Circulating branched-chain amino acids and incident heart failure in type 2 diabetes: The Hong Kong Diabetes Register

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Funding information
Hong Kong Association for the Study of Obesity; Hong Kong Society of Endocrinology, Metabolism and Reproduction

Aim: Levels of branched-chain amino acids (BCAAs, namely, isoleucine, leucine, and valine) are modulated by dietary intake and metabolic/genetic factors. BCAAs are associated with insulin resistance and increased risk of type 2 diabetes (T2D). Although insulin resistance predicts heart failure (HF), the relationship between BCAAs and HF in T2D remains unknown.

Methods: In this prospective observational study, we measured BCAAs in fasting serum samples collected at inception from 2139 T2D patients free of cardiovascular-renal diseases. The study outcome was the first hospitalization for HF.

Results: During 29 103 person-years of follow-up, 115 primary events occurred (age: 54.8 ± 11.2 years, 48.2% men, median [interquartile range] diabetes duration: 5 years [1–10]). Patients with incident HF had 5.6% higher serum BCAAs than those without HF (median 639.3 [561.3-756.3] vs 605.2 [524.8-708.7] μmol/L; P = .01). Serum BCAAs had a positive linear association with incident HF (per-SD increase in logarithmically transformed BCAAs: hazard ratio [HR] 1.22 [95% CI 1.07-1.39]), adjusting for age, sex, and diabetes duration. The HR remained significant after sequential adjustment of risk factors including incident coronary heart disease (1.24, 1.09-1.41); blood pressure, low-density lipoprotein cholesterol, and baseline use of related medications (1.31, 1.14-1.50); HbA1c, waist circumference, triglyceride, and baseline use of related medications (1.28, 1.11-1.48); albuminuria and estimated glomerular filtration rate (1.28, 1.11-1.48). The competing risk of death analyses showed similar results.

Conclusions: Circulating levels of BCAAs are independently associated with incident HF in patients with T2D. Prospective cohort analysis and randomized trials are needed to evaluate the long-term safety and efficacy of using different interventions to optimize BCAAs levels in these patients.

KEYWORDS
branched-chain amino acids, energy metabolism, heart failure, metabolic pathways, prevention, type 2 diabetes
1 | INTRODUCTION

Balanced nutritional intake and regular physical activity form the pillars of health promotion and disease prevention. Circulating levels of branched-chain (essential) amino acids (BCAAs, namely, isoleucine, leucine, and valine) are influenced by dietary protein intake and metabolism, with the latter being influenced by genetics. In individuals with high-intensity resistance training or poor nutritional status, increased BCAAs intake may confer health benefits, although its indiscriminate or excessive use as health supplements may potentially cause harm. Dysregulation of energy metabolism due to excessive calorie intake and/or reduced energy expenditure can lead to obesity and type 2 diabetes (T2D), which are the leading causes of atherosclerotic cardiovascular disease (ASCVD). With better control of cardiometabolic-renal risk factors, use of cardioprotective drugs, and coronary interventions, we and others have witnessed declining trends of myocardial infarction and its complications, especially in high-income countries. In these countries with rapidly ageing populations, heart failure (HF) is now a leading cause of morbidity, especially in people with diabetes in whom the occurrence of HF is similar or more frequent than ASCVD.

Traditionally, HF is considered a consequence of long-standing hypertension and coronary heart disease (CHD), although people with T2D may have subclinical cardiomyopathy for years due to predominantly metabolic causes. Given the high energy demand of myocardium, HF can be caused by abnormal utilization of energy substrate. In T2D and obesity, insulin resistance and beta-cell dysfunction can lead to inefficient uptake of glucose for energy production and non-suppression of lipolysis with increased release of free fatty acids (FFAs). Reduced glucose oxidation can shift myocardial energy metabolism from utilization of glucose to that of FFAs, ketone bodies, and BCAAs, which if dysregulated, may lead to adverse biological consequences.

Circulating levels of BCAAs are determined by dietary intake (such as dairy, meat, and grain products) and rate of intracellular BCAAs metabolism. Effective BCAAs metabolism involves two processes: (a) degradation of BCAAs into branched-chain α-keto acids (BCKAs) by branched-chain aminotransferase, which is a reversible process; and (b) degradation of BCKAs by BCKAs dehydrogenase (BCKDH) into acetyl-CoA and succinyl-CoA, which will enter the tricarboxylic acid (TCA) cycle for ATP synthesis. This process is irreversible with BCKDH being a rate-limiting enzyme. Metabolic stress such as insulin resistance and glucolipotoxicity can decrease BCKDH activity, which if accompanied by increased BCAAs intake, may saturate the limited BCKDH activity, resulting in accumulation of BCAAs and BCKAs. The latter two metabolites can cause mitochondrial dysfunction, oxidative stress, pancreatic beta-cell apoptosis, and impaired insulin signalling, culminating in systemic vasculopathy and inflammation to cause multiple organ dysfunction and a vicious cycle.

Given the potential adverse effects of high circulating levels of BCAAs on the development of cardiometabolic diseases, unravelling the complex relationships between BCAAs and cardiometabolic-renal risk factors may provide insights into the preventive and therapeutic approaches for HF. In this study, we hypothesized that elevated levels of circulating BCAAs might contribute to incident HF in Chinese adults with T2D, independent of CHD status and cardiometabolic-renal risk factors.

2 | MATERIALS AND METHODS

2.1 | Study population

Between 1995 and 2007, the Hong Kong Diabetes Register (HKDR) consecutively enrolled Chinese adults aged ≥18 years with T2D from the Diabetes Mellitus and Endocrine Centre, Prince of Wales Hospital, Hong Kong. After fasting for 10 hours, all patients underwent structured assessment including the collection of blood and urine samples. Upon entry into the HKDR, all patients provided written informed consent for banking of blood samples for future research purposes. Details of the HKDR have been published elsewhere. Figure 2 shows the study flow. This study complied with the Declaration of Helsinki and was approved by the local institutional review board.

2.2 | Measurement of serum BCAAs

From 1995 to 2007, we collected fasting blood samples from all consented patients. Serum samples were stored at -80°C from inception until retrieval for assay and analysis of serum total BCAAs concentration in 2018. In 2139 patients, we measured their BCAAs concentrations using a commercially available BCAAs colorimetric assay kit (BioVision cat no. K564-100, Milpitas, California), after excluding those with prior history of ASCVD, HF, or chronic kidney disease (CKD; defined as estimated glomerular filtration rate [eGFR] < 60 mL/min/1.73 m2 calculated using the creatinine-based CKD-Epidemiology Collaboration equation). Using a standard curve ranging from zero to 10 nmol per well, the kits have a sensitivity of ~0.2 nmol. We used DMEM medium (ThermoFisher Scientific cat no. 11885-084) with defined BCAAs concentrations (0.8 mM each, total 2.4 mM) as a positive control. The mean BCAAs concentration of 36 assay plates was 2.47 mM with a coefficient of variation (CV) of 7.2%. We also included intra-plate duplicate serum samples that yielded an average intra-plate CV of 4.88% (range: 0%-20.9%).

2.3 | Outcome

All patients were referred from community- or hospital-based clinics with a territory-wide Clinical Management System operating under the Hospital Authority, a public health system of 43 hospitals in Hong Kong, which captured all laboratory, drug, and hospitalization data. All hospital discharge diagnoses were retrieved from the Clinical Management System and coded by trained staff at the Hospital Authority.
according to the International Classification of Diseases, Ninth Revision. We defined clinical outcomes using the principal diagnosis with data censored on 30 June 2017. The study outcome was the first occurrence of HF requiring hospitalization (referred hereafter as HF; code 428), using clinical definitions including new or worsening signs and/or symptoms (such as dyspnoea, orthopnoea, paroxysmal nocturnal dyspnoea, pulmonary/peripheral oedema, jugular venous distension, gallop rhythm, and typical radiographic evidence of worsening
HF), and initiation or up-titration of oral or intravenous diuretics with appropriate response. These events were independently adjudicated by an endocrinologist (L.L.L.) and a cardiologist (E.F.). Follow-up time was calculated from the date of enrolment into the HKDR to the date of first HF, death, or 30 June 2017, whichever came first.

2.4 Statistical analysis

All data are presented as mean ± SD, median (interquartile range), or number (percentage), as appropriate. We used independent t test, Wilcoxon rank-sum test, \( \chi^2 \) test, or Fisher’s exact test for between-group comparisons, as appropriate. Spearman’s rho test was used to examine correlations between serum BCAAs and established cardiometabolic-renal risk factors.

Serum BCAAs were natural log (ln)-transformed and standardized. Triglyceride and urinary albumin:creatinine ratio (ACR) were ln-transformed to approximate normality in all regression analyses. We performed multivariable Cox proportional hazards models to estimate the adjusted hazard ratios (HRs) with 95% confidence interval (CI) for HF risk. HRs were scaled to per-SD (equivalent to 0.37) increment in ln-transformed BCAAs. We generated sequential models to adjust for multiple risk factors that were selected based on prior knowledge on predictors for HF\(^{21}\): model 1 (age, sex, and diabetes duration); model 2 (model 1 plus CHD incidence); model 3 (model 2 plus systolic and diastolic blood pressure [BP], low-density lipoprotein cholesterol [LDL-C], and the use of BP- and lipid-lowering drugs at baseline); model 4 (model 3 plus insulin resistance markers [HbA1c, waist circumference, ln-transformed triglyceride], and the use of insulin and oral glucose-lowering drugs at baseline); and model 5 (model 4 plus eGFR and ln-transformed ACR). We also explored the modifying effect of sex, diabetes duration (stratified by the median duration of the present cohort; <5 vs ≥5 years), CHD incidence, and obesity status (body mass index <25 vs ≥25 kg/m\(^2\))\(^{22}\) using each cross-product term in the Cox models, followed by stratified regression analysis.

To account for competing risk of death in the time-to-event analyses, we performed cumulative incidence function and subdistribution hazards models to estimate the subdistribution hazard ratio with 95% CI.\(^{23}\) In the fully adjusted model, we examined the non-linear relationship between standardized, ln-transformed BCAAs and HF using restricted cubic spline analysis with three knots located at 10th, 50th, and 90th percentiles. All analyses were performed using R 3.5.1 (www.r-project.org). A two-tailed \( P \) value <.05 denoted statistical significance.

3 RESULTS

3.1 Baseline characteristics

Table 1 shows the baseline characteristics of all patients, stratified by the status of study outcome. In this cohort (mean age 54.8 ± 11.2 years, 48.2% men, median diabetes duration 5 years [1-10]), patients with incident HF had 5.6% higher levels of serum BCAAs at baseline than those without HF (median 639.3 [561.3-756.3] vs 605.2 [524.8-708.7] \( \mu \)mol/L; \( P = .01 \)). There were weak correlations (\( r_s \) ranged from −0.285 to 0.364) between serum BCAAs and established risk factors (Table S1).

Patients with incident HF were older, more likely to be men, had longer diabetes duration, higher systolic BP, larger waist circumference, worse renal function, higher usage of insulin, and higher incidence of CHD and death rate than those without HF (Table 1).

3.2 Outcome

During 29 103 person-years of follow-up (mean 13.6 ± 4.2 years, \( n = 2139 \)), 115 incident cases of HF occurred. After adjusting for age, sex, and diabetes duration (Table 2, model 1), serum BCAAs remained positively associated with increased HF risk (HR per-SD increase in ln-transformed BCAAs: 1.22 [CI 1.07-1.39]). The risk association was significant after sequential adjustment for CHD incidence, systolic and diastolic BP, LDL-C, and related medication use at baseline (model 2: 1.24, 1.09-1.41 and model 3: 1.31, 1.14-1.50). This association remained robust even after adjusting for HbA1c, waist circumference, ln-transformed triglyceride, and related medication use (model 4: 1.28, 1.11-1.48), as well as eGFR and ln-transformed ACR (model 5: 1.28, 1.11-1.48). The BCAA-HF relationship did not differ by sex, diabetes duration, CHD incidence, and obesity status (all \( P_{interaction} > .05 \); Figure S1).

On restricted cubic spline analysis, incident HF was linearly related to standardized, ln-transformed BCAAs after adjusting for age, sex, diabetes duration, CHD incidence, cardiometabolic-renal risk factors, and medication use at baseline (Figure 3). Subdistribution hazards models to adjust for competing risk of death showed similar associations between BCAAs and incident HF (Table S2). Compared to those without BCAAs measurement, patients with BCAAs measurement were older and more likely to be men. They had worse renal function and increased usage of insulin, oral glucose-, BP- and lipid-lowering drugs but with lower incidence of CHD and all-cause death (Table S3).

4 DISCUSSION

In this prospective cohort of middle-aged Chinese adults with T2D observed for an average of 14 years, both men and women who developed HF had nearly 6% higher circulating levels of BCAAs at baseline than those who remained free of HF. To our knowledge, this is the first report of the positive linear relationship between BCAAs and incident HF in people with T2D, which was independent of established risk factors, medication use, and CHD incidence. This association remained robust even after adjusting for central obesity and renal function (reflected by both eGFR and ACR). The observed linear relationship between BCAAs and hazards of HF, together with the growing body of experimental data showing the detrimental effects of BCAAs on cellular metabolism, suggests a potential causal
relationship between BCAAs and HF. People with diabetes characterized by abnormal energy metabolism may have subclinical HF detectable only by advanced echocardiography or cardiac magnetic resonance imaging. Our observations suggest that BCAAs levels may have prognostic significance and if validated in other populations, may have utility in detecting high-risk individuals for interventions.

The health effects of high circulating levels of BCAAs, due to alterations in dietary intake and/or cellular metabolism, have been conflicting. In high-fat diet-fed mice, high dietary BCAAs intake for 8-10 weeks reduced adiposity and improved insulin resistance. In a 10-year prospective study of 13,525 apparently healthy Japanese, women in the highest tertile of BCAAs intake (median: 17.8% of total protein intake), mainly from grains and fish, had 43% reduced risk of T2D than those in the lowest tertile (median: 16.9%), with men having a similar but non-significant trend. By contrast, in a meta-analysis involving 8,000 individuals from eight Western population-based studies, every SD increase in circulating levels of BCAAs increased the risk of T2D by 40%. These conflicting results might be due to differences in study population, concurrent risk factors, patterns of animal and plant protein intake, genetic differences, or other factors yet to be identified.

TABLE 1  Baseline characteristics and incidence of cardiac events of Chinese patients with T2D after an observation period of 14 years, stratified by the status of study outcome

<table>
<thead>
<tr>
<th></th>
<th>Entire cohort (n = 2139)</th>
<th>Incident heart failure</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Yes (n = 115)</td>
<td>No (n = 2024)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>2139</td>
<td>54.8 ± 11.2</td>
<td>62.5 ± 9.9</td>
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<tr>
<td>Men, n (%)</td>
<td>2139</td>
<td>1030 (48.2%)</td>
<td>70 (60.9%)</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>2139</td>
<td>5 (1-10)</td>
<td>7 (3-11)</td>
</tr>
<tr>
<td>Serum branched-chain amino acids (umol/L)</td>
<td>2139</td>
<td>607.0 (526.7-710.3)</td>
<td>639.3 (561.3-756.3)</td>
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<tr>
<td>HbA1c (NGSP, %)</td>
<td>2139</td>
<td>7.6 ± 1.8</td>
<td>7.8 ± 1.8</td>
</tr>
<tr>
<td>HbA1c (IFCC, mmol/mol)</td>
<td>2139</td>
<td>60 ± 19.7</td>
<td>62 ± 19.7</td>
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<tr>
<td>Systolic BP (mmHg)</td>
<td>2137</td>
<td>131.6 ± 18.2</td>
<td>140.0 ± 17.2</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>2136</td>
<td>74.5 ± 10.3</td>
<td>75.0 ± 9.9</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>2123</td>
<td>5.1 ± 1.1</td>
<td>5.2 ± 1.1</td>
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<td>LDL-C (mmol/L)</td>
<td>2058</td>
<td>3.0 ± 0.9</td>
<td>3.0 ± 0.9</td>
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<td>HDL-C (mmol/L)</td>
<td>2130</td>
<td>1.4 ± 0.4</td>
<td>1.4 ± 0.4</td>
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<tr>
<td>Men</td>
<td>1024</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>Women</td>
<td>1106</td>
<td>1.4 ± 0.4</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>2136</td>
<td>1.3 (0.9-2.0)</td>
<td>1.4 (1.0-1.9)</td>
</tr>
<tr>
<td>Waist circumference (men; cm)</td>
<td>1027</td>
<td>88.5 ± 10.9</td>
<td>92.8 ± 12.4</td>
</tr>
<tr>
<td>Waist circumference (women; cm)</td>
<td>1106</td>
<td>83.1 ± 10.4</td>
<td>86.2 ± 11.1</td>
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<td>Urinary ACR (mg/mmol)</td>
<td>2110</td>
<td>1.4 (0.7-4.7)</td>
<td>3.7 (1.1-9.0)</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>2139</td>
<td>89.9 ± 14.8</td>
<td>83.5 ± 13.9</td>
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<td>Current smoker, n (%)</td>
<td>2131</td>
<td>321 (15.1%)</td>
<td>20 (17.5%)</td>
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<tr>
<td>Use of BP-lowering drugs, n (%)</td>
<td>2139</td>
<td>894 (41.8%)</td>
<td>70 (60.9%)</td>
</tr>
<tr>
<td>Use of lipid-lowering drugs, n (%)</td>
<td>2139</td>
<td>377 (17.6%)</td>
<td>22 (19.1%)</td>
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<tr>
<td>Use of oral glucose-lowering drugs, n (%)</td>
<td>2139</td>
<td>1647 (77.0%)</td>
<td>94 (81.7%)</td>
</tr>
<tr>
<td>Use of insulin, n (%)</td>
<td>2139</td>
<td>324 (15.1%)</td>
<td>30 (26.1%)</td>
</tr>
<tr>
<td>Complications after 14 years of follow-up, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>2139</td>
<td>243 (11.4%)</td>
<td>41 (35.7%)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>2139</td>
<td>115 (5.4%)</td>
<td>-</td>
</tr>
<tr>
<td>All-cause death</td>
<td>2139</td>
<td>429 (20.1%)</td>
<td>61 (53.0%)</td>
</tr>
</tbody>
</table>

Note: We used independent t test, Wilcoxon rank-sum test, χ² test, or Fisher’s exact test for between-group comparisons, as appropriate. Conversion factor: to convert HDL-C and LDL-C to mg/dL, multiply by 38.67. Abbreviations: ACR, albumin:creatinine ratio; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; IFCC, The International Federation of Clinical Chemistry and Laboratory Medicine; LDL-C, low-density lipoprotein cholesterol; NGSP, National Glycohaemoglobin Standardization Program; T2D, type 2 diabetes.

aData are presented as mean ± SD, median (interquartile range), or number (percentage), as appropriate.
People with T2D and/or obesity often have unhealthy or imbalanced diet, which together with beta-cell dysfunction and other neurohormonal dysregulation (such as leptin resistance and activation of sympathetic nervous system and renin-angiotensin system [RAS]) may contribute to abnormal energy metabolism. Despite the importance of protein in cellular metabolism, there is a paucity of data on the risk association of BCAAs and diabetes-related complications such as HF. In Pp2cM-knockout mice, high BCAAs and BCKA impaired myocardial contractile function which decompensated to symptomatic HF after exposure to metabolic stress. In a prospective, population-based Finnish cohort of 2441 middle-aged men (5% with T2D), during a 22-year follow-up period, individuals in the highest quartile of total protein intake at baseline (median: 109.1 g/day), mainly from animal sources as assessed by 4-day dietary recall, tended to have an excess HF risk (HR 1.33, 0.95-1.85) than those in the lowest quartile (median: 78.4 g/day). By contrast, in a post hoc, nested case-control analysis of the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial involving 3587 patients with T2D of predominantly European ancestry, every SD increase in non-fasted plasma BCAAs at baseline was associated with 10%-21% decreased risk of all-cause death. Apart from survival
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Apart from the small sample size, it was difficult to determine if this benefit was due to glycaemic improvement or direct influence of BCAAs. Besides, the differential amino acids concentrations were not described and it remained possible that the effect might be driven by other amino acids such as tyrosine. To this end, there are some reports suggesting an inverse association of plasma tyrosine with cardiovascular complications in the aforementioned ADVANCE cohort, although increased risk association of plasma tyrosine with coronary atherosclerosis has also been reported in a nested case-control analysis of the prospective, population-based Malmo Diet and Cancer cohort (11% with T2D).31

Dietary assessment can be complex including quality and quantity of nutrients and their interactions with host factors (such as risk factors and complications). Together with differences in study design, settings, and populations, these may contribute to the conflicting reports on the risk association of BCAAs. In a placebo-controlled, randomized trial involving 212 patients with T2D and left ventricular dysfunction, systolic and diastolic BP, LDL-C, waist circumference, ln-transformed triglyceride, HbA1c, ln-transformed urinary ACR, eGFR, and medication use at baseline (insulin, oral glucose-, BP-, and lipid-lowering drugs); ACR, albumin:creatinine ratio; BCAAs, branched-chain amino acids; BP, blood pressure; CI, confidence interval; eGFR, estimated glomerular filtration rate; LDL-C, low-density lipoprotein cholesterol bias, it remains plausible that these high-risk patients might have benefited most from intensive glucose lowering including the use of insulin which improved glucose utilization in a clinical trial setting.36

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In this context, one of the hypotheses underlying the cardioprotective effects of sodium-glucose cotransporter-2 inhibitors is a switch in the energy substrate utilization in the failing heart from glucose toward ketone bodies, FFAs, and BCAAs.7,11,42 In a non-diabetic animal model with HF due to myocardial infarction, 2 months of empagliflozin treatment was associated with decreased left ventricular hypertrophy and dilatation with improved ejection fraction.42 This was driven by enhanced myocardial utilization of ketone bodies, FFA, and BCAAs, in which the latter was due to increased BCKDH activity, resulting in lower circulating levels of BCAAs, which further supports our observations.42 To this end, there were reports which suggested the adverse impact of prolonged ketone bodies oxidation in the failing heart including mitochondrial proton hyperacetylation, mitochondrial dysfunction, and metabolic perturbations, which require further in-depth research.12,15

Albuminuria and eGFR are common risk factors of ASCVD and HF, probably due to the interlinking nature of these biological pathways such as systemic inflammation, left ventricular hypertrophy, vascular calcification, and fluid retention.44 In CKD, metabolic acidosis can stimulate BCKDH and BCAAs metabolism to promote renal ammonia and bicarbonate synthesis for maintaining acid-base homeostasis.55 This may result in disproportionately low circulating levels of BCAAs in CKD.45 In this analysis, we have excluded patients with CKD at baseline and adjusted for eGFR in the multivariable analyses.

5 | STRENGTHS AND LIMITATIONS

Strengths of the present study included the prospective design involving 2139 well-characterized Chinese adults of Southern Han ancestry
followed up for 29,103 person-years. The single-care provider system with a territory-wide Clinical Management System in Hong Kong, together with structured documentation of risk factors and treatment in the HKDR, enabled near complete data capture. The use of fasting serum to measure BCAAs had better reliability and reproducibility than that of non-fasted or plasma samples. Dietary protein is the main source of BCAAs and 80% of dietary BCAAs intake enter blood circulation, albeit other factors can cause intra- and inter-individual variability in circulating levels of BCAAs. Although we did not document dietary intake in this cohort, other reports suggested good correlations of dietary total protein and animal protein intake with serum BCAAs, which lent support to our inferences that excessive protein/BCAAs intake may trigger the pathological cascade, especially in people with T2D and/or obesity with impaired BCAAs metabolism.

Our study has several limitations. First, the association of HF and BCAAs was based on a single measurement on banked samples in a repository for years, although many studies have also used samples with storage time ranging from 6 months to 22 years. The HKDR was established as a research-driven quality improvement program using standard protocols for procedures, data collection, and sample processing/storage to minimize analytical errors. Although mass spectrometry is the gold standard for measuring BCAAs, we have used a validated commercial assay with internal control and yielded results comparable to that reported in other Chinese cohorts (6.5%-17.6% patients with T2D: median BCAAs 607 vs 413.4-502.7 μmol/L, respectively). Second, although 48.5% of eligible HKDR enrollees did not have complete clinical data and biosamples for analysis after excluding patients with prior HF, ASCVD, or CKD, 83% of them had biosample collection (compared to 26.6% of the present study cohort) prior to the introduction of territory-wide diabetes risk assessment program in 2000 which has begun to improve the standards of care in Hong Kong. Although our results are more reflective of diabetes care in recent years, these may have limited generalizability to high-risk patients, given the higher risk profile in our study cohort compared to those without BCAA measurements. Third, as in most public health facilities, the usage of echocardiography in the inpatient setting is not a routine procedure for diagnosing HF at our hospital. Furthermore, routine echocardiographic examination in the outpatient setting in asymptomatic patients is currently not recommended. Thus, the diagnosis of HF was based on hospital discharge codes without classification into HF with reduced or preserved ejection fraction. Nevertheless, we had adjudicated these events to improve accuracy. Since CHD is common in patients with HF with preserved ejection fraction, we only included patients without prior ASCVD and adjusted for incident CHD, although there might be under-reporting of silent or atypical CHD. Last, despite our careful adjustment for risk factors and competing risk of death analysis, the sample size was modest and other factors such as changing dietary patterns, physical activity, and gut microbiome were not captured.

6 CONCLUSIONS

High circulating levels of BCAAs were linearly associated with incident HF in Chinese patients with T2D, independent of established risk factors, medication use, and incident CHD. Given the known experimental effects of BCAAs on cellular metabolism, we deduced that high circulating levels of BCAAs might negatively affect endothelial function and systemic inflammation to impair myocardial energetics and haemodynamic function. While more confirmatory mechanistic studies and randomized controlled trials are needed to evaluate the long-term safety and efficacy of using different interventions to optimize circulating levels of BCAAs in patients with T2D, our findings did not support the use of BCAAs as health supplements or intake of high protein/ketogenic diets in these patients who are at risk of abnormal energy metabolism.

ACKNOWLEDGEMENTS

This work was supported by the Hong Kong Society of Endocrinology, Metabolism and Reproduction and the Hong Kong Association for the Study of Obesity. The funders had no role in the study design, data collection, data analysis, data interpretation, and writing of the manuscript. We thank all medical, nursing, and research staff at the Diabetes Mellitus and Endocrine Centre, Prince of Wales Hospital, Hong Kong for conducting this study.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

L.L.L., J.C.N.C., and A.P.S.K.: design the research; L.L.L., E.F., H.M.L., W.K.K.W., A.C.W.N., R.C.W.M., E.C., A.O.Y.L., J.C.N.C., and A.P.S.K.: acquired the data and conducted the research; L.L.L., E.S.H.L., C.H.T.T., and J.C.N.C.: analysed the data; L.L.L.: drafted the manuscript; J.C.N.C. and A.P.S.K. had primary responsibility for final content; R.C.W.M., A.O.Y.L., E.F., and A.J.: critically revised the manuscript for important intellectual content; and all authors: read and approved the final manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. J.C.N.C. and A.P.S.K. are the guarantors of this work and, as such, have full access to all the data in the study and take full responsibility for the integrity and accuracy of the data.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Lim L-L, Lau ESH, Fung E, et al. Circulating branched-chain amino acids and incident heart failure in type 2 diabetes: The Hong Kong Diabetes Register. Diabetes Metab Rev. 2019;e3253. https://doi.org/10.1002/dmrr.3253