Clinical Biochemistry 102 (2022) 26–33

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Branched chain amino acids are associated with metabolic complications in liver transplant recipients

Marco Böhler [a], Eline H. van den Berg [a], Maria C.T. Almanza [a], Margery A. Connelly [b], Stephan J. L. Bakker [c], Vincent E. de Meijer [d], Robin P.F. Dullaart [e], Hans Blokzijl [a,*], TransplantLines Investigators

[a] Department of Gastroenterology and Hepatology, University of Groningen, University Medical Center Groningen, The Netherlands
[b] Laboratory Corporation of America Holdings (Labcorp), Morrisville, NC 27560, United States
[c] Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[d] Department of Surgery, Division of Hepato-Pancreato-Biliary Surgery and Liver Transplantation, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[e] Department of Endocrinology, University of Groningen, University Medical Center Groningen, The Netherlands

ARTICLE INFO

Keywords:
Branched chain amino acids
Type 2 diabetes
Dyslipidemia
Metabolic syndrome
Liver transplantation
Sirolimus

ABSTRACT

Background: Obesity, dyslipidemia and type 2 diabetes (T2D) contribute substantially to increased cardiovascular morbidity and mortality in patients after orthotopic liver transplantation (OLTx). Elevated plasma branched chain amino acids (BCAA) are linked to metabolic disturbances and cardiovascular disease (CVD) risk profiles in several non-OLTx populations.

Methods: Cross-sectional analysis of liver transplant recipients from TransplantLines, a single-center biobank and cohort study. BCAA plasma levels were measured by means of nuclear-magnetic resonance spectroscopy. CVD and cardiometabolic factors were collected by using data from electronic patient records. Associations were determined between BCAA plasma levels and T2D, Metabolic Syndrome (MetS), CVD as well as mTOR inhibition in liver transplant recipients.

Results: 336 Patients were divided into sex-stratified tertiles of total BCAA. MetS ($P < 0.001$) and T2D ($P = 0.002$) were significantly more frequent in subjects in the highest BCAA tertile. In logistic regression analyses, the multivariable adjusted odds ratio (OR) per 1 standard deviation increase in BCAA was 1.68 (95%CI: 1.18–2.20, $P = 0.003$) for MetS and 1.60 (95%CI: 1.14–2.23, $P = 0.006$) for T2D. Use of Sirolimus (mTOR inhibitor) was significantly associated with higher BCAA plasma levels, independent of age, sex, time after OLTx, MetS and other immunosuppressive medication (adjusted $P = 0.002$).

Conclusion: Elevated BCAA plasma levels are associated with T2D, MetS and use of Sirolimus in liver transplant recipients. BCAA plasma levels may represent a valuable biomarker for cardiometabolic complications after OLTx.

Abbreviations: AAA, Aromatic Amino Acids; ALP, alkaline phosphatase; ALT, alanine aminotransferase; BCAA, Branched Chain Amino Acids; BMI, Body Mass Index; CI, Confidence Interval; CMV, Cytomegalovirus; CRP, C-Reactive Protein; CVD, Cardiovascular Disease; EBV, Epstein-Barr Virus; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyl transferase; HbA1c, glycated hemoglobin; HCC, hepatocellular carcinoma; HDL, high density lipoprotein; IRAS, Insulin Resistance and Atherosclerosis Study; LDL, low density lipoprotein; MAFLD, Metabolic Associated Fatty Liver Disease; MELD, Model for End-stage Liver Disease; MetS, Metabolic Syndrome; NMR, Nuclear Magnetic Resonance Spectroscopy; OR, Odds Ratio; PBC, Primary Biliary Cholangitis; PSC, Primary Sclerosing Cholangitis; T2D, Type 2 Diabetes Mellitus; TG, triglycerides; WHO, World Health Organization.

* Corresponding author.
E-mail addresses: m.t.bohler@umcg.nl (M. Böhler), h.blokzijl@umcg.nl (E.H. van den Berg), e.h.wouters@umcg.nl (M.C.T. Almanza), connem5@labcorp.com (M.A. Connelly), s.j.l.bakker@umcg.n (S.J.L. Bakker), v.e.de.meijer@umcg.nl (V.E. de Meijer), dull.fam@12move.nl (R.P.F. Dullaart), h.blokzijl@umcg.nl (H. Blokzijl).

https://doi.org/10.1016/j.clinbiochem.2022.01.009
Received 5 December 2021; Received in revised form 24 January 2022; Accepted 31 January 2022
Available online 7 February 2022

1. Introduction

End stage liver disease is a major cause of morbidity and mortality worldwide and orthotopic liver transplantation (OLTx) is the only curative treatment [1]. However, OLTx leads to its own risks and adverse sequelae. An increased rate of cardiovascular and cardiometabolic morbidities, including obesity, hypertension, dyslipidemia, metabolic syndrome (MetS), type 2 diabetes (T2D), as well as a greater risk of cardiovascular death, are common among liver transplant recipients [2–4]. Furthermore, cardiovascular disease (CVD) and renal failure are frequently observed non-hepatic long-term causes of morbidity and mortality after OLTx [4]. Immunosuppressive medication, primary liver disease and lifestyle have, among others, been identified as factors contributing to the pathogenesis of these morbidities [4–8]. However, alterations of amino acid and free fatty acid metabolism also remain present after OLTx [9,10]. The role of amino acids and their metabolism have recently gained more attention in this field of research. Specifically, branched-chain amino acids (BCAA) have repeatedly been associated with multiple cardiovascular and cardiometabolic morbidities that are observed in liver transplant recipients [11].

The BCAA, valine, leucine and isoleucine, are essential amino acids [12]. Besides being important building blocks for proteins, they are involved in various physiological processes during normal growth of cells and organs[11]. Particularly leucine promotes cell growth by overt nutrient signaling activity via the mTOR pathway [11,13]. In general, amino acid metabolism takes place in the liver, however, BCAA in particular are largely catabolized in extra hepatic tissues, due to the low activity of branched-chain amino acid transferase in the liver [14]. It is well established that the plasma concentration of BCAA decreases while the concentration of aromatic amino acids (AAA) increases in patients with advanced cirrhosis and that the BCAA/AAA ratio (Fischer's ratio) has been considered to be prognostic in liver cirrhosis [15,16]. However, little is known about plasma BCAA levels and their possible implications when studied after OLTx.

Plasma BCAA are elevated in individuals with obesity, insulin-resistance and T2D [17–20]. Moreover, plasma BCAA levels have been identified as strong risk predictors for CVD and T2D [21,22]. Although the role of BCAA metabolism in cardiometabolic disorders is not yet fully understood, rodent models suggest that mTOR signaling may be involved [23]. In concordance with elevated BCAA levels, an increase in mTORC1, a functional multiprotein complex of mTOR signaling, has been observed in the context of obesity and metabolic syndrome [23,24]. Increased mTOR signaling has been implicated in suppression of autophagy in the heart, as well as in endothelial senescence in the vasculature, contributing to cardiac hypertrophy and ischemic injury, respectively. Interestingly, rapamycin, an mTOR-inhibitor, has been shown to reverse these effects [25,26]. In liver transplant recipients, mTOR inhibitors, in particular sirolimus, are usually given as a non-nephrotoxic alternative to calcineurin inhibitors [27]. However, the relationship between sirolimus and BCAA plasma levels has not yet been investigated, and the role of BCAA plasma levels in metabolic and cardiovascular comorbidities of liver transplant recipients still remains poorly understood. In the present cross-sectional study, we aimed to investigate the associations of BCAA plasma levels with MetS, T2D and mTOR inhibition treatment in liver transplant recipients.

2. Materials and methods

2.1. Study population

TransplantLines is a single-center prospective biobank and cohort study that comprises recipients of solid organ transplantations, such as, heart, lungs, kidney, liver and small bowel [28]. The study was approved by the local ethics committee of the University Medical Center Groningen (METc 2014/077) and all procedures were performed in accordance with the Declaration of Helsinki. Subjects were included if they were older than 18 years at the time of transplantation and had provided written informed consent. Exclusion criteria were no mastery of the Dutch language or intellectual incapability to comprehend questionnaires or physical tests.

The present study is a cross-sectional analysis of patients in the liver transplant subgroup of TransplantLines. In all patients that underwent OLTx between February 1982 and July 2018 analyses were performed at the University Medical Center Groningen. Subjects with data collected $\leq$ 1 year after OLTx or absent BCAA measurements were excluded since most of the weight gain and therefore metabolic changes occur within the first year after transplantation [7].

2.2. Data collection

Data was collected between June 2015 and September 2019. During visits at the outpatient clinic, questionnaires, blood samples, 24-hour urine, feces, nails and hair were collected from all participants according to the TransplantLines protocol. Tissue samples and transplant biopsies were taken during transplant surgery. A standardized protocol was used to obtain blood pressure and anthropometric measurements (height, weight, hip-, waist - circumference and body mass index [BMI]) [28]. All medical records of the study participants were reviewed in order to retrieve all relevant additional data from electronic patient records, such as, medical (discharge) letters, operation-, anesthesiology- and intensive care unit (ICU) reports, pre-transplant and explant histology reports as well as biochemical testing results.

2.3. Clinical procedures, definitions and laboratory measurements

BMI was calculated as weight (kg) divided by height squared (m^2). Subjects with a BMI between 25 kg/m^2 and 30 kg/m^2 were classified as overweight and subjects with a BMI $\geq$ 30 kg/m^2 were classified as obese. A diagnosis of T2D was confirmed when a subject had either self-reported T2D, used glucose lowering medication, had a fasting glucose >7.0 mmol/L or had a HbA1c $\geq$ 48 mmol/mol. Blood pressure (mmHg) was measured according to a standard clinical protocol using an automatic device (Philips Suresign VS2+, Andover, Massachusetts, USA). The median of 4 measurements was used for further data analysis. At the time of measurement, patients continued their regular medication, including antihypertensive drugs. High blood pressure was defined as systolic blood pressure $\geq$130 mmHg or diastolic blood pressure $\geq$85 mmHg and/or the use of antihypertensive medication. MetS was defined by the revised diagnostic criteria from the American Heart Association by the National Cholesterol Education Program Adult Treatment Panel III [29]. Chronic impaired renal function was defined by an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m2, calculated by applying the combined creatinine cystatin C-based Chronic Kidney Disease Epidemiology Collaboration equation [30].

For the assessment of the severity of the underlying liver disease the model of end-stage liver disease (MELD) score was used [31].

The presence of CVD was confirmed if a patient had a history of myocardial infarction, coronary artery disease, angina pectoris, congestive heart failure, stroke or transient ischemic attack (TIA) between the timepoint of transplantation and the drawing of blood.

Blood samples were drawn after an overnight fasting period of 8–12 h. 24-hour urine specimens were collected at the same time. Both procedures were performed by experienced nurses at the outpatient clinic. Subsequently, all blood samples were centrifuged by qualified technicians and stored alongside the urine samples at −80 °C until further analysis. C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), albumin, HbA1c, fasting glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides (TG), cytomegalovirus (CMV), Epstein Barr virus (EBV), eGFR, 24-hour urine protein and

creatinine clearance were analyzed with standardized laboratory measurements and quality assessment control at the department of laboratory medicine of the University Medical Center Groningen.

Valine, leucine and isoleucine concentrations were measured in EDTA anticoagulated plasma samples using a Vantera Clinical Analyzer (Labcorp, Morrisville, NC), a fully automated, high-throughput, 400 MHz proton (1H) nuclear magnetic resonance (NMR) spectroscopy platform; in a standalone assay that has been optimized to quantify BCAA concentrations. Plasma samples were prepared on board the instrument and automatically delivered to the flow probe in the NMR spectrometer's magnetic field. The NMR method for quantification of BCAA has been previously described. Briefly, coefficients of variation for inter- and intra-assay precision ranged from 1.8% to 6.0%, 1.7% to 5.4%, 4.4% to 9.1%, and 8.8% to 21.3%, for total BCAA, valine, leucine, and isoleucine, respectively. The fairly high inter-assay variation of isoleucine is caused by the low sensitivity of NMR to detect small molecule analytes circulating at lower concentrations and to quantify them from spectra that include overlapping NMR signals from proteins and lipoproteins. Nonetheless, BCAA quantified from the same samples using NMR and LC-MS/MS were highly correlated, showing a $r^2 = 0.97$, 0.95 and 0.90 for valine, leucine, and isoleucine, respectively [17,19].

2.4. Statistical analysis

Statistical analyses were performed with SPSS (version 25.0, SPSS Inc., Chicago, IL, USA). Normality of distribution was assessed and checked for skewness. Continuous variables with normal distribution were reported as means with standard deviations, non-normally distributed variables were reported as medians with interquartile ranges. Categorical variables were expressed as numbers with percentages. Linear associations across sex-stratified tertiles were determined using one-way ANOVA for normally distributed data, Kruskal-Wallis tests for skewed data and Pearson Chi-Square tests for categorical data. Skewed distributed data were log-transformed when appropriate. Missing values were labeled as user-missing values and excluded from statistical analyses by pairwise-deletion. Univariate relationships were calculated using Spearman's rank correlation analyses. Subsequently, multivariate linear regressions were employed to assess independent associations of the study outcomes with BCAA plasma levels, while accounting for relevant clinical covariates. The selection of the covariates was based on statistically significant correlations with BCAA values in univariate analyses ($P < 0.05$) and in view of our study objectives. The models were built in a stepwise fashion adjusting for age, sex, time since OLTx, BMI, smoking, renal function, primary liver disease, medication use and immunosuppressive therapy. Results are presented as *P*-values with their corresponding beta standardized regression coefficients (ß), defined as the degree of change in the outcome variable for every 1-unit of change in the predictor variable. Total BCAA plasma levels were log-transformed to achieve normal distribution. In order to avoid multicollinearity, variables with correlations > 0.5 were excluded from the analyses. Furthermore, all variables in the equations defining MetS (waist circumference, triglycerides, use of antihypertensive medication or statins) were excluded to preclude interaction with the dependent variable MetS. Finally, binary logistic regression analyses were performed to identify independent associations with MetS and T2D. For the continuous variables eGFR and BCAA, z-transformation was applied. Results are presented by odds ratios (OR) with 95% confidence intervals (CI).

3. Results

After applying exclusion criteria, data from 336 participants were eligible for this study. The study group had a median age of 59 (IQR 46.0–67.0) years and was predominantly male (n = 157; 57%). The median BMI was 26.1 (IQR 23.4–29.9) kg/m^2 and the median time between OLTx and measurement was 10 (IQR 4.0–19.0) years. Baseline characteristics of the population are presented in total numbers and sex-stratified tertiles of total BCAA values (Table 1). The median of the total BCAA values was 358.5 (IQR 298.5–407.5) μmol/L in males and 300.0 (IQR 268.0–351.0) μmol/L in females. Subjects in the highest tertile of BCAA values were more likely to have a higher BMI, higher systolic blood pressure (both $P < 0.05$) and higher prevalence of T2D, high blood pressure and MetS (all $P < 0.01$). Accordingly, antihypertensive medication, statins and glucose-lowering medication were prescribed more frequently in participants with higher BCAA values. Plasma BCAA amounted to 367.5 (IQR 300.3–420.5) μmol/L in subjects with T2D (n = 88) vs. 319.5 (IQR 272.3–374.8) μmol/l in subjects without T2D (n = 249) ($P < 0.001$), and to 369.5 (IQR 311.5–424.0) μmol/L in subjects with MetS (n = 120) vs. 309.0 (IQR 271.3–366.0) μmol/l in subjects without MetS (n = 216) ($P < 0.001$). Additionally, subjects with higher BCAA values had higher TG and lower HDL-cholesterol concentrations. Elevated HbA1c and fasting glucose values were also more frequently present in patients with higher BCAA values, while their eGFR was lower. Age, time since OLTx, smoking status, MELD score and the occurrence of HCC did not differ significantly between BCAA tertiles. The MELD score could only be calculated for 155 subjects that underwent OLTx between January 2009 and September 2019, due to the lack of data in electronic patient files before 2009. Participants with higher BCAA values were significantly less likely to take immunosuppressive medication such as glucocorticoids ($P < 0.001$), azathioprine ($P = 0.007$) and cyclosporine ($P = 0.02$). Conversely, tacrolimus ($P = 0.009$) and sirolimus ($P = 0.001$) were associated with higher BCAA values. Metabolic-dysfunction associated fatty liver disease (MAFLD) and viral disease were more frequently the underlying etiology of liver disease requiring OLT in participants with the highest BCAA levels.

Univariate analyses were performed to identify linear correlations of BCAA levels with clinical parameters in the total study population as well as between group differences in subjects with and without MetS or T2D, respectively (Table 2). In the total population as well as all subgroups, male sex, and TG were positively associated with BCAA plasma levels. Conversely, HDL-cholesterol levels were inversely associated with BCAA plasma levels among all subgroups. BMI was positively associated with BCAA in all groups except in the MetS subgroup. While we observed a positive correlation between MAFLD as the primary liver disease and BCAA plasma levels in the total study population and the T2D group, no significant correlation was seen in subjects with and without MetS or T2D. In subjects with viral liver disease a significant correlation with BCAA plasma levels could be observed in the total population, the group without metabolic syndrome and the T2D subgroup. The use of corticosteroids was inversely correlated with BCAA plasma levels in the total population and the subgroups with and without T2D. Also, the use of azathioprine was inversely associated with BCAA in all groups, except the MetS and T2D subgroup. While the use of sirolimus was correlated with BCAA levels in the total population, MetS, non-MetS and non-diabetic groups, this was not seen in the T2D subgroup. There were no statistically significant correlations between eGFR, smoking status, high blood pressure, CVD and BCAA plasma levels.

Subsequently, we determined the extent to which the associations between BCAA and the variables MetS, the individual components of MetS and T2D were modified by the inclusion of other clinical parameters in multivariable linear regression models (Table 3, Table 4 and Supplementary Table 1). In the first model adjusted for age, sex and time since OLTx, BCAA plasma levels were positively associated with MetS (Table 3, Model 1, ß = 0.25, 95% CI: 0.03–0.08, $P < 0.001$). This association remained significant in all subsequent models, adjusting in a stepwise fashion for eGFR, smoking status, MAFLD as the primary liver disease (Table 3, Model 2, ß = 0.23, $P < 0.001$), use of corticosteroids, tacrolimus, sirolimus (Table 3, Model 3, ß = 0.25, $P < 0.001$), as well as when adjusting for all variables simultaneously (Table 3, Model 4, ß = 0.23, $P < 0.001$).

In consecutive analyses the individual components of MetS were

Table 1
Baseline characteristics of 336 participants after OLTx, divided into sex-stratified tertiles of BCAA plasma levels.

Population	Total study	BCAA tertile (range)			*P*-Value
		T1	T2	T3	
BCAA plasma levels					
Total BCAA (μmol/L)					
Male, median (IQR)					
Female, median (IQR)	327.0 (277.5–388.0)	262.0 (237.8–279.3)	327.0 (302.5–365.0)	420.0 (384.5–469.5)	<0.001
	358.5 (298.5–407.5)	273.0 (248.0–298.0)	358.5 (334.0–374.8)	436.5 (408.8–482.8)	<0.001
	300.0 (268.0–351.0)	252.0 (231.0–268.0)	300.0 (289.5–316.0)	373.0 (351.0–431.0)	<0.001
Valine (μmol/L)	189.0 (158.0–220.0)	150.5 (133.8–162.8)	190.0 (175.0–209.0)	231.0 (209.5–260.0)	<0.001
Male, median (IQR)	203.0 (168.5–230.0)	158.0 (141.0–175.0)	204.0 (189.3–219.5)	243.5 (228.8–266.0)	<0.001
Female, median (IQR)	176.0 (150.0–203.0)	141.0 (125.0–155.0)	176.0 (161.5–183.5)	211.0 (200.0–237.0)	<0.001
Leucine (μmol/L)	95.0 (76.0–118.8)	72.5 (58–85.3)	95.0 (81.0–107.0)	131.0 (111.0–150.0)	<0.001
Male, median (IQR)	99.0 (80.0–126.0)	73.0 (60.0–85.0)	99.5 (90–112.8)	137.0 (116.0–154.0)	<0.001
Female, median (IQR)	88.0 (73.0–108.0)	72.0 (58.0–87.0)	84.0 (73–96)	123.0 (101.0–135.0)	<0.001
Isoleucine (μmol/L)	47.0 (36.0–57.0)	36.5 (30.8–44.3)	47.0 (38.5–55.5)	59.0 (48.5–69.5)	<0.001
Male, median (IQR)	51.0 (39.5–62.0)	39.0 (32.0–48.0)	50.0 (42.0–57.8)	65.0 (54.8–73.3)	<0.001
Female, median (IQR)	42.0 (34.0–49.0)	34.0 (29.0–38.0)	43.0 (37.5–48.5)	51.0 (42.0–64.0)	<0.001
Baseline characteristics					
Participants, n (%)	336	110 (32.7)	117 (34.8)	109 (32.4)	
Sex: males, n (%)	193 (57.0%)	63 (57.3)	68 (58.1)	62 (56.9)	0.982
Age at BCAA measurement (year), median (IQR)	59 (46.0–67.0)	58 (43.0–66.3)	58 (41.5–66.0)	61 (52.0–67.0)	0.090
Time since OLTx (year), median (IQR)	10 (4.0–19.0)	14.5 (5.0–21.0)	9 (4.0–18.5)	9 (4.0–16.5)	0.711
Primary liver disease					
Storage disease, n (%)	36 (10.7)	15 (13.6)	13 (11.1)	8 (7.3)	0.317
Autoimmune hepatitis, n (%)	16 (4.8)	11 (10.0)	2 (1.7)	3 (2.8)	0.603
PSC/PBC, n (%)	92 (27.4)	31 (28.2)	34 (29.1)	27 (24.8)	0.750
Viral Hepatitis, n (%)	42 (12.5)	9 (8.2)	10 (8.5)	23 (21.1)	0.004
Alcohol, n (%)	34 (10.1)	13 (11.8)	12 (10.3)	9 (8.3)	0.682
MAFLD, n (%)	27 (8.0)	1 (0.9)	12 (10.3)	14 (12.9)	0.030
Vascular, n (%)	3 (0.9)	0	1 (0.9)	2 (1.8)	0.352
Other, n (%)	86 (25.6)	30 (27.3)	33 (28.2)	23 (21.1)	0.420
MELD score *, median (IQR)	17.5 (12.0–23.0)	18 (13.0–23.0)	18 (12.0–23.3)	16 (11.0–22.3)	0.525
Smoking, n (%)	34 (10.1)	15 (13.6)	10 (8.5)	9 (8.3)	0.344
BMI (kg/m^2), median (IQR)					
- Normal; ≤25 (kg/m^2), n (%)					
- Overweight; 25–30 (kg/m^2), n (%)					
Obese; ≥30 (kg/m^2), n (%)	26.1 (23.4–29.9)	23.9 (22.1–27.1)	25.8 (23.2–29.9)	27.5 (25.6–31.0)	<0.001
	132 (39.2)	61 (55.5)	48 (41.0)	23 (20.9)	<0.001
	119 (35.3)	30 (27.3)	40 (34.2)	49 (44.5)	0.026
	82 (24.3)	16 (14.5)	28 (23.9)	38 (34.5)	0.003
Waist circumference (cm)					
- Male, median (IQR)					
Female, median (IQR)					
	100.5 (91.2–110.0)	94.5 (85.7–105.3)	100.8 (91.9–111.2)	104 (97.6–114)	0.001
	90.1 (81.5–101.9)	88.3 (81.0–94.1)	85 (77.0–96.6)	100.3 (89.8–113.3)	0.000
Systolic blood pressure(mmHg), mean ± SD	132.3 ± 16.7	129.7 ± 16.2	130.8 ± 16.1	136.4 ± 17.3	0.006
Diastolic blood pressure(mmHg), mean ± SD	79.8 ± 10.6	80.2 ± 11.5	78.9 ± 9.5	80.4 ± 11.0	0.537
Laboratory tests					
hsCRP (mg/L), median (IQR)	2.1 (0.9–4.8)	1.9 (0.7–5.0)	1.9 (0.9–4.5)	2.5 (1.3–4.8)	0.151
ALT (U/L), median (IQR)	25.0 (18.0–35.0)	24.0 (18.0–35.0)	24.0 (18.0–35.0)	25.0 (19.0–33.0)	0.942
AST (U/L), median (IQR)	26.0 (20.0–33.0)	28.0 (22.0–34.0)	26.0 (20.0–33.0)	24.0 (20.0–33.0)	0.078
GGT (U/L), median (IQR)	39.0 (21.0–81.0)	36.0 (19.0–92.5)	39.0 (19.0–77.0)	42.0 (25.0–69.0)	0.556
ALP (U/L), median (IQR)	86.0 (69.0–122.0)	86.0 (63.3–122.8)	88.0 (67.0–129.0)	84.0 (72.0–112.0)	0.814
Albumin (g/L), median (IQR)	44.0 (42.0–46.0)	44.0 (41.0–46.0)	44.0 (43.0–47.0)	45.0 (43.0–46.0)	0.100
HbA1c (mmol/mol), median (IQR)	35.0 (31.8–42.0)	34.0 (31.0–38.0)	35.0 (31–39)	38.0 (33.0–48.0)	<0.001
HbA1c (%), median (IQR)	5.4 (5.0–6.0)	5.3 (5.0–5.6)	5.3 (5.0–5.7)	5.7 (5.2–6.5)	<0.001
Fasting glucose (mmol/L), median (IQR)	5.6 (-5.2–6.6)	5.5 (5.0–6.2)	5.5 (5.1–6.4)	6.1 (5.4–7.9)	<0.001
Total cholesterol (mmol/L), median (IQR)	4.4 (3.8–5.2)	4.5 (3.7–5.3)	4.4 (3.7–5.2)	4.4 (4.0–5.1)	0.811
HDL cholesterol (mmol/L), median (IQR)	1.4 (1.1–1.8)	1.7 (1.4–2.0)	1.4 (1.0 –1.8)	1.3 (1.0–1.6)	<0.001
LDL cholesterol, median (IQR)	2.8 (2.2–3.3)	2.7 (2.2–3.2)	2.8 (1.0–3.3)	2.8 (2.2–3.3)	0.460
Triglycerides, median (IQR)	1.2 (0.9–1.8)	1.0 (0.8–1.3)	1.0 (0,7–1.7)	1.5 (1.1–2.4)	<0.001
eGFR, mean ± SD	72 (54.0–90.0)	74 (55.8–91.3)	74 (55.0–92.0)	65.5 (51.0–81.5)	0.049
Comorbidities					

(*continued on next page*)

Table 1 (*continued*)

Population	Total study	BCAA tertile (range)			*P*-Value
		T1	T2	T3	
Type 2 diabetes, n (%)	88 (26.2)	21 (19.1)	25 (21.4)	42 (38.5)	0.002
High blood pressure, n (%)	240 (71.4)	75 (68.2)	75 (64.1)	90 (82.6)	0.006
Metabolic Syndrome, n (%)	120 (35.7)	25 (22.7)	37 (31.6)	58 (53.2)	0.004
Cardiovascular disease, n (%)	24 (7.1)	8 (7.3)	4 (3.4)	12 (11.0)	0.086
Impaired renal function, n (%)	112 (33.3)	33 (30.0)	34 (29.1)	45 (41.3)	0.099
Medication use					
Antihypertensive medication, n (%)	158 (47.0)	46 (41.8)	50 (42.7)	62 (56.9)	0.043
Statins, n (%)	76 (22.6)	19 (17.3)	23 (19.7)	34 (31.2)	0.031
Glucose-lowering medication, n (%)	57 (17.0)	11 (10.0)	17 (14.5)	29 (26.6)	0.030
Post-operative immunosuppressive therapy					
Prednisone, n (%)	148 (44.0)	65 (59.1)	38 (32.5)	45 (41.3)	<0.001
Cellcept, n (%)	84 (25.0)	20 (18.2)	30 (25.6)	34 (31.2)	0.083
Cyclosporine, n (%)	37 (11.0)	20 (18.2)	6 (5.1)	11 (10.1)	0.007
Azathioprine, n (%)	87 (25.9)	39 (35.5)	25 (21.4)	23 (21.1)	0.020
Tacrolimus, n (%)	202 (60.1)	57 (51.8)	83 (70.9)	62 (56.9)	0.009
Whole blood levels, median (IQR)	3.3 (2.3–4.7)	3.5 (2.3–4.5)	3.3 (2.4–5.0)	2.9 (2.0–5.0)	0.529
Sirolimus, n (%)	35 (10.4)	3 (2.7)	12 (10.3)	20 (18.3)	0.001
Whole blood levels, median (IQR)	4.2 (3.1–4.9)	4.3	4.0 (3.1–4.4)	4.2 (2.8–5.4)	0.424

Data are represented as mean ± standard deviation (SD) for normally distributed data, median and interquartile range (IQR) for non-normally distributed data or number with percentages (%) for categorical data. P-values among the BCAA tertiles were obtained by using Kruskal-Wallis tests for continuous data and Chi-Square tests for categorical data. Abbreviations: BCAA, Branched Chain Amino Acid, BMI, Body Mass Index, MELD, Model for End-stage Liver Disease, HCC, Hepatocellular Carcinoma, CMV, Cytomegalovirus, EBV, Epstein-Barr Virus, PSC, Primary Sclerosing Cholangitis, PBC, Primary Biliary Cholangitis, MAFLD, Metabolic Associated Fatty Liver Disease, CRP, C-Reactive Protein, ALT, alanine aminotransferase, AST, aspartate aminotransferase, GGT, gamma-glutamyl transferase, ALP, alkaline phosphatase, HbA1c, glycated hemoglobin, HDL, high density lipoprotein, LDL, low density lipoprotein, eGFR, estimated glomerular filtration rate.
* Participants with available MELD score data (OLTx between 2009 and 2019): n = 142 (89 males, 53 females)

Table 2
Univariate correlations of BCAA values with clinical parameters in subjects with and without MetS and with and without T2D.

	Total Population (n = 336)	MetS (n = 120)	No MetS (n = 216)	T2D (n = 88)	No T2D (n = 248)
Sex: male	0.28***	0.31***	0.26***	0.28**	0.28**
Age	0.09	−0.09	0.03	−0.02	0.03
Time since OLTx	−0.14*	−0.19*	−0.06	−0.00	−0.17**
BMI	0.31***	0.17	0.251***	0.24*	0.29***
Primary liver disease: MAFLD	0.20***	0.18	0.13	0.25*	0.12
Primary liver disease: Viral	0.16**	0.09	0.16**	0.32**	0.06
eGFR	−0.10	−0.04	0.01	−0.08	−0.05
Smoking	−0.03	−0.07	0.02	0.04	−0.02
Triglycerides	0.34***	0.37***	0.18*	0.34**	0.25***
HDL-Cholesterol	−0.39***	−0.36***	−0.24***	−0.34**	−0.36***
Overweight/Adiposity	0.28***	0.15	0.20**	0.26*	0.26***
High blood pressure	0.10	−0.04	0.02	0.02	0.09
MetS	0.28***	–	–	0.07	0.28***
T2D	0.20***	0.03	0.15*	–	–
CVD	0.04	−0.02	0.01	0.10	−0.03
Statins	0.12*	−0.01	0.05	0.10	0.02
Antihypertensive medication	0.10	0.03	0.03	0.05	0.05
Glucose-lowering medication	0.20***	0.19	0.19**	0.12	–
Corticosteroids	−0.15**	−0.13	−0.13	−0.27*	−0.13*
Cyclosporine	−0.07	−0.04	−0.04	−0.01	−0.08
Azathioprine	−0.17**	−0.16	−0.14*	−0.08	−0.19**
Tacrolimus	0.05	0.08	−0.02	−0.01	0.06
Sirolimus	0.21***	0.24**	0.16*	0.20	0.20**

Abbreviations: See Table 1. Results of Spearman's rank correlation coefficients are depicted.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

included, in order to determine their individual contribution to the association of MetS with BCAA plasma levels (Supplementary Table 1). BCAA plasma levels were positively associated with elevated waist circumference and elevated TG in all models (all P-values <0.05) and with hyperglycemia in models 1, 2 and 3. The association of BCAA with reduced HDL-cholesterol and elevated blood pressure did not reach statistical significance.

Regarding T2D, we observed positive associations with BCAA plasma levels in the first model (Table 4, Model 1, ß = 0.18, P = 0.002), after adjusting for BMI and eGFR (Table 4, Model 2, ß = 0.14, P = 0.021), after adjusting for smoking status, MAFLD as the primary liver disease and the use of statins (Table 4, Model 3, ß = 0.13, P = 0.039), as well as after adjusting for the use of corticosteroids, tacrolimus and sirolimus (Table 4, Model 4, ß = 0.17, P = 0.002). Remarkably, we observed that the use of sirolimus was significantly correlated with BCAA plasma levels in all models except in Model 4 of Supplementary Table 1.

Table 3
Multivariable linear regression analyses demonstrating the positive associations of MetS with BCAA plasma levels.

	Model 1		Model 2		Model 3		Model 4	
	ß	*P*	ß	*P*	ß	*P*	ß	*P*
Age	−0.01	0.886	−0.07	0.268	−0.01	0.799	−0.06	0.370
Sex (male vs. female)	0.26	<0.001	0.27	<0.001	0.25	<0.001	0.26	<0.001
Time since OLTx	−0.04	0.438	−0.01	0.845	0.02	0.731	0.03	0.615
MetS	0.25	<0.001	0.23	<0.001	0.25	<0.001	0.23	<0.001
eGFR			−0.08	0.230			−0.06	0.380
Smoking			−0.06	0.260			−0.05	0.370
Primary liver disease: MAFLD			0.15	0.011			0.10	0.083
Corticosteroids					−0.10	0.104	−0.09	0.188
Tacrolimus					−0.04	0.601	−0.03	0.683
Sirolimus					0.17	0.002	0.13	0.031

Abbreviations: See Table 1. ß: standardized regression coefficient. BCAA plasma levels were log_{10}-transformed. All models are adjusted for age, sex and time since OLTx. In Model 2 eGFR, smoking status and MAFLD as primary liver disease are added to Model 1. In Model 3 corticosteroids, Tacrolimus and Sirolimus are added to Model 1. In Model 4 all variables were included into one Model.

Table 4
Multivariable linear regression analyses demonstrating associations between T2D and BCAA plasma levels.

	Model 1		Model 2		Model 3		Model 4		Model 5	
	ß	*P*	ß	*P*	ß	*P*	ß	*P*	ß	*P*
Age	0.03	0.660	−0.06	0.407	−0.01	0.815	0.01	0.863	−0.06	0.381
Sex (male vs. female)	0.25	<0.001	0.26	<0.001	0.26	<0.001	0.25	<0.001	0.26	<0.001
Time since OLTx	−0.09	0.114	−0.05	0.408	−0.05	0.355	0.00	0.996	0.03	0.658
T2D	0.18	0.002	0.14	0.021	0.13	0.039	0.17	0.002	0.10	0.092
BMI			−0.12	0.069					0.21	<0.001
eGFR			0.21	<0.001					−0.07	0.278
Smoking					−0.03	0.579			−0.03	0.661
Primary liver disease: MAFLD					0.16	0.008			0.08	0.210
Statins					0.04	0.564			0.01	0.857
Corticosteroids							−0.12	0.061	−0.07	0.288
Tacrolimus							−0.03	0.692	−0.01	0.856
Sirolimus							0.17	0.003	0.15	0.018

Abbreviations: See Table 1. ß: standardized regression coefficient. BCAA plasma levels were log_{10}-transformed. All models are adjusted for age, sex and time since OLTx. In Model 2 BMI and eGFR are added to Model 1. In Model 3 smoking status, MAFLD as primary liver disease and the use of statins are added to Model 1. In Model 4 Corticosteroids, Tacrolimus and Sirolimus are added to Model 1. In Model 5 all variables were included into one Model

Finally, stepwise multivariable logistic regression analyses were applied in order to disclose the independent associations of MetS and T2D with BCAA plasma levels and other clinical characteristics (Table 5). In age-, sex- and time since OLTx- adjusted models BCAA plasma levels (OR: 1.68, 95% CI: 1.23–2.27, P = 0.001) and age were significantly associated with MetS. Likewise, BCAA plasma levels (OR: 1.60, 95% CI: 1.14–2.23, P = 0.006) as well as age, smoking status, MAFLD as primary liver disease and statins were associated with T2D.

4. Discussion

In the present study we demonstrated a positive association of BCAA plasma levels with MetS and T2D in liver transplant recipients. The prevalence of MetS increased by 67.5% and the prevalence of T2D increased by 59.5% per 1 SD increase in BCAA plasma levels, even after adjusting for traditional CVD risk factors, such as age, sex, renal function and smoking. Of the individual MetS components waist circumference, elevated TG and hyperglycemia showed the strongest associations with BCAA levels. Although such associations have been observed in other

Table 5
Multivariable logistic regression analyses demonstrating independent associations of MetS and T2D with BCAA plasma levels.

	Model 1: MetS			Model 2: T2D		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Age (years)	1.05	1.02–1.08	<0.001	1.05	1.01–1.08	0.010
Sex (male vs. female)	1.10	0.61–1.99	0.743	1.52	0.76–3.03	0.233
Time since OLTx (years)	1.04	0.99–1.09	0.140	1.03	0.98–1.08	0.247
Total BCAA (per SD)	1.68	1.23–2.27	0.001	1.60	1.14–2.23	0.006
eGFR (per SD)	0.70	0.48–1.00	0.052	0.98	0.41–1.02	0.061
Smoking (yes/no)	0.55	0.23–1.28	0.164	4.00	1.18–13.55	0.026
Primary liver disease: MAFLD (yes/no)	0.44	0.15–1.28	0.133	0.20	0.06–0.62	0.005
Statins (yes/no)				3.67	1.78–7.59	<0.001
Corticosteroids (yes/no)	0.98	0.49–1.99	0.963	1.71	0.75–3.90	0.204
Tacrolimus (yes/no)	0.66	0.31–1.41	0.282	2.08	0.88–4.93	0.098
Sirolimus (yes/no)	1.77	0.60–5.21	0.299	0.55	0.15–1.98	0.357

Abbreviations: See Table 1. OR: Odds Ratio, CI: Confidence Interval. For time variables ORs are expressed per year increase, other continuous variables are expressed per SD increase. Binary logistic regression analyses were used for all models. Model 1: Dependent variable is MetS. The use of statins is excluded. Model 2: Dependent variable is T2D. The use of statins is included.

observational studies before, to the best of our knowledge, they have not been documented in liver transplant recipients yet. Furthermore, we demonstrated a positive relationship between the use of sirolimus and BCAA plasma levels.

Liver transplant recipients are known to be at high risk of developing T2D [4]. In addition to general adverse effects of T2D, liver transplant recipients frequently suffer from poor graft survival and increased mortality [3,32]. Recently, elevated levels of circulating BCAA have been repeatedly associated with insulin resistance, T2D and the future development of T2D [14,17–19,21,22]. Notably, dividing the study population according to sex-stratified tertiles of BCAA values demonstrated that T2D prevalence was almost 2-fold higher in the highest BCAA tertile compared with the lowest tertile. Subsequent analyses confirmed this trend by demonstrating that BCAA plasma levels are positively associated with T2D, independently of traditional T2D risk factors, such as age, sex, smoking and immunosuppressive medication.

The prevalence of MetS among liver transplant recipients of our study (35.7%) was considerably elevated when compared to the estimated age-adjusted prevalence in the general western population (23.7%) [33]. Previously, BCAA plasma levels have been associated with MetS in non-OLTx populations [34]. Resembling the trend in T2D, we found a more than two-fold increase in MetS prevalence when comparing the third to the first BCAA tertile. Subsequent analyses revealed a positive and independent association between plasma BCAA and MetS, even after adjusting for MetS-associated factors, such as age, sex, MAFLD as primary liver disease, and immunosuppressive medication. We repeated the analyses with the individual components of MetS. Hyperglycemia, elevated TG and enlarged waist circumference showed the strongest independent associations with BCAA. Our findings are consistent with those seen in non-OLTx patient groups [18,20,35]. Although we could not demonstrate a statistically significant association of reduced HDL-cholesterol with BCAA plasma levels in multivariable linear regression analyses (Supplementary Table 1), we were able to observe a statistically significant trend of HDL-cholesterol values being lower in tertiles of higher BCAA values (Table 1). This trend is consistent with other data comprising associations of BCAA with HDL-cholesterol values [36]. This may underscore a role for establishing BCAA levels as a new biomarker in the T2D and MetS risk assessment in liver transplant recipients.

Interestingly, a direct association between BCAA plasma levels and incident CVD has been demonstrated [21,37]. However, we were not able to reproduce such an association. Primarily, we attribute this to differences in study design. Hu et al. [37] used a cross-section of the general Chinese population, whereas Tobias et al. [21] analyzed a prospective cohort of U.S. women. In contrast, our single center study was carried out in liver transplant recipients from the Netherlands. Another important difference is that subjects with pre-existing CVD, which may strongly affect patient survival after OLTx, were excluded from transplantations in our medical center. Moreover, liver transplant recipients receive regular follow-up and more frequent medical examinations than the general population and most other patient groups. Consequently, this may have led to earlier recognition of adverse cardiovascular risk profiles and thereby more efficient prevention of CVD in our study group. Besides, our sample size, as well as the varying timespan of individual participant follow-up may have contributed to the small number of CVD cases in our study group.

Treatment with immunosuppressive medication is widely known to inflict adverse effects upon human metabolism. In particular, mTOR inhibitors (sirolimus) are known to be associated with dyslipidemia and new onset diabetes after transplantation [27,38]. Notably, in the present study associations of T2D and MetS with BCAA plasma levels remained statistically significant, despite adjusting for concomitant use of other immunosuppressives. Consequently, it appears that a relationship between BCAA and metabolic derangements is present beyond the effects of immunosuppressive therapy. Also, in our study we found significant associations between BCAA plasma levels and the use of sirolimus, even after adjustment for age, sex, BMI, MetS, T2D and other immunosuppressive medications. BCAA levels are currently not used to guide treatment decisions in immunosuppressive therapy, suggesting that the use of sirolimus may lead to elevated circulating BCAA. The mechanism by which means sirolimus may raise BCAA levels remains subject of discussion. Newgard proposes that a rise in circulating BCAA can be driven by an obesity-related decline in BCAA catabolism. Accordingly, the abundance of glucose as well as lipid substrates in overnutrition or obesity obviates the need for utilizing amino acids as a source of energy, thereby leading to their accumulation [39]. Consistently, we observed that almost 2/3 (62.9%) of the subjects undergoing sirolimus treatment were either overweight or obese. Moreover, Sipula et al. demonstrated that the use of sirolimus, similarly to a high fat diet, causes a metabolic shift from glucose utilization to fatty-acid oxidation [40]. We hypothesize that this switch may in part be due to insulin resistance induced by the use of sirolimus. Subsequently, this may mimic a state of overnutrition by liberating excess energy sources from the breakdown of free fatty acids, despite the abundance of glucose, resulting in elevated circulating BCAA as proposed in Newgard's model.

Remarkably, our study group of liver transplant recipients exhibited lower overall BCAA values when compared with another Dutch study population of non-OLTx patients. While we found a median BCAA concentration of 358.5 μmol/L in males and of 300.0 μmol/L in females, Flores-Guerrero et al. reported a mean of 405.4 ± 90 μmol/L in males and 366.11 ± 72.43 μmol/L in females in a group of 6244 non-diabetic subjects of the PREVEND cohort recruited from the same region of The Netherlands [22]. In non-diabetic Japanese adults even higher BCAA levels, assayed by liquid chromatography and triple quadrupole mass spectrometry have been reported (431 μmol/L in males and females combined) [41]. However, BCAA plasma levels in non-diabetic US participants from the Insulin Resistance and Atherosclerosis Study (IRAS), assayed using the same NMR technique as in the current study, averaged 337 μmol/L [17]. Thus, no overall conclusions can be drawn as to whether BCAA levels remain low >1 year after OLT compared to the general population [10].

The present study has several strengths. Firstly, this is the first study to investigate relationships between BCAA plasma levels and CVD risk factors in liver transplant recipients. Secondly, extensive data on clinical endpoints provided by the TransplantLines Biobank and Cohort study allowed us to describe our patient population in close detail and thoroughly address potential confounding factors. Limitations should also be considered. First, the single-center study design limits the external validity of our study. Second, the number of CVD cases was relatively small, thereby limiting the generalizability of our results concerning CVD. Third, the cross-sectional study design does not allow for the establishment of cause-effect relationships between BCAA plasma levels and CV risk factors over time.

In conclusion, elevated BCAA plasma levels are associated with T2D, MetS and the use of Sirolimus in stable OLTx recipients. BCAA plasma levels may be considered a biomarker that reflects an unfavorable metabolic status in liver transplant recipients.

Data availability statement

Data of this study are available upon reasonable request.

6. Financial support

Generation of the TransplantLines Food and Nutrition Biobank and Cohort Study (clinical trial reg. no. NCT02811835, Clinical-Trials.gov) was funded by the Top Institute Food and Nutrition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2022.01.009.

References

[1] Collaborators GBD 2017 C. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. The LancetGastroenterology & Hepatology 2020;5:245–66. S2468-1253(19)30349-8.

[2] S.D. Johnston, J.K. Morris, R. Cramb, B.K. Gunson, J. Neuberger, Cardiovascular morbidity and mortality after orthotopic liver transplantation, Transplantation 73 (6) (2002) 901–906, https://doi.org/10.1097/00007890-200203270-00012.

[3] I. Laish, M. Braun, E. Mor, J. Sulkes, Y. Harif, Z.B. Ari, Metabolic syndrome in liver transplant recipients: Prevalence, risk factors, and association with cardiovascular events, Liver Transpl. 17 (1) (2011) 15–22.

[4] M.R. Lucey, N. Terrault, L. Ojo, J.E. Hay, J. Neuberger, E. Blumberg, L. W. Teperman, Long-term management of the successful adult liver transplant: 2012 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation, Liver Transpl. 19 (1) (2013) 3–26, https://doi.org/10.1002/lt.23566.

[5] P. Marchetti, R. Navalesi, The metabolic effects of cyclosporin and tacrolimus, J. Endocrinol. Invest. 23 (7) (2000) 482–490, https://doi.org/10.1007/BF03343761.

[6] L.R. Anastacio, S.R. Hde, L.G. Ferreira, A.S. Lima, E.G. Vilela, T.D. Correia, Mi., Incidence and risk factors for diabetes, hypertension and obesity after liver transplantation, Nutr. Hosp. 28 (2013) 643–648, https://doi.org/10.3305/nh.2013.28.3.6193.

[7] J. Richards, B. Gunson, J. Johnson, J. Neuberger, Weight gain and obesity after liver transplantation, Transp. Int. 18 (4) (2005) 461–466.

[8] D.L. Bigam, J.J. Pennington, A. Carpentier, I.R. Wanless, A.W. Hemming, R. Croxford, P.D. Greig, L.B. Lilly, J.E. Heathcote, G.A. Levy, M.S. Cattral, Hepatitis C-related cirrhosis: a predictor of diabetes after liver transplantation, Hepatology (Baltimore, MD) 32 (1) (2000) 87–90.

[9] L. Luzi, G. Perseghin, E. Regalia, L.P. Sereni, A. Battezzati, D. Baratti, E. Bianchi, I. Terruzzi, H. Hilden, L.C. Groop, A. Pulvirenti, M.R. Taskinen, L. Gennari, V. Mazzaferro, Metabolic effects of liver transplantation in cirrhotic patients, J. Clin. Investig. 99 (4) (1997) 692–700, https://doi.org/10.1172/JCI119213.

[10] U.J.F. Tietge, M.J. Bahr, M.P. Manns, K.H.W. Boker, K.H.W. Boker, Hepatic amino-acid metabolism in liver cirrhosis and in the long-term course after liver transplantation, Transp. Int. 16 (1) (2003) 1–8.

[11] Y. Huang, M. Zhou, H. Sun, Y. Wang, Branched-chain amino acid metabolism in heart disease: an epiphenomenon or a real culprit? Cardiovasc. Res. 90 (2) (2011) 220–223, https://doi.org/10.1093/cvr/cvr070.

[12] E.S. Tai, M.L.S. Tan, R.D. Stevens, Y.L. Low, M.J. Muehlbauer, D.L.M. Goh, O. R. Ilkayeva, B.R. Wenner, J.R. Bain, J.J.M. Lee, S.C. Lim, C.M. Khoo, S.H. Shah, C. B. Newgard, Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men, Diabetologia 53 (4) (2010) 757–767, https://doi.org/10.1007/s00125-009-1637-8.

[13] J.L. Jewell, Y.C. Kim, R.C. Russell, F.-X. Yu, H.W. Park, S.W. Plouffe, V. S. Tagliabracci, K.-L. Guan, Differential regulation of mTORC1 by leucine and glutamine, Science 347 (6218) (2015) 194–198.

[14] M. Holeček, Branched-chain amino acids in health and disease: metabolism, alterations in blood plasma, and as supplements, Nutr. Metab. 15 (1) (2018), https://doi.org/10.1186/s12986-018-0271-1.

[15] U.JF. Tietge, M.J. Bahr, M.P. Manns, K.HW. Böker, Plasma amino acids in cirrhosis and after liver transplantation: influence of liver function, hepatic hemodynamics and circulating hormones, Clin. Transplant. 16 (1) (2002) 9–17, https://doi.org/10.1034/j.1399-0012.2002.160101.x.

[16] O. Campollo, D. Sprengers, N. McIntyre, The BCAA/AAA ratio of plasma amino acids in three different groups of cirrhotics, Revista de Investigacion Clinica; Organo Del Hospital de Enfermedades de La Nutricion 44 (1992) 513–518.

[17] J. Wolak-Dinsmore, E.G. Gruppen, I. Shalaurova, S.P. Matyus, R.P. Grant, R. Gegen, S.J.L. Bakker, J.D. Otvos, M.A. Connelly, R.P.F. Dullaart, A novel NMR-based assay to measure circulating concentrations of branched-chain amino acids: Elevation in subjects with type 2 diabetes mellitus and association with carotid intima media thickness, Clin. Biochem. 54 (2018) 92–99.

[18] van den Berg EH, Flores-Guerrero JL, Gruppen EG, de Borst MH, Wolak-Dinsmore J, Connelly MA, et al. Non-Alcoholic Fatty Liver Disease and Risk of Incident Type 2 Diabetes: Role of Circulating Branched-Chain Amino Acids. Nutrients 2019;11: 10.3390/nu11030705. E705.

[19] M.A. Connelly, J. Wolak-Dinsmore, R.P.F. Dullaart, Branched chain amino acids are associated with insulin resistance independent of leptin and adiponectin in subjects with varying degrees of glucose tolerance, Metab. Syndr. Related Disorders 15 (4) (2017) 183–186, https://doi.org/10.1089/met.2016.0145.

[20] C.B. Newgard, J. An, J.R. Bain, M.J. Muehlbauer, R.D. Stevens, L.F. Lien, A. M. Haqq, S.H. Shah, M. Arlotto, C.A. Slentz, J. Rochon, D. Gallup, O. Ilkayeva, B. R. Wenner, W.S. Yancy, H. Eisenson, G. Musante, R.S. Surwit, D.S. Millington, M. D. Butler, L.P. Svetkey, A Branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance, Cell Metab. 9 (4) (2009) 311–326, https://doi.org/10.1016/j.cmet.2009.02.002.

[21] D.K. Tobias, P.R. Lawler, P.H. Harada, O.V. Demler, P.M. Ridker, J.E. Manson, S. Cheng, S. Mora, Circulating branched-chain amino acids and incident cardiovascular disease in a prospective cohort of US women, Circ: Genomic Precis. Med. 11 (4) (2018), https://doi.org/10.1161/CIRCGEN.118.002157.

[22] Flores-Guerrero JL, Oste MCJ, Kieneker LM, Gruppen EG, Wolak-Dinsmore J, Otvos JD, et al. Plasma Branched-Chain Amino Acids and Risk of Incident Type 2 Diabetes: Results from the PREVEND Prospective Cohort Study. Journal of Clinical Medicine 2018;7:10.3390/jcm7120513. E513.

[23] S. Sciarretta, M. Volpe, J. Sadoshima, Mammalian target of rapamycin signaling in cardiac physiology and disease, Circ. Res. 114 (3) (2014) 549–564, https://doi.org/10.1161/CIRCRESAHA.114.302022.

[24] Mao Z, Zhang W. Role of mTOR in Glucose and Lipid Metabolism. International Journal of Molecular Sciences 2018;19:10.3390/ijms19072043. E2043.

[25] C.-Y. Wang, H.-H. Kim, Y. Hiroi, N. Sawada, S. Salomone, L.E. Benjamin, K. Walsh, M.A. Moskowitz, J.K. Liao, Obesity increases vascular senescence and susceptibility to ischemic injury through chronic activation of Akt and mTOR, Sci. Signal. 2 (62) (2009), https://doi.org/10.1126/scisignal.2000143.

[26] S. Sciarretta, A. Ferrucci, G. Ciavarella, P. Depaolis, V. Venturelli, G. Tocci, L. Debiase, S. Rubattu, M. Volpe, Markers of inflammation and fibrosis are related to cardiovascular damage in hypertensive patients with metabolic syndrome, Am. J. Hypertens. 20 (7) (2007) 784–791.

[27] O. Massoud, R.H. Wiesner, The use of sirolimus should be restricted in liver transplantation, J. Hepatol. 56 (1) (2012) 288–290, https://doi.org/10.1016/j.jhep.2011.06.012.

[28] M.F. Eisenga, A.W. Gomes-Neto, M. van Londen, A.L. Ziengs, R.M. Douwes, S. P. Stam, M.C.J. Osté, T.J. Knobbe, N.R. Hessels, A.M. Buunk, C. Annema, M. J. Siebelink, E. Racz, J.M. Spikman, F.A.J.A. Bodewes, R.A. Pol, S.P. Berger, G. Drost, R.J. Porte, H.G.D. Leuvenink, K. Damman, E.A.M. Verschuuren, V.E. de Meijer, H. Blokzijl, S.J.L. Bakker, Rationale and design of TransplantLines: a prospective cohort study and biobank of solid organ transplant recipients, BMJ Open 8 (12) (2018) e024502, https://doi.org/10.1136/bmjopen-2018-024502.

[29] S.M. Grundy, J.I. Cleeman, S.R. Daniels, K.A. Donato, R.H. Eckel, B.A. Franklin, D. J. Gordon, R.M. Krauss, P.J. Savage, S.C. Smith, J.A. Spertus, F. Costa, Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement, Circulation 112 (17) (2005) 2735–2752.

[30] L.A. Inker, C.H. Schmid, H. Tighiouart, J.H. Eckfeldt, H.I. Feldman, T. Greene, J. W. Kusek, J. Manzi, F. Van Lente, Y.L. Zhang, J. Coresh, A.S. Levey, Estimating Glomerular Filtration Rate from Serum Creatinine and Cystatin C, N. Engl. J. Med. 367 (1) (2012) 20–29, https://doi.org/10.1056/NEJMoa1114248.

[31] R. Wiesner, E. Edwards, R. Freeman, A. Harper, R. Kim, P. Kamath, W. Kremers, J. Lake, T. Howard, R.M. Merion, R.A. Wolfe, R. Krom, Model for end-stage liver disease (MELD) and allocation of donor livers, Gastroenterology 124 (1) (2003) 91–96, https://doi.org/10.1053/gast.2003.50016.

[32] J.I. Moon, R. Barbeito, R.N. Faradji, J.J. Gaynor, A.G. Tzakis, Negative impact of new-onset diabetes mellitus on patient and graft survival after liver transplantation: Long-term follow up, Transplantation 82 (2006) 1625–1628, https://doi.org/10.1097/01.tp.0000250361.60415.96.

[33] E.S. Ford, W.H. Giles, W.H. Dietz, Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey, JAMA 287 (3) (2002) 356, https://doi.org/10.1001/jama.287.3.356.

[34] P. Reddy, J. Leong, I. Jialal, Amino acid levels in nascent metabolic syndrome: A contributor to the pro-inflammatory burden, J. Diabetes Complications 32 (5) (2018) 465–469.

[35] K. Fukushima, S. Harada, A. Takeuchi, A. Kurihara, M. Iida, K. Fukai, K. Kuwabara, S. Kato, M. Matsumoto, A. Hirata, M. Akiyama, M. Tomita, A. Hirayama, A. Sato, C. Suzuki, M. Sugimoto, T. Soga, D. Sugiyama, T. Okamura, T. Takebayashi, Association between dyslipidemia and plasma levels of branched-chain amino acids in the Japanese population without diabetes mellitus, J. Clin. Lipidol. 13 (6) (2019) 932–939.e2, https://doi.org/10.1016/j.jacl.2019.09.002.

[36] J.L. Flores-Guerrero, D. Groothof, M.A. Connelly, J.D. Otvos, S.J.L. Bakker, R.P. F. Dullaart, Concentration of branched-chain amino acids is a strong risk marker for incident hypertension, Hypertension (Dallas, Tex) 2019 (74) (1979) 1428–1435, https://doi.org/10.1161/HYPERTENSIONAHA.119.13735.

[37] W. Hu, L. Sun, Y. Gong, Y. Zhou, P. Yang, Z. Ye, J. Fu, A. Huang, Z. Fu, W. Yu, Y. Zhao, T. Yang, H. Zhou, Relationship between branched-chain amino acids, metabolic syndrome, and cardiovascular risk profile in a Chinese population: a cross-sectional study, Int. J. Endocrinol. 2016 (2016) 1–10, https://doi.org/10.1155/2016/8173905.

[38] O. Bamgbola, Metabolic consequences of modern immunosuppressive agents in solid organ transplantation, Therap. Adv. Endocrinol. Metab. 7 (3) (2016) 110–127, https://doi.org/10.1177/2042018816641580.

[39] C. Newgard, Interplay between lipids and branched-chain amino acids in development of insulin resistance, Cell Metab. 15 (5) (2012) 606–614, https://doi.org/10.1016/j.cmet.2012.01.024.

[40] I.J. Sipula, N.F. Brown, G. Perdomo, Rapamycin-mediated inhibition of mammalian target of rapamycin in skeletal muscle cells reduces glucose utilization and increases fatty acid oxidation, Metab. Clin. Exp. 55 (12) (2006) 1637–1644.

[41] R. Katagiri, A. Goto, S. Budhathoki, T. Yamaji, H. Yamamoto, Y. Kato, M. Iwasaki, S. Tsugane, Association between plasma concentrations of branched-chain amino acids and adipokines in Japanese adults without diabetes, Sci. Rep. 8 (1) (2018), https://doi.org/10.1038/s41598-018-19388-w.