PREDIM index (PREDicted M-value).

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Results: In type 1 diabetes, postprandial total BCAA, valine, and leucine levels were 25%, 18%, and 19% higher vs control, and total as well as individual postprandial BCAA were related inversely to C-peptide levels. In type 2 diabetes, postprandial isoleucine was 16% higher vs the respective controls, while neither total nor individual BCAA correlated with C-peptide levels. Whole-body insulin sensitivity was lower in both diabetes groups than in corresponding controls.

Conclusion: Insulin deficiency associates with sustained high BCAA concentrations, which could contribute to exhausting the insulin secretory reserve in early type 1 diabetes.

Key Words: postprandial insulin secretion, BCAA, recent-onset diabetes

Maintaining residual β-cell function is a key target of treating diabetes mellitus, in order to prevent hyperglycemia and delay diabetes-related complications [1]. In type 1 diabetes, dietary habits play a substantial role in preserving the residual β-cell function in the early course of the disease [2]. In type 2 diabetes, the β-cell plasticity determines the responsiveness to lifestyle modifications aiming at the prevention of further β-cell loss [3].

Amino acids (AA) enhance β-cell function via higher insulin biosynthesis and insulin secretion [4]. These short-term effects are predominantly due to branched-chain amino acids (BCAA) (leucine [LEU], isoleucine [ILE] and valine [VAL]), accounting for approximately 20% of dietary protein intake, which also relate to metabolic diseases [5]. Increased AA levels associate with insulin resistance [6], and particularly, BCAA may impair insulin signaling through the persistent activation of the mechanistic target of rapamycin (mTOR) [7], or through alterations of gut microbiota and affecting nutrient absorption [8]. In this context, a short-term dietary reduction of BCAA resulted in lower postprandial insulin secretion and improved postprandial insulin sensitivity in volunteers with type 2 diabetes [9]. However, elevated BCAA, and especially LEU, not only stimulate insulin release [10] but also jeopardize β-cell integrity and may promote its intrinsic dysfunction [11]. BCAA are responsible for activation of several key enzymes and upregulation of genes [12] that coordinate the β-cell adaptation to dysglycemia, thereby exerting both stimulatory and inhibitory input to rise an adequate secretory response [13].

Nevertheless, the relationship of circulating BCAA with β-cell function is still controversial, which at least partly results from the use of different tests in humans. Here, we employed the mixed meal tolerance test (MMTT), which is a highly reproducible method, has excellent tolerability, and is a reliable tool for the assessment of residual β-cell function [14]. Moreover, the intake of a standardized liquid meal induces a strong β-cell response independent of individual chewing and digestive efficiency and enables tracking of the physiological responses after a load of mixed nutrients [14] and in addition allowed tracking of acute BCAA load. We tested the hypothesis that circulating BCAA associate negatively with β-cell function from C-peptide in people with recently diagnosed type 1 diabetes, who demonstrated at least 20% rise in C-peptide in glucagon stimulation test, or with type 2 diabetes, who demonstrated at least 40% rise in C-peptide.

Research Design and Methods

This cross-sectional study was performed in participants with type 1 diabetes (T1D group) or type 2 diabetes (T2D group) with known diabetes duration of less than 1 year and glucose-tolerant control persons of similar age, sex, and body mass index (BMI) (CON-T1D and CON-T2D) recruited from the German Diabetes Study (GDS) [15]. Participants with type 1 diabetes (n = 10) were treated with combination of different types of insulin (long-acting insulin detemir, n = 5 or glargine, n = 3, short-acting human insulin, n = 2 and rapid-acting insulin aspart, n = 6 or insulin lispro, n = 3, insulin glulisine, n = 1), participants with type 2 diabetes (n = 10) were treated with lifestyle modification and/or metformin but no other glucose-lowering drugs. Volunteers withdrew insulin (10 hours) and metformin (3 days) prior to examination. All participants gave their informed consent to the study protocol, which was approved by the Ethics Board of the Medical Faculty of the Heinrich Heine University Düsseldorf (Clinicaltrials.gov registration number: NCT01055093) and performed according to the Declaration of Helsinki [15]. The MMTT was performed after a 10-hour overnight fast. The study participants were given 378 g of the standardized commercial liquid meal Boost High Protein 15 g protein (Nestlé S.A., Vevey, Switzerland), (365.8 kcal; 9.1 g fat, 50.1 g carbohydrates, and 22.8 g protein). Blood samples were taken at −10, −1, +10, +20, +30, +60, +90, +120, and +180 minutes for measurements of concentrations of blood glucose, serum insulin, serum C-peptide, free fatty acids (FFA), and triglycerides (TG) as previously described [15]. The glucagon stimulation test was performed as reported [15].
modeling allows analysis of biphasic insulin secretion with data computed separately for the first (0-60 minutes) and second phase (61-180 minutes) of insulin secretion after meal administration [16]. Insulin sensitivity was assessed by the PREDIM index (PREDicted M-value index) [17]. Adipose tissue insulin resistance index (Adipo-IR) was calculated as the product of fasting or postprandial (180 min) plasma FFA (mmol·l⁻¹) and insulin (pmol·l⁻¹) [18]. Measurements of AA levels were performed as previously described [19]. Total LDL-cholesterol, HDL-cholesterol, TG, and FFA as well as transaminases were measured on a Cobas c311 analyzer (Roche, Diagnostics, Darmstadt, Germany) [15]. Data are presented as mean ± SD or median (first and third quartile) for continuous and percentages for categorical variables. Values of \( P < 0.05 \) were considered significant. Skewed data were log-transformed before analyses to approximate normal distribution. Differences between insulin secretion parameters and AA levels were assessed by ANOVA-like regression models, where residual variances were allowed to be different between groups. The area under the curve (AUC) and the incremental area under the concentration time curve (iAUC) were calculated using the trapezoidal method. Correlations between insulin secretion parameters and BCAA levels (iAUC of individual and total) were assessed in each group using Spearman correlation coefficients \( r \) and corresponding \( P \) values. To control for age, sex, and BMI as potential confounders the compared groups were matched for these factors. All statistical analyses were performed using SAS (version 9.4; SAS Institute, Cary, NC, USA).

### Results

Table 1 shows anthropometric parameters of the participants and their metabolic characteristics, indicating no clinically relevant impairment of liver or kidney function, excellent blood glucose control, and residual C-peptide secretion in participants with type 1 diabetes. Levels of BCAA and VAL were 16% and 15% higher during the last 2 hours of MMTT in type 1 diabetes vs CON-T1D, respectively (all \( P < 0.05 \), Figure 1A and 1G), whereas LEU and ILE were not different (Figure 1C and 1E). In participants with type 1 diabetes, the AUC of BCAA, LEU, and VAL were higher vs CON-T1D by 25%, 19%, and 18% (all \( P < 0.05 \), respectively (Table 2). In participants with type 2 diabetes, serum levels of BCAA, LEU, and ILE in the last 2 hours of MMTT were 16% \( (P < 0.05 \), 15% \( (P < 0.05 \), and 9% \( (P < 0.01 \) (Figure 1B, 1D, and 1F) higher vs CON-T2D. In participants with type 2 diabetes, there were no differences in AUC of total and individual BCAA vs CON-T2D except for ILE, which was 12% higher in participants with type 2 diabetes \( (P < 0.05 \) (Table 2). Participants with type 1 diabetes had higher blood glucose, but lower insulin and C-peptide levels than the corresponding controls. Participants with type 2 diabetes also had higher blood glucose levels, but delayed peaks of insulin and C-peptide levels compared to the corresponding control group (Figure 2). Accordingly, in participants with type 1 diabetes, postprandial serum insulin and serum C-peptide levels were lower vs CON-T1D \( (P < 0.05 \) (Table 2).

#### Table 1. Anthropometric and metabolic characteristics of study participants

| Sex, n (male/female) | T1D       | CON-T1D     | T2D       | CON-T2D     |
|----------------------|-----------|-------------|-----------|-------------|
| (S/5)                | (S/5)     | (S/5)       | (S/5)     | (S/5)       |
| Age [years]          | 36.6 ± 3.3| 35.0 ± 1.8  | 57.4 ± 1.1| 55.5 ± 1.9  |
| BMI [kg/m²]          | 26.2 ± 1.2| 25.4 ± 0.7  | 31.6 ± 0.6| 30.6 ± 0.4  |
| HbA1c [mmol/mol]     | 48 ± 9    | 32 ± 3      | 52 ± 9    | 35 ± 3      |
| HbA1c [%]            | 6.6 ± 0.9 | 5.1 ± 0.3   | 6.9 ± 1.1 | 5.3 ± 0.3   |
| Fasting blood glucose [mg/dL] | 120.3 ± 37.8 | 87.9 ± 11.3 | 138.8 ± 21.4 | 92.3 ± 6.4 |
| Fasting C-peptide [ng/mL] | 0.7 ± 0.4 | 1.7 ± 0.5   | 3.9 ± 0.9 | 2.6 ± 1.1   |
| Triglycerides [mg/dL] | 70 [54; 100] | 117 [60; 146] | 172 [143; 225] | 93 [85; 148] |
| LDL-cholesterol [mg/dL] | 127.4 ± 28.1 | 103 ± 28.4  | 121.5 ± 38.7 | 151.3 ± 43.5 |
| HDL-cholesterol [mg/dL] | 71.5 ± 15.9 | 67.5 ± 21.9 | 44.5 ± 16.9 | 62.2 ± 14.9 |
| ALT [U/L]            | 18 [15; 28]| 17 [16; 27] | 36 [21; 50] | 22 [18; 30] |
| AST [U/L]            | 23 [17; 36]| 23 [18; 24] | 21 [19; 30] | 21 [18; 24] |
| eGFR mL/min/1.73 m²  | 107 [95; 117] | 105 [92; 110] | 77 [69; 86] | 83 [80; 96] |
| GGT [U/L]            | 22 [13; 28]| 14 [10; 18] | 44 [27; 59] | 35 [20; 54] |
| Fasting BCAA [µmol/L] | 170.4 ± 46.1 | 152.4 ± 30.5 | 171.6 ± 26.2 | 146.4 ± 29.0 |

Data are shown as mean ± SD or median (first and third quartile).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCAA, branched-chain amino acids; BMI, body mass index; CON-T1D, control group for T1D group; CON-T2D, control group for T2D group; GGT, gamma-glutamyltransferase; HbA1c, glycated hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T1D, type 1 diabetes; T2D, type 2 diabetes.
PREDIM was lower in type 1 diabetes $3.3 \pm 1.1$ vs CON-T1D $7.0 \pm 1.4$ mg$\text{kg}^{-1}\text{min}^{-1}$, $P < 0.01$ and in type 2 diabetes $2.3 \pm 0.5$ vs CON-T2D $5.0 \pm 0.7$ mg$\text{kg}^{-1}\text{min}^{-1}$, $P < 0.01$. Fasting and postprandial Adipo-IR were not different in type 1 diabetes vs CON-T1D, while both were higher in type 2 diabetes vs CON-T2D (both $P < 0.001$, data not shown). To test for associations between BCAA and insulin sensitivity, we analyzed in detail the correlations between total and individual BCAA with PREDIM and Adipo-IR at fasting and in the postprandial state.
Neither PREDIM nor Adipo-IR related to total or individual BCAA levels (Table 3).

Participants with type 1 diabetes showed an inverse association between AUC C-peptide and iAUC of total BCAA as well as iAUC of individual BCAA (Table 4). Furthermore, total insulin secretion associated negatively with the total and individual BCAA. In the corresponding matched CON-T1D, no associations were detected. In participants with type 2 diabetes and in CON-T2D, there were no associations of BCAA levels and parameters of insulin secretion (data not shown). Comparison of the time courses of BCAA (Figure 1) with glucose, insulin, and C-peptide concentrations (Figure 2) revealed that their peaks occurred later than those of BCAA in both groups of participants with diabetes (all P < 0.01). On the other hand, in the respective control groups, BCAA peaks coincided with those of glucose, insulin, and C-peptide concentrations (all P > 0.05).

**Discussion**

In persons with well-controlled recent-onset type 1 diabetes, impaired insulin secretion relates to elevated postprandial BCAA levels. Insulin deficiency could foster higher appearance of BCAA; in turn, sustained circulating BCAA might promote β-cell dysfunction in recent-onset type 1 diabetes.

In previous studies in persons with type 1 diabetes, the association between BCAA levels and β-cell function was assessed from oral glucose tolerance test (OGTT) and related to fasting but not to postprandial BCAA [20]. In our study, β-cell function and BCAA levels were assessed from MMTT, which reflects the physiological postprandial interdependence of circulating AA and insulin secretion. We further expand on previous observations regarding the relationship of BCAA and β-cell function in the fasting state, by exploring their dynamics in the postprandial state in humans with type 1 and type 2 diabetes. The design of the current study allows an accurate assessment of pancreatic secretory capacity, as β-cell sensitivity is higher during the meal [21]. Compared with the glucagon stimulation test, the MMTT induces a stronger postprandial C-peptide release and therefore represents the most suitable method to estimate residual β-cell function. In the present study, all tested persons with diabetes duration of < 1 year had measurable C-peptide values and excellent blood glucose control. This is in agreement with a study describing detectable C-peptide values and fasting blood glucose levels between 70 and 200 mg/dL in more than 85% of individuals with type 1 diabetes over up to 4 years after diagnosis [14].

The meal-induced insulin response is typically biphasic, with the amount of insulin released also depending on
factors other than β-cell function, such as the meal size and composition, prevailing glycemia, and insulin sensitivity [2]. Interestingly, in participants with type 1 diabetes, BCAA and VAL concentrations remained higher in the second phase of the MMTT compared with the matched control group. This was likely the result of limited secretory capacity of the β-cell in type 1 diabetes leading to less persistent uptake of BCAA, which is in line with previously shown data in humans with type 1 diabetes [22]. Of note, in our study population, total BCAA, but also all individual BCAA, LEU, ILE, and VAL, were each negatively associated with in vivo β-cell function as assessed by C-peptide levels.

In addition to β-cell function, we assessed insulin sensitivity from MMTT. Insulin sensitivity was lower in the participants with type 1 diabetes compared with glucose-tolerant participants of similar weight. The study participants with type 1 diabetes were metabolically well-controlled according to glycated hemoglobin (HbA1c) levels and TG levels, but they proved to be insulin-resistant shortly after diagnosis. While fasting BCAA levels were comparable to CON-T1D, postprandial levels

Figure 2. Postprandial concentrations of blood glucose, serum insulin, and C-peptide assessed during MMTT in participants with type 1 diabetes (T1D) and matched metabolically healthy control participants (T1D-CON), and type 2 diabetes (T2D) and matched metabolically healthy control participants (T2D-CON). Postprandial levels of blood glucose (A, B), serum insulin (C, D) and serum C-peptide (E, F) during MMTT. Data were compared by unpaired t-test. Data are shown as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001. Symbols above data points indicate significance compared to the corresponding data points of the control group. Symbols above lines indicate significance during last 2 hours of MMTT.
of total BCAA, as well as VAL and LEU, were increased, likely contributing to the development of insulin resistance in early type 1 diabetes. Elevated circulating LEU and ILE levels result in increased β-oxidation and acetyl CoA formation, while inhibiting lipid-derived acetyl CoA entry in the tricarboxylic acid cycle, thus contributing to impaired glycolysis and glucose uptake in skeletal muscle [23]. Furthermore, increased BCAA levels led to mTOR activation and disturbed insulin signaling through inhibitory phosphorylation of ribosomal protein S6 kinase 1 [24]. Moreover, BCAA elevation may jeopardize β-cell survival possibly due to chronic mTOR activation with enhanced endoplasmic reticulum stress [25, 26] ultimately favoring β-cell apoptosis [27] and altered mitochondrial function [24]. Furthermore, it might be speculated that long-term exposure to sustained circulating BCAA could promote β-cell dysfunction. However, deteriorating effects of BCAA on β-cells may require further elucidation, as recent evidence implies lack of association between BCAA levels, risk of insulin resistance and β-cell dysfunction in clinical [28] and experimental settings [29]. Interestingly, we found no association between BCAA levels and insulin sensitivity, as assessed from PREDIM or Adipo-IR. These findings suggest that in type 1 diabetes lower insulin levels and in type 2 diabetes impaired insulin sensitivity might promote lower postprandial BCAA uptake and protein synthesis. However, as BCAA levels and insulin sensitivity did not correlate, our current data do not allow us to draw any conclusion regarding a causal relationship. In the postabsorptive state of participants with type 2 diabetes, only ILE remained elevated throughout the MMTT compared with CON-T2D. Increased levels of total or individual BCAA associate with obesity observed in participants with type 2 diabetes and CON-T2D as proven in previous works [24]. This finding might result from the accumulation of incompletely oxidized intermediates of fatty acids and BCAA oxidation [23]. In type 2 diabetes, no correlations between circulating BCAA and insulin secretion were observed. In these participants, glucose-lowering medication could contribute to β-cell preservation and thereby conceal the relation between BCAA and insulin secretion. As expected, insulin sensitivity was lower and insulin secretion was higher in type 2 diabetes compared to CON-T2D. Moreover, Adipo-IR was higher in type 2 diabetes compared with the control group, a possible indication of beginning impairment of adipose tissue energy metabolism [18].

In the present study, participants with recently diagnosed type 1 diabetes had partially preserved insulin secretory capacity. Detectable residual β-cell function in the early course of the disease has strong long-term beneficial effects [30]. However, residual effects of exogenous insulin in participants with type 1 diabetes may still represent a limitation of the study, as they may affect fasting plasma insulin levels. Residual effects of metformin therapy in participants with type 2 diabetes may affect the findings. However, discontinuation of treatment for 3 days prior to the study in these volunteers with largely preserved kidney function renders this interference with the results unlikely. Further limitations are the relatively small study population and interindividual differences of premenopausal females. However, the study was designed as a proof-of-concept pilot study with unknown effect size of BCAA on

| Table 3. Correlations of PREDIM index and Adipo-IR with BCAA levels |
|-------------------------------|------------------|-----------------|------------------|
|          | T1D                  |                 | T2D                  |                 |
|          | PREDIM | Adipo-IR fasted | Adipo-IR postp | PREDIM | Adipo-IR fasted | Adipo-IR postp |
| Total BCAA | -0.14  | 0.31             | 0.05             | -0.05  | 0.15             | -0.20           |
|            | 0.70   | 0.38             | 0.88             | 0.88   | 0.68             | 0.61            |
|            | 0.62   | 0.37             | 0.50             | 0.48   | 0.11             | 0.49            |
| LEU       | -0.47  | 0.28             | 0.05             | -0.08  | 0.12             | -0.18           |
|            | 0.17   | 0.43             | 0.88             | 0.83   | 0.75             | 0.64            |
|            | 0.58   | 0.37             | 0.41             | 0.46   | 0.06             | 0.35            |
| ILE       | -0.27  | 0.25             | 0.04             | -0.13  | 0.25             | -0.12           |
|            | 0.45   | 0.49             | 0.91             | 0.73   | 0.49             | 0.77            |
|            | 0.64   | 0.78             | 0.80             | 0.38   | 0.03             | 0.21            |
| VAL       | -0.16  | 0.27             | 0.01             | 0.03   | 0.05             | -0.32           |
|            | 0.65   | 0.45             | 0.99             | 0.93   | 0.88             | 0.41            |
|            | 0.68   | 0.35             | 0.52             | 0.57   | 0.31             | 0.92            |

N = 10/group; P of comparisons between individual and total BCAA and PREDIM index, fasted and postprandial Adipo-IR. Correlations are assessed in every group using Spearman correlation coefficients r and corresponding P. P* of comparisons between the differences of the corresponding regression slopes of the 2 diabetes groups and their controls analyzed by a general linear mixed model approach, where the residual variances were allowed to be different between the groups. Abbreviations: BCAA, branched-chain amino acids; VAL, valine; LEU, leucine; ILE, isoleucine.
Table 4. Associations of insulin secretion parameters and BCAA during MMTT in participants with type 1 diabetes and CON-T1D

| Parameter                  | T1D     | CON-T1D  | P       |
|----------------------------|---------|----------|---------|
| iAUC BCAA                  | -0.65   | 0.08     | 0.05    |
| iAUC C-peptide             | -0.37   | 0.28     | 0.37    |
| AUC C-peptide              | -0.07   | 0.01     | 0.03    |
| iAUC glucose               | 0.99    | 0.01     | 0.01    |
| AUC glucose               | 2.02    | 0.01     | 0.01    |
| Total insulin secretion    | -0.91   | 0.01     | 0.01    |
| Blood glucose basal        | -2.31   | 0.01     | 0.01    |
| N = 10/group; r of comparisons between changes of blood glucose, serum insulin, serum C-peptide, and BCAA levels during MMTT. Correlations are assessed in every group using Spearman correlation coefficients ρ and of comparisons between the differences of the corresponding regression slopes of the 2 diabetes groups and their controls analyzed by a general linear mixed model approach, where the residual variances were allowed to be different between the groups.

Abbreviations: AUC, area under the curve; iAUC, incremental area under the curve; ILE, isoleucine; LEU, leucine; VAL, valine.

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Author Contributions: Y.K. conceived the experiments, researched data, contributed to the discussion, and wrote the manuscript. K.S. performed statistical analyses and generated allocation sequence. D.M. performed laboratory analyses. O.P.Z., K.B., and T.K. researched data and contributed to the discussion. G.P. and A.T. analyzed and interpreted the data with mathematical modeling. V.B., M.R., and J.S. conceived the experiments and contributed to the discussion. All authors reviewed and edited the manuscript. J.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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