Received: 4 December 2023 | Revised: 10 January 2024 | Accepted: 15 January 2024

DOI: 10.1111/dom.15475

ORIGINAL ARTICLE

WILEY

Branched-chain amino acid levels are inversely associated with incident and prevalent chronic kidney disease in people with type 2 diabetes

Juliette A. de Klerk BSc[1,2] | Roel Bijkerk PhD[2] | Joline W. J. Beulens PhD[3,4,5] | Anton Jan van Zonneveld PhD[2] | Mirthe Muilwijk PhD[3,5] | Peter P. Harms PhD[3,6] | Marieke T. Blom PhD[3,6] | Leendert M. 't Hart PhD[1,3,5,7] | Roderick C. Slieker PhD[1,3,5]

[1]Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, the Netherlands

[2]Department of Internal Medicine (Nephrology) and the Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, the Netherlands

[3]Amsterdam Public Health, Health Behaviors & Chronic Diseases, Amsterdam, the Netherlands

[4]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands

[5]Department of Epidemiology and Data Science, Amsterdam UMC, Location Vrije Universiteit Amsterdam, Amsterdam, the Netherlands

[6]Department of General Practice Medicine, Amsterdam UMC, Location Vrije Universiteit Amsterdam, Amsterdam, the Netherlands

[7]Department of Biomedical Data Sciences, Section Molecular Epidemiology, Leiden University Medical Center, Leiden, the Netherlands

Correspondence
Juliette A. de Klerk, BSc and Roderick C. Slieker, PhD, Department of Cell and Chemical Biology, Leiden University Medical Center, P.O. Box 9600, 2300RC Leiden, the Netherlands.
Emails: j.a.de_klerk@lumc.nl; r.c.slieker@lumc.nl

Funding information
Parelsnoer Initiative (PSI); BBMRI-NL, Grant/Award Numbers: 184021.007, 184033111; ZonMW Priority Medicines Elderly, Grant/Award Number: 113102006; Dutch Federation of University Medical Centres; The Netherlands Cardiovascular Research Initiative, Dutch Heart Foundation

[Correction added on 15 April 2024, after first online publication: The author's name 'Mirthe Muilwijk' was previously misspelt and has been corrected in this version]

Abstract

Aim: To investigate the association of plasma metabolites with incident and prevalent chronic kidney disease (CKD) in people with type 2 diabetes and establish whether this association is causal.

Materials and Methods: The Hoorn Diabetes Care System cohort is a large prospective cohort consisting of individuals with type 2 diabetes from the northwest part of the Netherlands. In this cohort we assessed the association of baseline plasma levels of 172 metabolites with incident ($N_{total} = 462/N_{case} = 81$) and prevalent ($N_{total} = 1247/N_{case} = 120$) CKD using logistic regression. Additionally, replication in the UK Biobank, body mass index (BMI) mediation and causality of the association with Mendelian randomization was performed.

Results: Elevated levels of total and individual branched-chain amino acids (BCAAs)—valine, leucine and isoleucine—were associated with an increased risk of incident CKD, but with reduced odds of prevalent CKD, where BMI was identified as an effect modifier. The observed inverse effects were replicated in the UK Biobank. Mendelian randomization analysis did not provide evidence for a causal relationship between BCAAs and prevalent CKD.

Conclusions: Our study shows the intricate relationship between plasma BCAA levels and CKD in individuals with type 2 diabetes. While an association exists, its

manifestation varies based on disease status and BMI, with no definitive evidence supporting a causal link between BCAAs and prevalent CKD.

KEYWORDS
cohort study, diabetic nephropathy, observational study, type 2 diabetes

1 | INTRODUCTION

Chronic kidney disease (CKD) is a complication affecting approximately 40% of people with type 2 diabetes.[1] CKD is defined by albuminuria (urine albumin-creatinine ratio [UACR] > 3 mg/mmol) and/or a reduced estimated glomerular filtration rate (eGFR < 60 mL/min/1.73m^2).[2] Previous studies have highlighted the association between various metabolites in plasma and CKD, as well as diabetic kidney disease (DKD).[3–6] However, the relationship between different metabolites and the development of CKD, as well as their correlation with existing cases of CKD in type 2 diabetes, remains unclear. Consequently, this study aims to investigate the potential association between plasma metabolites and incident and prevalent CKD in people with type 2 diabetes. We also investigate the genetic influences on these metabolites and the extent to which they play a suggestive causal role in CKD, using two-sample Mendelian randomization (MR).

2 | MATERIALS AND METHODS

2.1 | The Hoorn Diabetes Care System study cohort

The Hoorn Diabetes Care System (DCS) cohort is an open prospective cohort that started in 1998 with individuals with type 2 diabetes from the northwest part of the Netherlands. People visit the DCS annually to monitor their type 2 diabetes. Repeated measurements are collected as part of routine care during this visit, including anthropometric and laboratory measurements. Individuals in the Hoorn DCS cohort were asked to participate in the Hoorn DCS biobank, from which, after obtaining informed consent, we also collected and stored serum, plasma and urine for future research. Blood samples were collected during two phases of biobanking in 2008/2009 and 2012/2013/2014. The Ethical Review Committee of the VU University Medical Center, Amsterdam, approved the study. All participants provided written informed consent and this study was conducted in accordance with the Declaration of Helsinki.

2.2 | Study design

We used two subcohorts from the Hoorn DCS cohort biobank,[7] encompassing incident CKD ($N_{control} = 381/N_{case} = 81$) and prevalent CKD ($N_{control} = 1127/N_{case} = 120$) (Figure S1). CKD risk stage was defined based on the Kidney Disease: Improving Global Outcomes (KDIGO) criteria that combines eGFR and UACR (0 = low risk; 3 = very high risk). However, the source of CKD has not been verified by kidney biopsies. Incident cases and controls were identified during an average follow-up of 9 years. Cases had CKD risk stage (KDIGO) 0 at baseline and at least the year before and developed CKD (risk stage ≥ 2) in at least two consecutive years ($n = 81$), whereas controls remained stable in stage 0 during at least 3 years of follow-up ($n = 381$). For the prevalent CKD study, the following inclusion criteria were used: cases are those that have CKD (risk stage ≥ 2 [high risk] at the moment of sample collection [baseline]) ($n = 120$). Controls did not have CKD (risk stage 0 [low risk]) at the moment of sample collection (baseline) ($n = 1127$). Baseline was the date of blood sampling. The incident and prevalent CKD study included 375 overlapping controls (Figure S1).

2.3 | Statistical methods

With NMR spectrometry (Nightingale Health blood analysis platform), 172 metabolites were measured in plasma, as previously described (Table S1).[8] Metabolites were z-scaled before analysis. Logistic regression analyses were conducted adjusted for age and sex (model 1). For the incident CKD study, the model was further adjusted for baseline eGFR, UACR, HbA1c and systolic blood pressure (SBP) (model 2). For prevalent CKD, the fully adjusted model included age, sex, HbA1c and SBP (model 2). Metabolites were considered differentially expressed if an observed difference between two conditions was statistically significant based on a P value of less than .05. Multiple testing threshold was set at P of .0006 or less (similar to Bonferroni adjustment for 83 independent tests[9]), as previously suggested for these types of data. Also, secondary analyses with eGFR lower than 60 mL/min/1.73m^2 and UACR higher than 3 mg/mmol were performed using logistic regression. Effect modification by body mass index (BMI) and triglycerides was investigated by stratifying models in four equal strata. All analyses were performed using R statistics (v. 4.2.1) and the figures were produced with *ggplot2* (v. 3.4.2).

2.4 | UK Biobank

Significantly associated metabolites from the incident and prevalent CKD study were compared with the results from the UK Biobank Nightingale Health Biomarker-Disease Atlas, which also measured metabolomics with the Nightingale blood analysis platform. The UK Biobank Nightingale Biomarker-Disease Atlas included 118 461

participants (with and without diabetes), of whom 4075 participants had incident CKD and 181 had prevalent CKD. Metabolite biomarkers were measured from baseline plasma samples using the Nightingale Health NMR platform. Disease outcomes were derived from UK Hospital Episode Statistics data, with hospital diagnoses forming the basis of the disease endpoint definition. Cox proportional hazard estimated biomarker associations were used with incident disease outcomes, adjusting for sex and UK Biobank assessment centre and using age as the time scale. Logistic regression models adjusted for age, sex and assessment centre were used for biomarker association testing with prevalent diseases. Details of the UK Biobank study have been published.[10]

2.5 | Two-sample MR

A two-sample MR analysis was performed using the TwoSampleMR R package (v. 0.5.6). Branched-chain amino acid (BCAA) expression quantitative trait loci (eQTLs) were obtained from a publicly available dataset by Borges et al. at a genome-wide level ($P < 5 \times 10^{-8}$).[11] To obtain independent SNPs, clumping was performed, removing SNPs in linkage disequilibrium ($r^2 < 0.001$). The instrumental strength of each SNP was assessed using the F statistics $= (\beta/SE)^2$.All the available outcomes from the TwoSampleMR R package were used, except for eQTLs, because they are beyond the scope of the current study. To estimate the causal effect between BCAA and traits, a two-sample MR analysis was performed. Causal associations based on a single instrument (Wald ratio) were omitted. Causal associations based on multiple instruments (inverse variance weighting) were calculated by dividing the SNPs-outcome by the SNPs-exposure. A causal association was statistically significant based on a false discovery rate-adjusted P value below .05. Heterogeneity was calculated with the mr_heterogeneity() test: a high heterogeneity indicates a high variance across instruments suggestive of invalid instruments. For three or more instruments, the Egger's intercept was used to estimate horizontal pleiotropy. The TwoSampleMR R package was also used to test for reverse causality on the suggested causal associations. For visualization of the MR results, a scatterplot for the effect of the SNPs on the exposure against the effect of the SNPs on the outcome is shown. A forest plot for visualization of the estimates from multiple instruments is also shown.

3 | RESULTS

3.1 | Baseline characteristics

For the incident CKD subcohort ($N_{control} = 381/N_{case} = 81$), the mean (standard deviation [SD]) age of the individuals was 59.7 (9.0) years and 46.1% were females (Table 1). On average, the population developed CKD after 6.9 (2.5) years and was obese (mean BMI: 30.8 [5.4] kg/m^2). For the prevalent CKD subcohort ($N_{control} = 1127/N_{case} = 120$), the mean (SD) age of the individuals was 63.3 (9.8) years and 43.3% were females (Table 1). On average, the time since CKD diagnosis until blood sampling for metabolomics was 2.9 (2.8) years. The population was obese (mean BMI: 30.4 [5.3] kg/m^2).

3.2 | BCAA levels are associated with increased incident CKD risk in contrast to prevalent CKD

Six out of 172 metabolites were nominally significantly associated with incident CKD (Table S2, Figure 1A). These included albumin (odds ratio [OR] = 0.74, 95% confidence interval [CI] = 0.55-0.99, $P = 4.7 \times 10^{-2}$), glycine (OR = 1.41, 95% CI = 1.07-1.84, $P = 1.3 \times 10^{-2}$), total BCAA (OR = 1.49, 95% CI = 1.10-2.02, $P = 9.9 \times 10^{-3}$) and the three individual BCAAs, valine (OR = 1.48, 95% CI = 1.10-1.99, $P = 9.7 \times 10^{-3}$), leucine (OR = 1.45, 95% CI = 1.07-1.97, $P = 1.6 \times 10^{-2}$) and isoleucine (OR = 1.43, 95% CI = 1.06-1.94, $P = 2.0 \times 10^{-2}$) (Figure 1B–E). This effect was comparable for eGFR less than 60 mL/min/1.73m^2 and UACR more than 3 mg/mmol as endpoint, although it was slightly stronger for eGFR (Table S3). Although not significant after multiple testing in our study (multiple testing threshold was set at $P \leq .0006$[9]), five metabolites nominally significant in our study were significantly associated in the UK Biobank (Figure 1F–I), including albumin (hazard ratio [HR] = 0.80, 95% CI = 0.77-0.82, $P = 8.8 \times 10^{-53}$), total and individual BCAAs, valine (HR = 1.11, 95% CI = 1.08-1.14, $P = 4.3 \times 10^{-11}$), leucine (HR = 1.10, 95% CI = 1.06-1.13, $P = 7.1 \times 10^{-9}$) and isoleucine (HR = 1.12, 95% CI = 1.09-1.15, $P = 2.4 \times 10^{-14}$) (Table S4).

Interestingly, for prevalent CKD, the associations for BCAAs were opposite compared with incident CKD in both our study and the UK Biobank (Figures 1B–E and 2A, Tables S4 and S5). This was the case for total and individual BCAAs (valine and leucine) in the DCS (total BCAA: OR = 0.72, 95% CI = 0.56-0.93, $P = 1.2 \times 10^{-2}$; valine: OR = 0.67, 95% CI = 0.53-0.85, $P = 1.3 \times 10^{-3}$; leucine: OR = 0.72, 95% CI = 0.56-0.93, $P = 1.4 \times 10^{-2}$) and valine in the UK Biobank (OR = 0.68, 95% CI = 0.68-0.94, $P = 6.9 \times 10^{-3}$; Figure 1F–H). In the UK Biobank, total BCAAs and leucine were not significant (total BCAA: OR = 0.87, 95% CI = 0.74-1.02, $P = .09$; leucine: OR = 0.88, 95% CI = 0.75-1.03, $P = .12$), probably because of the low number of prevalent cases ($n = 181$). Furthermore, isoleucine did not show this opposite association in prevalent CKD in our study (OR = 0.96, 95% CI = 0.75-1.20, $P = .72$), or in the UK Biobank (OR = 1.06, 95% CI = 0.92-1.23, $P = .44$) (Figure 1I).

3.3 | Poor kidney function is associated with lower BCAA levels

In the UK Biobank, the strongest association with BCAAs after type 2 diabetes is overweight and obesity.[10] There was no difference in average baseline BMI across the groups (Figure 2B). However, BCAA levels increased with increasing baseline BMI (Figure S2). The interaction term (BMI) was significant for incident CKD ($P \leq .04$). However, adjusting for BMI was not enough, because this resulted in similar effect sizes (Figure S3). In addition, stratifying the results by BMI into four quartiles showed that the effects were primarily present in the

TABLE 1 Patient characteristics.

	Incident			Prevalent		
	Cases ($n = 81$)	Controls ($n = 381$)	Total ($n = 462$)	Cases ($n = 120$)	Controls ($n = 1127$)	Total ($n = 1247$)
Age (y)	64.4 (± 5.6)	58.7 (± 9.3)	59.7 (± 9.0)	71.1 (± 8.3)	62.5 (± 9.5)	63.3 (± 9.8)
Female (%)	$n = 34$ 42.0	$n = 179$ 47.0	$n = 213$ 46.1	$n = 56$ 46.7	$n = 484$ 42.9	$n = 540$ 43.3
BMI (kg/m^2)	31.6 (± 6.1)	30.6 (± 5.2)	30.8 (± 5.4)	30.0 (± 4.9)	30.4 (± 5.3)	30.4 (± 5.3)
HbA1c (mmol/mol)	52.4 (± 9.5)	51.0 (± 11.2)	51.2 (± 10.9)	53.9 (± 12.6)	51.9 (± 11.5)	52.1 (± 11.6)
HbA1c (%)	6.9 (0.9)	6.8 (1.0)	6.8 (1.0)	7.1 (± 3.3)	6.9 (± 3.2)	6.9 (± 3.2)
Fasting glucose (mmol/L)	8.3 (± 1.8)	8.1 (± 2.2)	8.1 (± 2.2)	8.1 (± 2.6)	8.2 (± 2.2)	8.2 (± 2.3)
Age at diagnosis of type 2 diabetes (y)	55.5 (± 6.9)	52.8 (± 9.0)	53.3 (± 8.8)	61.6 (± 8.8)	56.0 (± 9.5)	56.5 (± 9.6)
Diabetes duration (y)	8.0 (5.3-11.7)	5.3 (2.9-8.3)	6.0 (3.1-8.8)	8.6 (5.7-12.1)	5.5 (2.9-9.1)	6.0 (3.0-9.5)
Smoking	$n = 9$ 11.1%	$n = 82$ 21.5%	$n = 91$ 19.7%	$n = 21$ 17.5%	$n = 216$ 19.2%	$n = 237$ 19.0%
HDL cholesterol (mmol/L)	1.1 (± 0.3)	1.2 (± 0.3)	1.2 (± 0.3)	1.2 (± 0.3)	1.2 (± 0.3)	1.2 (± 0.3)
LDL cholesterol (mmol/L)	2.7 (± 0.8)	2.6 (± 0.8)	2.6 (± 0.8)	2.6 (± 1.0)	2.5 (± 0.9)	2.6 (± 0.9)
Total cholesterol (mmol/L)	4.7 (± 1.0)	4.6 (± 1.0)	4.6 (± 1.0)	4.6 (± 1.2)	4.5 (± 1.0)	4.5 (± 1.0)
Triglycerides (mmol/L)	2.0 (± 1.2)	1.7 (± 0.9)	1.8 (± 1.0)	1.9 (± 0.9)	1.7 (± 1.0)	1.7 (± 1.0)
eGFR (mL/min/1.73m^2)	76.5 (69.5-87.7)	92.7 (84.2-98.8)	90.9 (80.2-98.2)	44.4 (35.3-57.9)	86.0 (74.3-95.8)	84.2 (71.6-94.9)
Creatinine blood (μmol/L)	81.9 (±18.0)	71.1 (±13.0)	73.0 (±14.6)	123.6 (±37.1)	75.2 (±14.4)	79.9 (±22.9)
UACR (mg/mmol)	0.9 (± 0.7)	0.6 (± 0.8)	0.6 (± 0.8)	24.6 (± 50.8)	0.7 (± 0.7)	3.0 (± 17.2)
SBP (mmHg)	143.0 (133.0-157.0)	137.0 (126.0-148.0)	138.0 (128.0-149.0)	145.0 (132.0-159.5)	140.0 (128.0-153.0)	141.0 (128.0-154.0)

Note: Data are in mean (SD), median (IQR) or in *n* (%).
Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; IQR, interquartile range; SBP, systolic blood pressure; SD, standard deviation; UACR, urine albumin-creatinine ratio.

lower BMI group (BMI < 27 kg/m^2) (Table S6, Figure 2C). However, BMI alone is not the primary or exclusive risk factor for CKD. Instead, insulin resistance has emerged as a more precise factor linked to the development of CKD. Because measures of insulin resistance were not measured at the time of the metabolomics measurement, we used triglycerides as a surrogate marker for insulin resistance. We observed the same pattern for triglycerides as for BMI. Higher levels of triglycerides result in higher levels of BCAAs (Figure S4). In addition, stratifying the results into four quartiles showed that the effects were primarily present in the lower triglycerides group (triglycerides strata = 0.32-1.11). BMI and triglycerides did not explain the inverse relationship between BCAA levels and incident and prevalent CKD. However, we did observe that the diagnosis of CKD was at a younger age for prevalent cases than for incident cases (Figure 2D). Most importantly, we show that poorer kidney function (lower eGFR) is associated with lower levels of BCAAs in prevalent CKD cases. Again, this effect was strongest in the low BMI group (Figure 2E,F).

3.4 | BCAA levels are not causally involved in prevalent CKD

To test whether BCAAs may have a causal effect on CKD relevant traits, we extracted 31 independent eQTLs that were associated with BCAA levels. The eQTLs had an F-statistic ranging from 30 to 673, indicating strong instruments (Table S7).[12] Using MR, we observed no causal relationship between BCAAs and eGFR, UACR or prevalent CKD. However, we could not test for the causal effect on incident CKD. BCAAs did have a suggestive causal effect on seven traits, including four metabolic intermediates of the metabolic pathway of BCAAs (6-12 SNPs, $P \leq 3.6 \times 10^{-2}$), antihypertensive medication use (16 SNPs, $P = 2.1 \times 10^{-4}$), circulating leptin (3-5 SNPs, $P \leq 1.1 \times 10^{-2}$) and creatinine levels (seven SNPs, $P = 2.9 \times 10^{-2}$) (Figures S5–S25). No evidence for reverse causality was found (Table S8).

4 | DISCUSSION

This study showed that higher levels of BCAAs, including valine, leucine and isoleucine, are associated with CKD in type 2 diabetes. This association exhibits an inverse relationship; individuals who will develop CKD in the future have higher levels of BCAAs, whereas individuals with prevalent CKD have lower levels of BCAAs compared with controls. Despite not having a definitive answer as to why this inverse relationship exists, we believe it is probably a consequence of kidney damage, resulting in impaired renal function and an inability of the kidneys to retain essential solutes such as amino acids in the

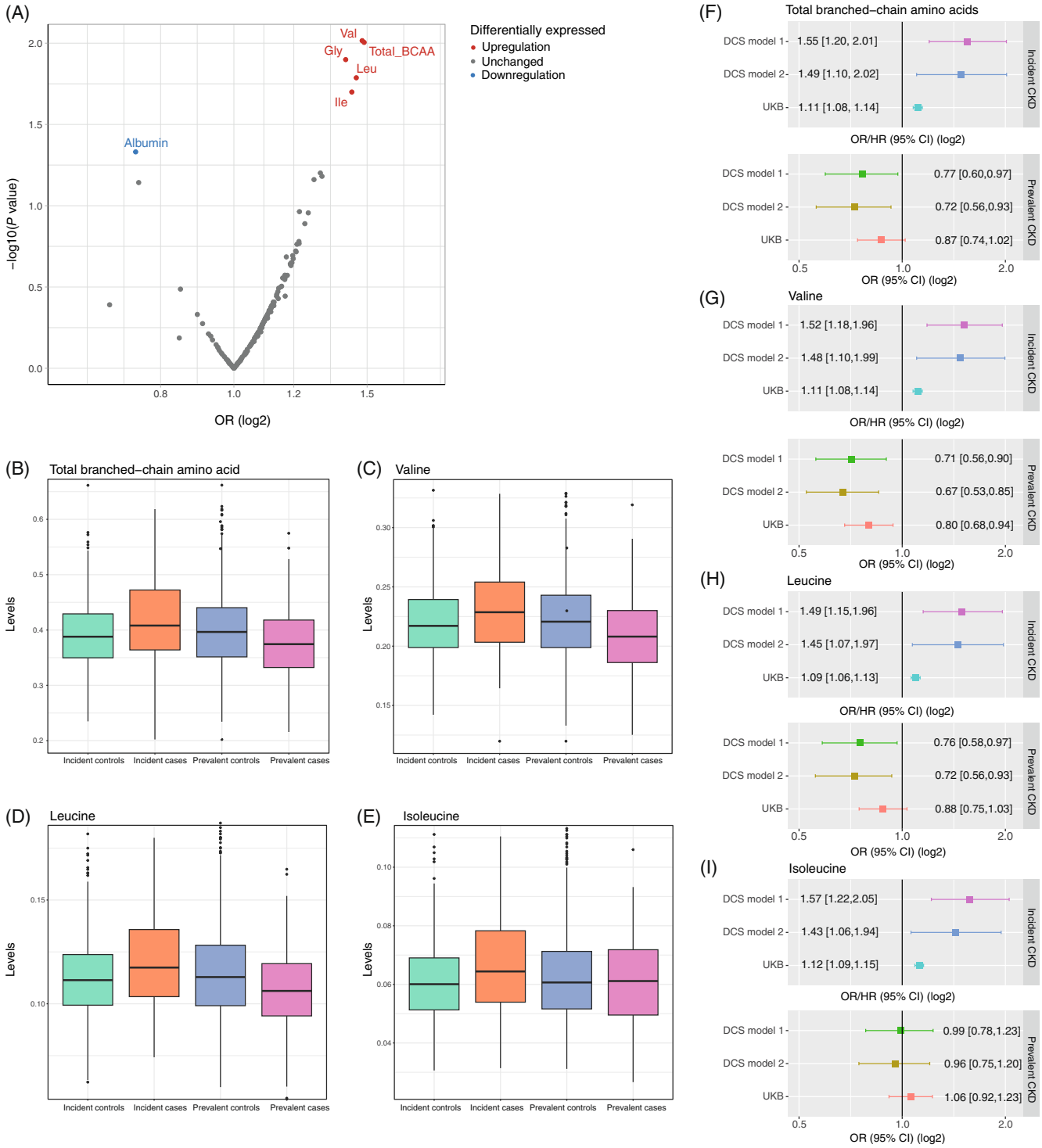

FIGURE 1 Branched-chain amino acid (BCAA) levels are associated with increased incident CKD risk but reduced prevalent CKD odds in type 2 diabetes. A, Volcano plot of differentially expressed metabolites for incident CKD for model 2 (age, sex, baseline eGFR, UACR, HbA1c and SBP). B–E, Boxplots of total branched-chain amino acid (valine, leucine and isoleucine) levels for incident and prevalent cases and controls. F–I, Plots with odds ratios (ORs) (hazard ratios [HRs] for incident CKD in the UK Biobank [UKB] study) and 95% confidence intervals (CIs) for total BCAAs (valine, leucine and isoleucine) for each study; incident CKD (model 1 [age and sex], model 2 [age, sex, eGFR, UACR, HbA1c and SBP] and the UKB) and prevalent CKD (model 1 [age and sex], model 2 [age, sex, HbA1c and SBP] and the UKB). CKD, chronic kidney disease; DCS, Diabetes Care System; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; UACR, urine albumin-creatinine ratio.

bloodstream (Figure 3). Consequently, there is an increased excretion of total BCAAs and other solutes in the urine, as supported by previous studies that observed elevated levels of urinary valine associated with a higher risk of end-stage renal disease.[13] The proximal tubule is responsible for amino acids reabsorption.[14] Mounting evidence strongly suggests the involvement of proximal tubular injury in the

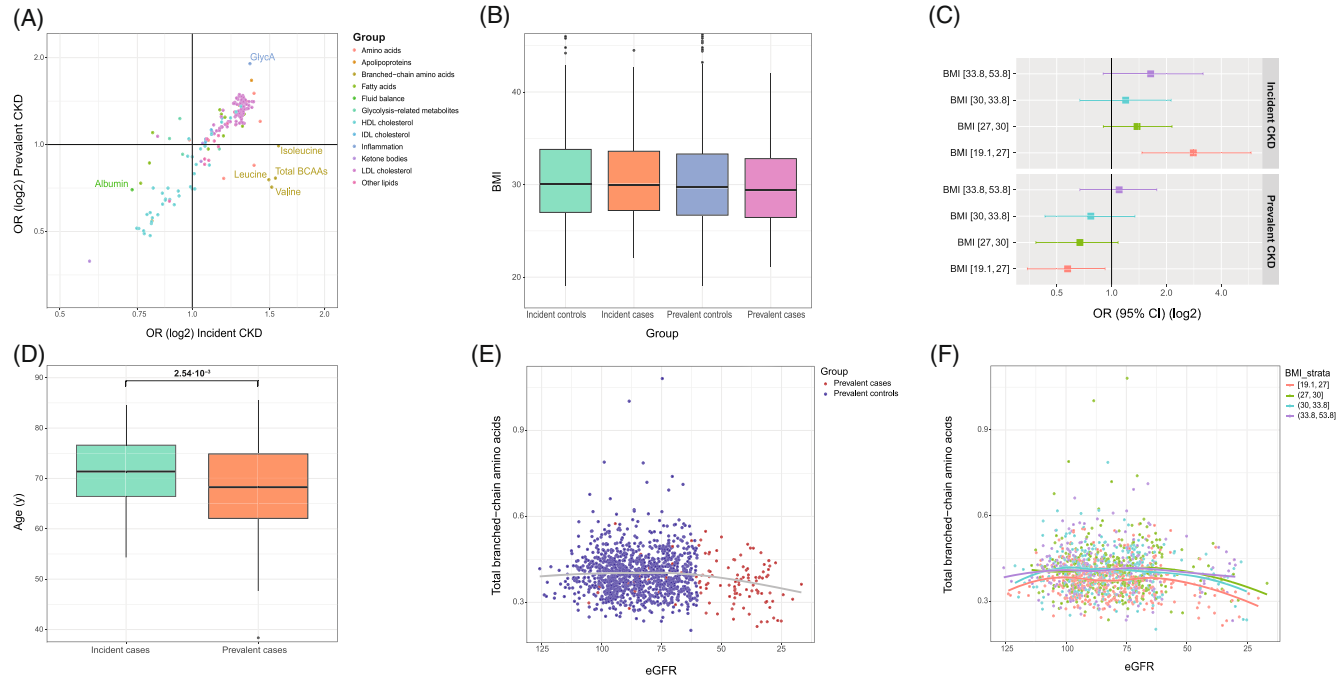

FIGURE 2 Different characteristics of the incident and prevalent cases in the DCS. A, Beta-beta plot of metabolites for the incident and prevalent CKD study. B, Boxplot BMI for incident and prevalent cases and controls. C, Plot with odds ratios (ORs) and 95% confidence intervals (CIs) for total branched-chain amino acids divided into BMI quantiles for incident and prevalent CKD study. D, Boxplot of age in years of CKD diagnosis for the incident and prevalent CKD cases used in this study. E, Plot of eGFR and branched-chain amino acid levels for prevalent cases and controls. F, Plot of eGFR and branched-chain amino acid levels for prevalent cases and controls divided by BMI quantiles. BMI, body mass index; CKD, chronic kidney disease; DCS, Diabetes Care System; eGFR, estimated glomerular filtration rate; IDL, intermediate density lipoprotein.

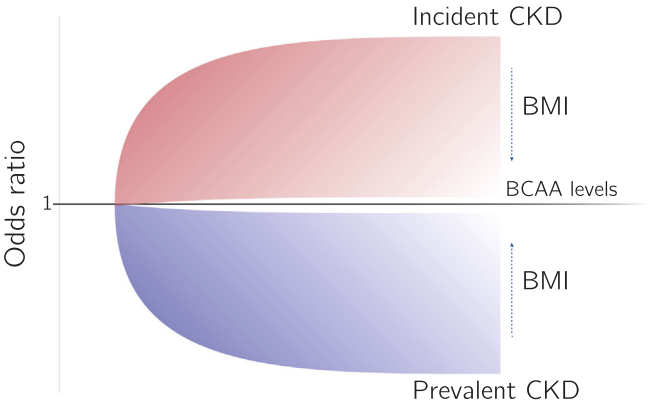

FIGURE 3 Proposed hypothesis. Incident and prevalent CKD cases occur on distinct time scales, yielding contrasting plasma levels of BCAAs dependent on BMI. Over time, incident cases exhibit higher plasma BCAA levels, while prevalent cases experience reduced levels because of impaired kidney function, resulting in elevated urine BCAA levels.[13] This effect is primarily seen in the lower BMI group. BCAAs, branched-chain amino acids; BMI, body mass index; CKD, chronic kidney disease.

advancement of DKD.[15] Furthermore, our study shows a noticeable trend towards decreased levels of BCAAs in individuals with impaired kidney function (low eGFR). However, in the incident CKD analyses, increased levels of BCAAs are associated with an increased CKD risk, particularly in the low BMI group. Perhaps BCAA levels are already increased in the high BMI group, which masks the increased levels associated with the increased kidney risk. Elevated BCAA levels have been consistently observed in obesity and type 2 diabetes.[16–18]

Interestingly, in our previous work we observed that BCAA levels were higher in the insulin-resistant type 2 diabetes cluster.[19] In addition, people in this group were also at an increased risk of developing CKD.[20] This reinforces the association between BCAAs and CKD. Some research has proposed that elevated BCAA levels might interfere with insulin signalling pathways in cells, which can contribute to insulin resistance.[21,22] This interference can affect the ability of insulin to stimulate glucose uptake in muscle and fat cells.[23,24] Additionally, as previously mentioned, BMI is not identified as the primary risk factor for CKD according to the literature.[25] Instead, insulin resistance exhibits a more substantial association with CKD.[19,26] Furthermore, numerous other risk factors have been pinpointed for CKD, including abdominal obesity, metabolic syndrome, hypertension and hyperlipidaemia.[27,28] While these risk factors tend to align somewhat with BMI, not all individuals with obesity have insulin resistance.[29,30] In our study, fasting triglyceride levels were utilized as a proxy for insulin resistance.[31] This revealed that elevated triglyceride levels (indicative of greater insulin resistance) corresponded to higher BCAA levels in both the incident and prevalent study. Moreover, akin to BMI, the analysis of incident CKD showed that higher BCAA levels were associated with an increased risk of CKD in the lowest triglyceride strata.

To investigate a possible causal effect of BCAAs, we used MR. With MR, we show an expected causal relationship between BCAAs and metabolic intermediates of the metabolic pathway of BCAAs.[32] Moreover, our study found a potential causal effect of

BCAAs on antihypertensive medication use. Recent research has suggested that elevated BCAAs levels might be linked to an increased risk of developing hypertension.[33,34] Furthermore, another study showed a direct causal relationship between higher circulating BCAAs levels and elevated blood pressure.[35] Here, we did not observe a causal effect for BCAAs on SBP and hypertension, but we did find a causal relationship between BCAAs and antihypertensive medication use. Based on MR, we also found that higher levels of BCAAs are suggested to reduce circulating leptin and creatinine levels. This may initially appear counterintuitive. Nevertheless, it is important to consider that the relationship of BCAAs can vary significantly across different diseases. Notably, we did not observe any causal association between BCAAs and insulin resistance, eGFR, UACR and prevalent CKD. Interestingly, in another study, the authors found that higher levels of BCAAs do not have a causal effect on insulin resistance, while increased insulin resistance drives higher circulating BCAA levels.[21] This suggests that elevated BCAA levels are just a marker of impaired insulin action and not a causative factor in the development of insulin resistance.

Our study has several limitations. First, the sample size was comparatively small, but similar results were observed in a large cohort of 118 461 participants with and without diabetes from the UK Biobank.[10] Second, the absence of urinary samples limited our ability to show that, in the prevalent cases, urinary BCAA levels are indeed increased. Third, we could not confirm the source of CKD to be type 2 diabetes, because of the absence of kidney biopsies. Kidney biopsies in patients with diabetes have shown that also a large number have non-diabetic renal disease.[36] Finally, the use of a genome-wide association study conducted in the general population, including both healthy individuals and those with type 2 diabetes, is not ideal.

Our study indicates that BCAAs exhibit opposite associations with incident and prevalent CKD in individuals with type 2 diabetes. This relationship seems to be different between people with low and high BMI, which might be attributable to a priori increased levels in people with a high BMI. Our results show lower levels of BCAAs in prevalent CKD, although this relationship is probably not causal. However, we observe higher levels of BCAAs in incident CKD for which a causal relationship cannot be excluded and should be explored further. In addition, this is the first study to shed light on the association of elevated levels of plasma BCAAs with future CKD in type 2 diabetes.

AUTHOR CONTRIBUTIONS

JAdK, LM'tH and RCS designed the study and drafted the manuscript. JAdK and RCS performed the analyses. JWJB and LM'tH contributed to the data acquisition and project logistics. JAdK, RCS, LM'tH, RB, AJvZ, JWJB, MM, PPH and MTB contributed to the data interpretation. All authors critically revised the manuscript and approved the final version. JAdK and RCS are the guarantors of the work.

ACKNOWLEDGEMENTS

We thank the participants of the Hoorn DCS cohort for their participation.

FUNDING INFORMATION

This work has been funded by BBMRI-NL (Complementary project, CP2013-69, NWO, grant nos. 184021.007 and 184033111). It was furthermore funded by ZonMW Priority Medicines Elderly (grant 113102006) and the Parelsnoer Initiative (PSI), the Netherlands Cardiovascular Research Initiative and the Dutch Heart Foundation (CVON2017-15 RESCUED. PSI is part of and is funded by the Dutch Federation of University Medical Centres and, from 2007 to 2011, received initial funding from the Dutch Government. RB and AJvZ were supported by funding of the European Foundation for the Study of Diabetes. The opinions expressed and arguments employed herein do not necessarily reflect the official views of these funding bodies. JAdK was supported by the Medical Genomics research theme at Leiden University Medical Centre.

CONFLICT OF INTEREST

All the authors declared no competing interests.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.15475.

DATA AVAILABILITY STATEMENT

Individual level data will be available upon request by contacting the corresponding author but access to data must be granted by the respective steering committee.

ORCID

Juliette A. de Klerk https://orcid.org/0000-0002-7018-3143
Roel Bijkerk https://orcid.org/0000-0002-6438-4133
Joline W. J. Beulens https://orcid.org/0000-0002-4181-0937
Anton Jan van Zonneveld https://orcid.org/0000-0002-1676-7738
Mirthe Muilwijk https://orcid.org/0000-0002-3686-5116
Peter P. Harms https://orcid.org/0000-0002-7721-6198
Marieke T. Blom https://orcid.org/0000-0002-8088-1105
Leendert M. 't Hart https://orcid.org/0000-0003-4401-2938
Roderick C. Slieker https://orcid.org/0000-0003-0961-9152

REFERENCES

1. Feng XS, Farej R, Dean BB, et al. CKD prevalence among patients with and without type 2 diabetes: regional differences in the United States. *Kidney Med*. 2021;4(1):100385. doi:10.1016/j.xkme.2021.09.003
2. Chen TK, Knicely DH, Grams ME. Chronic kidney disease diagnosis and management: a review. *JAMA*. 2019;322(13):1294-1304. doi:10.1001/jama.2019.14745
3. Lecamwasam A, Mansell T, Ekinci EI, Saffery R, Dwyer KM. Blood plasma metabolites in diabetes-associated chronic kidney disease: a focus on lipid profiles and cardiovascular risk. *Front Nutr*. 2022;9:821209. doi:10.3389/fnut.2022.821209
4. Yamaguchi Y, Zampino M, Moaddel R, et al. Plasma metabolites associated with chronic kidney disease and renal function in adults from the Baltimore longitudinal study of aging. *Metabolomics*. 2021;17(1):9. doi:10.1007/s11306-020-01762-3

5. Mahbub MH, Yamaguchi N, Nakagami Y, et al. Association of plasma branched-chain and aromatic amino acids with reduction in kidney function evaluated in apparently healthy adults. *J Clin Med*. 2021; 10(22):5234. doi:10.3390/jcm10225234
6. Kumar MA, Bitla AR, Raju KV, Manohar SM, Kumar VS, Narasimha SR. Branched chain amino acid profile in early chronic kidney disease. *Saudi J Kidney Dis Transpl*. 2012;23(6):1202-1207. doi: 10.4103/1319-2442.103560
7. van der Heijden AA, Rauh SP, Dekker JM, et al. The Hoorn diabetes care system (DCS) cohort. A prospective cohort of persons with type 2 diabetes treated in primary care in The Netherlands. *BMJ Open*. 2017;7(5):e015599. doi:10.1136/bmjopen-2016-015599
8. Soininen P, Kangas AJ, Wurtz P, et al. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *Analyst*. 2019;134(9):1781-1785. doi:10.1039/b910205a
9. Makinen VP, Karsikas M, Kettunen J, et al. Longitudinal profiling of metabolic ageing trends in two population cohorts of young adults. *Int J Epidemiol*. 2022;51(6):1970-1983. doi:10.1093/ije/dyac062
10. Julkunen H, Cichonska A, Tiainen M, et al. Atlas of plasma NMR biomarkers for health and disease in 118 461 individuals from the UK biobank. *Nat Commun*. 2023;14(1):604. doi:10.1038/s41467-023-36231-7
11. Borges MC, Haycock PC, Zheng J, et al. Role of circulating polyunsaturated fatty acids on cardiovascular diseases risk: analysis using Mendelian randomization and fatty acid genetic association data from over 114,000 UK biobank participants. *BMC Med*. 2022;20(1):210. doi:10.1186/s12916-022-02399-w
12. Brion MJ, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. *Int J Epidemiol*. 2013;42(5):1497-1501. doi:10.1093/ije/dyt179
13. Zhang J, Fuhrer T, Ye H, et al. High-throughput metabolomics and diabetic kidney disease progression: evidence from the chronic renal insufficiency (CRIC) study. *Am J Nephrol*. 2022;53(2–3):215-225. doi: 10.1159/000521940
14. Gonska T, Hirsch JR, Schlatter E. Amino acid transport in the renal proximal tubule. *Amino Acids*. 2000;19(2):395-407. doi:10.1007/s007260070019
15. Chang J, Yan J, Li X, Liu N, Zheng R, Zhong Y. Update on the mechanisms of tubular cell injury in diabetic kidney disease. *Front Med (Lausanne)*. 2021;8:661076. doi:10.3389/fmed.2021.661076
16. Bagheri M, Djazayery A, Farzadfar F, et al. Plasma metabolomic profiling of amino acids and polar lipids in Iranian obese adults. *Lipids Health Dis*. 2019;18(1):94. doi:10.1186/s12944-019-1037-0
17. Liu M, Huang Y, Zhang H, et al. Restricting branched-chain amino acids within a high-fat diet prevents obesity. *Metabolites*. 2022;12(4): 334. doi:10.3390/metabo12040334
18. Vanweert F, Schrauwen P, Phielix E. Role of branched-chain amino acid metabolism in the pathogenesis of obesity and type 2 diabetes-related metabolic disturbances BCAA metabolism in type 2 diabetes. *Nutr Diabetes*. 2022;12(1):35. doi:10.1038/s41387-022-00213-3
19. Slieker RC, Donnelly LA, Fitipaldi H, et al. Distinct molecular signatures of clinical clusters in people with type 2 diabetes: an IMI-RHAPSODY study. *Diabetes*. 2021;70(11):2683-2693. doi:10.2337/db20-1281
20. Ahlqvist E, Storm P, Karajamaki A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *Lancet Diabetes Endocrinol*. 2018;6(5): 361-369. doi:10.1016/S2213-8587(18)30051-2
21. Mahendran Y, Jonsson A, Have CT, et al. Genetic evidence of a causal effect of insulin resistance on branched-chain amino acid levels. *Diabetologia*. 2017;60(5):873-878. doi:10.1007/s00125-017-4222-6
22. Yao H, Li K, Wei J, Lin Y, Liu Y. The contradictory role of branched-chain amino acids in lifespan and insulin resistance. *Front Nutr*. 2023; 10:1189982. doi:10.3389/fnut.2023.1189982
23. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. *Nat Rev Endocrinol*. 2014;10(12):723-736. doi:10.1038/nrendo.2014.171
24. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. *Physiol Rev*. 2018;98(4):2133-2223. doi:10.1152/physrev.00063.2017
25. Stefansson VTN, Schei J, Solbu MD, Jenssen TG, Melsom T, Eriksen BO. Metabolic syndrome but not obesity measures are risk factors for accelerated age-related glomerular filtration rate decline in the general population. *Kidney Int*. 2019;93(5):1183-1190. doi:10.1016/j.kint.2017.11.012
26. Fritz J, Brozek W, Concin H, et al. The association of excess body weight with risk of ESKD is mediated through insulin resistance, hypertension and hyperuricemia. *J Am Soc Nephrol*. 2022;33(7):1377-1389. doi:10.1681/ASN.2021091263
27. Duan J, Wang C, Liu D, et al. Prevalence and risk factors of chronic kidney disease and diabetic kidney disease in Chinese rural residents: a cross-sectional survey. *Sci Rep*. 2019;9(1):10408. doi:10.1038/s41598-019-46857-7
28. Hu J, Yang S, Zhang A, et al. Abdominal obesity is more closely associated with diabetic kidney disease than general obesity. *Diabetes Care*. 2016;39(10):e179-e180. doi:10.2337/dc16-1025
29. Calori G, Lattuada G, Piemonti L, et al. Prevalence, metabolic features, and prognosis of metabolically healthy obese Italian individuals: the Cremona study. *Diabetes Care*. 2011;34(1):210-215. doi:10.2337/dc10-0665
30. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G. Insulin reistance and hypersecretion in obesity. *J Clin Invest*. 1997; 100(5):1166-1173. doi:10.1172/JCI119628
31. Ma M, Liu H, Yu J, et al. Triglyceride is independently correlated with insulin resistance and islet beta cell function: a study in population with different glucose and lipid metabolism states. *Lipids Health Dis*. 2020;19(121):121. doi:10.1681/ASN.2021091263
32. Biswas D, Duffley L, Pulinilkunnil T. Role of branched-chain amino acid-catabolizing enzymes in intertissue signaling, metabolic remodeling, and energy homeostasis. *FASEB J*. 2019;33(8):8711-8731. doi:10.1096/fj.201802842RR
33. Flores-Guerrero JL, Groothof D, Connelly MA, Otvos JD, Bakker SJL, Dullaart RPF. Concentration of branched-chain amino acids is a strong risk marker for incident hypertension. *Hypertension*. 2019; 74(6):1428-1435. doi:10.1161/HYPERTENSIONAHA.119.13735
34. Mahbub MH, Yamaguchi N, Hase R, et al. Plasma branched-chain and aromatic amino acids in relation to hypertension. *Nutrients*. 2020; 12(12):3791. doi:10.3390/nu12123791
35. Lin C, Sun Z, Mei Z, et al. The causal associations of circulating amino acids with blood pressure: a Mendelian randomization study. *BMC Med*. 2022;20(1):414. doi:10.1186/s12916-022-02612-w
36. Sharma SG, Bomback AS, Radhakrishnan J, et al. The modern spectrum of renal biopsy findings in patients with diabetes. *Clin J Am Soc Nephrol*. 2013;8(10):1718-1724. doi:10.2215/CJN.02510213

SUPPORTING INFORMATION

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

How to cite this article: de Klerk JA, Bijkerk R, Beulens JWJ, et al. Branched-chain amino acid levels are inversely associated with incident and prevalent chronic kidney disease in people with type 2 diabetes. *Diabetes Obes Metab*. 2024; 26(5):1706-1713. doi:10.1111/dom.15475