

Supplementary Materials

Divergent survival outcomes associated with elevated branched-chain amino acid levels among older adults with or without hypertension and diabetes: a validated, prospective, longitudinal follow-up study

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Table S1. Cardiovascular deaths classified by ICD-10 codes among 2997 older adults during a median follow-up of 17 years.

	Count (n)	% Cardiovascular deaths
Ischaemic heart disease (IHD)	109	41.4%
Stroke	77	29.3%
Heart failure (HF)	29	11.0%
IHD + stroke + HF	215	81.7%
Total cardiovascular deaths	263	100%

ICD-10 code	Description	Count (n)
D65	Disseminated intravascular coagulation [defibrination syndrome]	1
I09.9	Rheumatic heart disease, unspecified	2
I10	Essential (primary) hypertension	4
I11.0	Hypertensive heart disease with (congestive) heart failure	1
I11.9	Hypertensive heart disease without (congestive) heart failure	17
I12.0	Hypertensive renal disease with renal failure	1
I21.9	Acute myocardial infarction, unspecified	40
I24.9	Acute ischaemic heart disease, unspecified	15
I25.1	Atherosclerotic heart disease	4
I25.9	Chronic ischaemic heart disease, unspecified	50
I26.9	Pulmonary embolism without mention of acute cor pulmonale	1
I34.0	Mitral (valve) insufficiency	1
I35.0	Aortic (valve) stenosis	2
I38	Endocarditis, valve unspecified	3
I42.0	Dilated cardiomyopathy	2
I48.0	Atrial fibrillation and flutter	1
I50.0	Congestive heart failure	23
I50.9	Heart failure, unspecified	3
I60.9	Subarachnoid haemorrhage, unspecified	8
I61.3	Intracerebral haemorrhage in brain stem	1
I61.5	Intracerebral haemorrhage, intraventricular	1
I61.9	Intracerebral haemorrhage, unspecified	14
I62.0	Nontraumatic subdural haemorrhage	3
I62.9	Intracranial haemorrhage (nontraumatic), unspecified	13
I63.2	Cerebral infarction due to unspecified occlusion or stenosis of precerebral arteries	1
I63.9	Cerebral infarction, unspecified	2
I64	Stroke, not specified as haemorrhage or infarction	22
I67.9	Cerebrovascular disease, unspecified	6
I69.4	Sequelae of stroke, not specified as haemorrhage or infarction	5
I69.8	Sequelae of other and unspecified cerebrovascular diseases	1
I71.0	Dissection of aorta [any part]	3
I71.1	Thoracic aortic aneurysm, ruptured	1
I71.3	Abdominal aortic aneurysm, ruptured	4
I71.4	Abdominal aortic aneurysm, without mention of rupture	1
I72.8	Aneurysm and dissection of other specified arteries	1
I99	Other and unspecified disorders of circulatory system	1
K55.9	Vascular disorder of intestine, unspecified	4
Total number of cardiovascular deaths		263

Table S2. Non-cardiovascular deaths classified by ICD-10 codes among 2997 older adults during a median follow-up of 17 years.

	Count (n)	% Non-cardiovascular deaths
Cancer/neoplasia	375	38.6%
Infection	383	39.4%
Miscellaneous	214	22.0%
Total non-cardiovascular deaths	972	100%

ICD-10 code	Description	Count (n)
A09.0	Other and unspecified gastroenteritis and colitis of infectious origin	1
A09.9	Gastroenteritis and colitis of unspecified origin	2
A16.1	Tuberculosis of lung, bacteriological and histological examination not done	1
A16.2	Tuberculosis of lung, without mention of bacteriological or histological confirmation	1
A16.9	Respiratory tuberculosis unspecified, without mention of bacteriological or histological confirmation	1
A41.5	Sepsis due to other Gram-negative organisms	1
A41.9	Sepsis, unspecified	25
B34.8	Other viral infections of unspecified site	1
C02.9	Malignant neoplasm of tongue, unspecified	1
C11.9	Malignant neoplasm of nasopharynx, unspecified	3
C15.9	Malignant neoplasm of oesophagus, unspecified	8
C16.9	Malignant neoplasm of stomach, unspecified	16
C17.0	Malignant neoplasm of duodenum	2
C18.0	Malignant neoplasm of caecum	1
C18.2	Malignant neoplasm of ascending colon	1
C18.5	Malignant neoplasm of splenic flexure	1
C18.7	Malignant neoplasm of sigmoid colon	8
C18.9	Malignant neoplasm of colon, unspecified	53
C19	Malignant neoplasm of rectosigmoid junction	1
C20	Malignant neoplasm of rectum	12
C22.0	Malignant neoplasm of liver	12
C22.1	Malignant neoplasm of intrahepatic bile ducts	11
C22.9	Malignant neoplasm of liver and intrahepatic bile ducts, unspecified	5
C23	Malignant neoplasm of gallbladder	4
C24.1	Malignant neoplasm of ampulla of Vater	1
C24.9	Malignant neoplasm of other and unspecified parts of biliary tract, unspecified	1
C25.0	Malignant neoplasm of head of pancreas	1
C25.4	Malignant neoplasm of endocrine pancreas	1
C25.9	Malignant neoplasm of pancreas, unspecified	13
C26.9	Malignant neoplasm of ill-defined sites within the digestive system	1
C31.0	Malignant neoplasm of maxillary sinus	1
C34.9	Malignant neoplasm of bronchus and lung, unspecified	101
C49.9	Malignant neoplasm of other connective and soft tissue, unspecified	2
C50.9	Malignant neoplasm of breast, unspecified	5
C53.9	Malignant neoplasms of cervix uteri, unspecified	1
C55	Malignant neoplasm of uterus, part unspecified	1
C56	Malignant neoplasm of ovary	2
C61	Malignant neoplasm of prostate	24
C64	Malignant neoplasm of kidney, except renal pelvis	5
C67.9	Malignant neoplasm of bladder, unspecified	8
C73	Malignant neoplasm of thyroid gland	2
C78.0	Secondary malignant neoplasm of lung	1
C78.7	Secondary malignant neoplasm of liver and intrahepatic bile duct	1
C79.3	Secondary malignant neoplasm of brain and cerebral meninges	1
C80	Malignant neoplasm, primary site unknown, so stated	3
C80.9	Malignant neoplasm, primary site unspecified	13
C82.9	Follicular lymphoma, unspecified	1
C85.9	Other and unspecified types of non-Hodgkin lymphoma, unspecified	14
C90.0	Multiple myeloma	3
C90.1	Plasma cell leukaemia	1
C92.0	Acute myeloblastic leukaemia	6
C92.7	Other myeloid leukaemia	1
C95.0	Acute leukaemia of unspecified cell type	1
C95.9	Leukaemia, unspecified	2
C97	Malignant neoplasms of independent (primary) multiple sites	3
D37.6	Neoplasm of uncertain or unknown behaviour of liver, gallbladder and bile ducts	2
D38.1	Neoplasm of uncertain or unknown behaviour of trachea, bronchus and lung	1
D43.2	Neoplasm of uncertain or unknown behaviour of brain, unspecified	6
D46.9	Myelodysplastic syndrome, unspecified	3
D47.1	Chronic myeloproliferative disease	1
D48.3	Neoplasm of Retroperitoneum	1
E14.2	Unspecified diabetes mellitus with renal complications	2
E14.9	Unspecified diabetes mellitus without complications	2
E87.7	Fluid overload	1

F01.9	Vascular dementia, unspecified	2
F03	Unspecified dementia	25
G09	Sequelae of inflammatory diseases of central nervous system	1
G12.2	Motor neuron disease	1
G20	Parkinson disease	2
G30.9	Alzheimer disease, unspecified	1
G37.3	Acute transverse myelitis in demyelinating disease of central nervous system	1
G93.4	Encephalopathy, unspecified	1
G95.9	Disease of spinal cord, unspecified	1
J10.0	Influenza with pneumonia, seasonal influenza virus identified	2
J18.0	Bronchopneumonia, unspecified	1
J18.9	Pneumonia, unspecified	260
J44.0	Chronic obstructive pulmonary disease with acute lower respiratory infection	9
J44.8	Other specified chronic obstructive pulmonary disease	1
J44.9	Chronic obstructive pulmonary disease, unspecified	15
J45.9	Asthma, unspecified	3
J47	Bronchiectasis	9
J64	Unspecified pneumoconiosis	2
J69.0	Pneumonitis due to food and vomit	10
J80	Adult respiratory distress syndrome	1
J84.1	Other interstitial pulmonary diseases with fibrosis	8
J84.9	Interstitial pulmonary disease, unspecified	1
J98.4	Other disorders of lung	1
K25.4	Gastric ulcer	1
K26.3	Duodenal ulcer	1
K56.6	Other and unspecified intestinal obstruction	4
K63.1	Perforation of intestine (nontraumatic)	2
K65.0	Acute peritonitis	1
K65.9	Peritonitis, unspecified	2
K72.9	Hepatic failure, unspecified	6
K74.3	Primary biliary cirrhosis	1
K74.6	Other and unspecified cirrhosis of liver	5
K75.0	Abscess of liver	1
K81.0	Acute cholecystitis	1
K81.9	Cholecystitis, unspecified	1
K83.0	Cholangitis	11
K85.9	Acute pancreatitis, unspecified	3
K92.2	Gastrointestinal haemorrhage, unspecified	3
L12.0	Bullous pemphigoid	1
L89.9	Decubitus ulcer and pressure area, unspecified	3
M00.9	Pyogenic arthritis, unspecified	2
M35.1	Other systemic involvement of connective tissue	1
M41.9	Scoliosis, unspecified	1
M46.9	Inflammatory spondylopathy, unspecified	1
M81.9	Osteoporosis, unspecified	2
N03.9	Chronic nephritic syndrome	3
N17.9	Acute renal failure, unspecified	7
N18.0	Chronic kidney disease	2
N18.5	Chronic kidney disease, stage 5	27
N18.9	Chronic kidney disease, unspecified	10
N19	Unspecified kidney failure	7
N39.0	Urinary tract infection, site not specified	21
N40	Hyperplasia of prostate	1
N49.2	Inflammatory disorders of scrotum	1
R02	Gangrene, not elsewhere classified	1
R09.8	Other specified symptoms and signs involving the circulatory and respiratory systems	1
R54	Senility	31
R99	Other ill-defined and unspecified causes of mortality	4
S06.2	Diffuse brain injury	2
S06.5	Traumatic subdural haemorrhage	6
S09.9	Unspecified injury of head	5
S14.1	Other and unspecified injuries of cervical spinal cord	1
S32.5	Fracture of pubis	1
S42.4	Fracture of lower end of humerus	1
S72.0	Fracture of neck of femur	1
T07	Unspecified multiple injuries	4
T17.9	Foreign body in respiratory tract, part unspecified	3
T71	Asphyxiation	1
T75.1	Drowning and nonfatal submersion	2
	ICD-10 code unavailable	5
Total number of non-cardiovascular deaths		972

Table S3. Serum branched-chain amino acid levels were consistently higher in older men than women at both time points, 0Y and 14Y.

Baseline (0Y)

	Men	Women	p value
N (%)	1424 (47.5%)	1573 (52.5%)	-
Total BCAA, $\mu\text{mol/L}$	541.6 (491.3–600.4)	489.8 (440.6–543.7)	<0.0001
Isoleucine, $\mu\text{mol/L}$	83.4 (74.7–94.7)	74.7 (66.4–83.5)	<0.0001
Leucine, $\mu\text{mol/L}$	155.7 (140.3–173.3)	139.3 (124.9–153.7)	<0.0001
Valine, $\mu\text{mol/L}$	302.0 (272.9–335.6)	276.1 (248.2–308.5)	<0.0001

14Y

	Men	Women	p value
N (%)	408 (47.4%)	452 (52.6%)	-
Total BCAA, $\mu\text{mol/L}$	438.0 (397.2–478.0)	407.3 (363.6–459.7)	<0.0001
Isoleucine, $\mu\text{mol/L}$	59.3 (50.6–66.9)	49.7 (42.6–57.6)	<0.0001
Leucine, $\mu\text{mol/L}$	132.1 (120.2–145.8)	120.9 (107.1–136.4)	<0.0001
Valine, $\mu\text{mol/L}$	245.5 (224.9–268.8)	234.8 (212.7–266.4)	0.0005

BCAA, branched-chain amino acid. Median BCAA concentrations (IQR) are shown.

Table S4. Adjusted Cox regression modelling of baseline independent variables on incident all-cause mortality stratified by the combined status of hypertension (HT) and diabetes (DM) with adjustment for age, sex, BMI, smoking, frailty, eGFR, and use of antihypertensive and/or hypoglycaemic drugs.

Variable	HT ⁺ DM ⁺ (deaths, n=172/289)		HT ⁻ DM ⁺ (deaths, n=62/136)		HT ⁺ DM ⁻ (deaths, n=424/993)		HT ⁻ DM ⁻ (deaths, n=577/1579)	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Age	1.07 (1.03-1.1)	0.0006	1.19 (1.12-1.26)	<0.0001	1.1 (1.08-1.12)	<0.0001	1.1 (1.08-1.12)	<0.0001
Sex	1.59 (1.08-2.35)	0.020	1.29 (0.62-2.67)	0.50	1.45 (1.12-1.87)	0.0047	1.77 (1.42-2.2)	<0.0001
BMI	0.92 (0.87-0.97)	0.0033	0.96 (0.87-1.07)	0.50	1.03 (1-1.07)	0.062	0.98 (0.95-1.01)	0.14
Previous smoking	0.99 (0.67-1.46)	0.95	1.96 (1.05-3.64)	0.034	1.46 (1.15-1.87)	0.0022	1.23 (1-1.51)	0.052
Active smoking	1.24 (0.57-2.68)	0.59	6.23 (2-19.43)	0.0016	3.09 (2-4.77)	<0.0001	1.72 (1.29-2.3)	0.0003
Frailty index	3.62 (0.34-38.44)	0.29	0.98 (0.01-147.47)	0.99	3.22 (0.67-15.4)	0.14	4.18 (0.93-18.76)	0.062
PASE	1 (0.99-1)	0.15	1 (0.99-1)	0.48	1 (1-1)	0.66	1 (1-1)	0.22
eGFR	0.98 (0.97-0.99)	0.0011	1.01 (0.98-1.03)	0.62	0.99 (0.98-1)	0.0020	0.99 (0.98-1)	0.0066
log(BCAA) [‡]	1.52 (0.5-4.61)	0.46	0.3 (0.03-3.01)	0.30	0.39 (0.19-0.81)	0.012	0.41 (0.23-0.73)	0.0026
Antihypertensive drugs*	0.88 (0.58-1.35)	0.57	1.94 (1.02-3.71)	0.043	1.24 (0.97-1.59)	0.082	1.21 (0.93-1.58)	0.15
Hypoglycaemic drugs [†]	1.29 (0.87-1.91)	0.21	1.14 (0.63-2.08)	0.66	1.78 (0.73-4.38)	0.21	1.93 (0.27-13.95)	0.52

BCAA, branched-chain amino acid. BMI, body mass index. DM, diabetes mellitus. eGFR, estimated glomerular filtration rate by CKD-EPI formula. HR, hazard ratio. HT, hypertension. PASE, Physical Activity Scale for the Elderly

*Antihypertensive drugs include α -blocker, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, β -blocker, calcium channel blocker, diuretics and nitrate.

[†]Hypoglycaemic drugs include metformin, glibenclamide, gliclazide, glipizide, acarbose, insulin, and tolbutamide. See also Table 1.

[‡]HR represents 1 unit increase in log scale which is approximately equivalent to 2.718 times increase in BCAA level. See Methods for details.

Deaths (n) among total number of study participants in subgroup within parentheses.

Table S5. Details pertinent to the selection of best fitting models from restricted cubic spline and adjusted Cox regression of BCAA on all-cause, non-cardiovascular and cardiovascular mortality in older adults stratified by hypertension (HT) or diabetes mellitus (DM) status.

All-cause mortality

	Linear model		Overall p value	RCS model	
	p value	AIC		Non-linear p value	AIC
All	<0.0001	18392.62	<0.0001	0.076	18391.55*
HT ⁺	0.057	7880.64	0.010	0.057	7877.65*
HT ⁻	0.0002	8810.63*	0.0009	0.90	8812.62
DM ⁺	0.31	2577.85	0.032	0.017	2574.53*
DM ⁻	<0.0001	14629.18*	0.0001	0.47	14630.67

Non-cardiovascular mortality

	Linear model		Overall p value	RCS model	
	p value	AIC		Non-linear p value	AIC
All	0.0001	14453.21	<0.0001	0.027	14450.48*
HT ⁺	0.054	6102.83	0.018	0.045	6100.97*
HT ⁻	0.0008	7010.43*	0.0012	0.25	7011.15
DM ⁺	0.33	1938.11	0.047	0.025	1935.42*
DM ⁻	0.0001	11607.23*	0.0001	0.15	11607.25

Cardiovascular mortality

	Linear model		Overall p value	RCS model	
	p value	AIC		Non-linear p value	AIC
All	0.11	3943.2*	0.27	0.67	3945.02
HT ⁺	0.66	1786.94*	0.52	0.29	1787.88
HT ⁻	0.11	1803.6	0.051	0.050	1801.27*
DM ⁺	0.70	652.13*	0.66	0.42	653.51
DM ⁻	0.84	3024.78*	0.12	0.22	3025.2

AIC, Akaike information criterion. RCS, restricted cubic spline. *The best-fitting model was selected based on the lowest AIC value. p values and AIC were calculated using both linear and restricted cubic spline models (see also Figure 3).

Table S6. Details pertinent to the selection of best fitting models from restricted cubic spline and adjusted Cox regression of BCAA on all-cause, non-cardiovascular and cardiovascular mortality in older adults stratified by the combined status of hypertension (HT) and diabetes mellitus (DM).

All-cause mortality

	Linear model		Overall p value	RCS model	
	p value	AIC		Non-linear p value	AIC
All	<0.0001	18392.62	<0.0001	0.076	18391.55*
HT ⁺ DM ⁺	0.82	1773.34	0.0613	0.019	1770.19*
HT ⁻ DM ⁺	0.16	528.83*	0.3295	0.68	530.66
HT ⁺ DM ⁻	0.041	5399.2*	0.8594	0.45	5400.63
HT ⁻ DM ⁻	0.0002	7877.18*	0.001	0.84	7879.14

Non-cardiovascular mortality

	Linear model		Overall p value	RCS model	
	p value	AIC		Non-linear p value	AIC
All	0.0001	14453.21	<0.0001	0.027	14450.48*
HT ⁺ DM ⁺	0.44	1323.09	0.048	0.020	1320.13*
HT ⁻ DM ⁺	0.74	402.57*	0.94	0.93	404.56
HT ⁺ DM ⁻	0.098	4236.68*	0.17	0.41	4238.01
HT ⁻ DM ⁻	0.0003	6296.31*	0.0006	0.34	6297.41

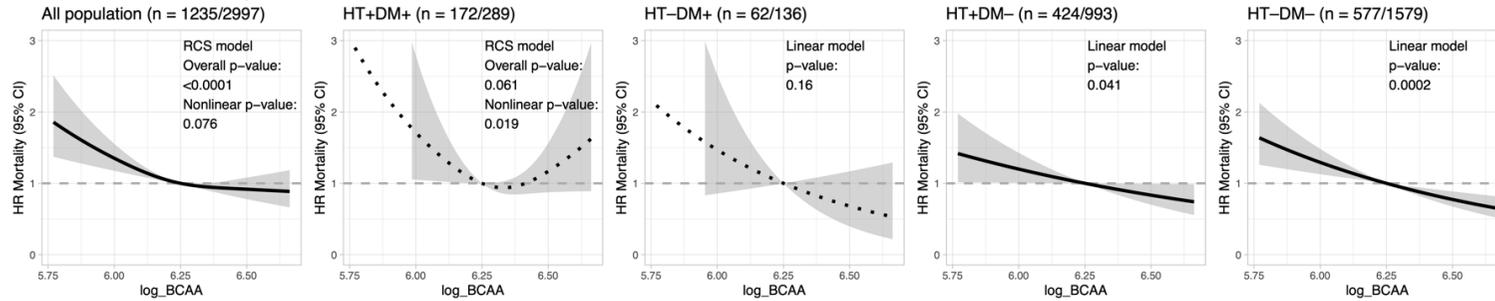
Cardiovascular mortality

	Linear model		Overall p value	RCS model	
	p value	AIC		Non-linear p value	AIC
All	0.11	3943.2*	0.27	0.67	3945.02
HT ⁺ DM ⁺	0.41	458.17*	0.65	0.68	460.01
HT ⁻ DM ⁺	0.03	134.62*	0.093	0.87	136.6
HT ⁺ DM ⁻	0.21	1171.58*	0.45	0.97	1173.58
HT ⁻ DM ⁻	0.33	1584.01	0.21	0.13	1583.55*

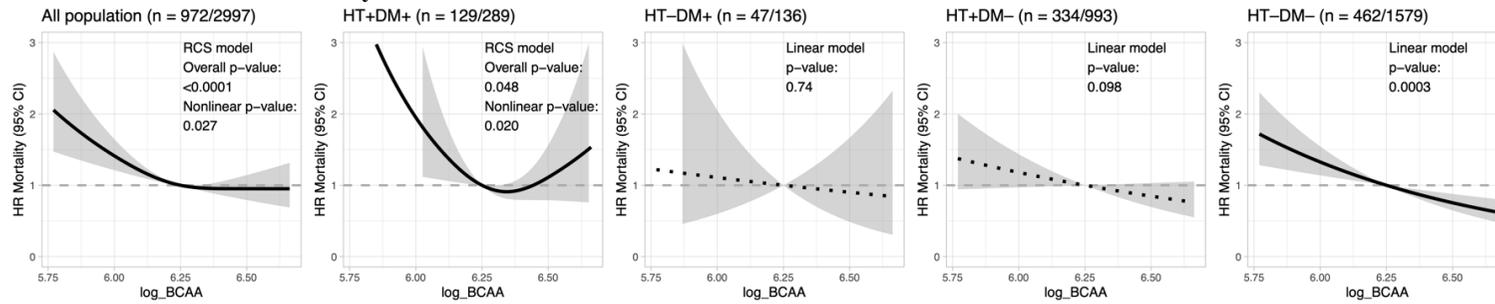
AIC, Akaike information criterion. RCS, restricted cubic spline. *The best-fitting model was selected based on the lowest AIC value. p values and AIC were calculated using both linear and restricted cubic spline models (see also Figure S1).

Figure S1. Restricted cubic splines of log(BCAA) from adjusted Cox regression were modelled on A) all-cause, B) non-cardiovascular and C) cardiovascular mortality in older adults stratified by the combined status of hypertension (HT) and diabetes mellitus (DM). The best fitting model with the lowest AIC value was selected. Models were adjusted for age, sex, smoking, frailty index, eGFR, and use of antihypertensive and hypoglycaemic drugs. HR, hazard ratio. ‘+’ and ‘-’ denote presence and absence, respectively, of disease.

A. All-cause mortality



B. Non-cardiovascular mortality



C. Cardiovascular mortality

