

Circulating amino acids and the risk of macrovascular, microvascular, and mortality outcomes in individuals with type 2 diabetes: results from the ADVANCE trial

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SUPPLEMENTARY DATA

ESM Table 1 Illustrative comparisons of mean or median concentrations of amino acids measured by comparable method (1), by study.

Study	Phenylalanine (mmol/l)	Isoleucine (mmol/l)	Glutamine (mmol/l)	Leucine (mmol/l)	Alanine (mmol/l)	Tyrosine (mmol/l)	Histidine (mmol/l)	Valine (mmol/l)
ADVANCE: participant with type 2 diabetes case-cohort study. 2955 who did not die during follow-up. Mean (SD)	0.061 (0.009)	0.063 (0.017)	0.381 (0.109)	0.082 (0.020)	0.372 (0.065)	0.053 (0.011)	0.050 (0.009)	0.176 (0.035)
SABRE (2): A community-based cohort of European, South Asian and African-Caribbean origin in London. 1279 Europeans without diabetes. Median (IQR)	0.092 (0.084,0.100)	0.057 (0.050,0.066)	0.384 (0.257,0.448)	0.091 (0.080,0.110)	0.326 (0.290,0.365)	0.054 (0.048,0.061)	0.077 (0.069,0.088)	0.179 (0.158,0.202)
SABRE (2): A community-based cohort of European, South Asian and African-Caribbean origin in London. 1007 South Asians without diabetes. Median (IQR)	0.094 (0.086,0.100)	0.060 (0.052,0.068)	0.410 (0.309,0.471)	0.093 (0.083,0.110)	0.335 (0.301,0.375)	0.060 (0.053,0.067)	0.078 (0.070,0.089)	0.178 (0.156,0.199)
FINRISK (3): Finnish general population cohort. 7610 with available serum sample. Mean (SD)	0.085 (0.014)	0.058 (0.019)	0.470 (0.070)	0.088 (0.021)	0.430 (0.067)	0.051 (0.013)	0.064 (0.010)	0.210 (0.043)
Young Finns Study (4): Cohort study of younger adults aged 34-49. 1630 with normal fatty liver score. Mean (SD)	0.074 (0.010)	0.051 (0.015)	0.595 (0.068)	0.080 (0.016)	0.399 (0.059)	0.050 (0.010)	0.065 (0.010)	0.199 (0.038)
CAMERA Trial (5): Randomised controlled trial of metformin in people with CHD but not diabetes. 173 randomised participants Mean (SD)	0.047 (0.008)	0.043 (0.013)	0.396 (0.064)	0.056 (0.012)	0.250 (0.062)	0.061 (0.012)	0.046 (0.010)	0.152 (0.031)

ESM Table 2 Baseline characteristics classified by quarters of the phenylalanine distribution

Characteristics	Phenylalanine (mmol/L)				p-value for trend
	1st(≤ 0.056) (N = 885)	2nd(0.056 to ≤ 0.061) (N = 886)	3rd(0.061 to ≤ 0.066) (N = 884)	4th(> 0.066) (N = 884)	
Sex					
Male	545/ 885 (61.6%)	527/ 886 (59.5%)	525/ 884 (59.4%)	553/ 884 (62.6%)	0.6999
Female	340/ 885 (38.4%)	359/ 886 (40.5%)	359/ 884 (40.6%)	331/ 884 (37.4%)	
Current smokers					
No	747/ 885 (84.4%)	763/ 886 (86.1%)	745/ 884 (84.3%)	757/ 884 (85.6%)	0.7314
Yes	138/ 885 (15.6%)	123/ 886 (13.9%)	139/ 884 (15.7%)	127/ 884 (14.4%)	
History of macrovascular event					
No	626/ 885 (70.7%)	580/ 886 (65.5%)	538/ 884 (60.9%)	556/ 884 (62.9%)	<0.001
Yes	259/ 885 (29.3%)	306/ 886 (34.5%)	346/ 884 (39.1%)	328/ 884 (37.1%)	
Age (years)					
N	885	886	884	884	<0.001
Mean (SD)	66.0 (6.4)	66.4 (6.4)	66.9 (6.5)	68.0 (6.8)	
Median (IRQ)	66.0 (61.0, 71.0)	67.0 (61.0, 71.0)	67.0 (62.0, 71.0)	68.0 (63.0, 73.0)	
Duration of diabetes (years)					
N	885	885	884	884	0.001
Mean (SD)	8.5 (6.6)	7.8 (6.3)	7.8 (6.3)	7.5 (6.6)	
Median (IRQ)	7.0 (3.0, 12.0)	6.0 (3.0, 11.0)	6.0 (3.0, 11.0)	6.0 (2.0, 11.0)	
BMI (Kg/m ²)					
N	885	885	883	884	<0.001
Mean (SD)	28.8 (4.5)	29.9 (5.1)	30.6 (5.6)	30.8 (5.5)	

Characteristics	Phenylalanine (mmol/L)				p-value for trend
	1st(<=0.056) (N = 885)	2nd(0.056 to <=0.061) (N = 886)	3rd(0.061 to <=0.066) (N = 884)	4th(>0.066) (N = 884)	
Median (IRQ)	28.4 (25.7, 31.3)	29.1 (26.5, 32.5)	29.7 (26.7, 33.5)	30.1 (27.0, 33.6)	
History of heart failure					
No	859/ 885 (97.1%)	855/ 886 (96.5%)	839/ 884 (94.9%)	824/ 884 (93.2%)	<0.001
Yes	26/ 885 (2.9%)	31/ 886 (3.5%)	45/ 884 (5.1%)	60/ 884 (6.8%)	
Participation in moderate or vigorous activity					
No	439/ 885 (49.6%)	431/ 886 (48.6%)	453/ 884 (51.2%)	499/ 884 (56.4%)	0.0021
Yes	446/ 885 (50.4%)	455/ 886 (51.4%)	431/ 884 (48.8%)	385/ 884 (43.6%)	
SBP (mmHg)					
N	885	886	883	884	0.4541
Mean (SD)	146.1 (21.1)	147.6 (21.9)	148.2 (21.8)	146.7 (21.0)	
Median (IRQ)	144.0 (131.5, 158.0)	145.0 (132.5, 159.5)	146.5 (133.5, 160.5)	145.5 (132.5, 159.0)	
DBP (mmHg)					
N	885	886	883	884	0.5481
Mean (SD)	81.3 (10.9)	81.9 (10.7)	82.4 (11.0)	80.8 (10.7)	
Median (IRQ)	80.5 (73.5, 88.5)	81.5 (74.5, 89.0)	82.0 (75.0, 89.0)	80.5 (73.5, 87.5)	
Total chol (mmol/l)					
N	885	884	884	884	0.1233
Mean (SD)	5.1 (1.1)	5.2 (1.2)	5.2 (1.3)	5.1 (1.1)	
Median (IRQ)	5.0 (4.3, 5.9)	5.1 (4.3, 5.9)	5.0 (4.4, 5.9)	4.9 (4.3, 5.8)	
HDL Chol (mmol/l)					
N	884	883	884	883	

Characteristics	Phenylalanine (mmol/L)				p-value for trend
	1st(<=0.056) (N = 885)	2nd(0.056 to <=0.061) (N = 886)	3rd(0.061 to <=0.066) (N = 884)	4th(>0.066) (N = 884)	
Mean (SD)	1.3 (0.3)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	<0.001
Median (IRQ)	1.2 (1.0, 1.5)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	
Triacylglycerol (mmol/l)					
N	885	884	882	883	
Mean (SD)	1.8 (1.1)	2.0 (1.3)	2.0 (1.1)	2.0 (1.3)	0.0017
Median (IRQ)	1.6 (1.2, 2.2)	1.7 (1.2, 2.3)	1.8 (1.3, 2.4)	1.7 (1.3, 2.4)	
HbA1c					
N	882	881	881	879	
Mean % (SD) [mmol/mol]	7.3 (1.3) [52]	7.4 (1.4) [57]	7.5 (1.5) [59]	7.4 (1.5) [57]	0.1989
Urinary albumin:creatinine ratio (mg/mmol)					
N	823	838	845	819	
Median (IRQ)	1.4 (0.7, 3.6)	2 (0.8, 5.3)	2 (0.8, 5.3)	2.2 (0.9, 6.5)	<0.001
Glucose (mmol/l)					
N	885	884	884	884	
Mean (SD)	8.5 (2.6)	8.5 (2.6)	8.6 (2.8)	8.3 (2.8)	0.1697
Median (IRQ)	8.1 (6.8, 9.7)	8.0 (6.6, 9.8)	8.0 (6.6, 9.9)	7.7 (6.4, 9.6)	
eGFR (mL/min/1.73 m ²)					
N	883	883	878	878	
Mean (SD)	75.4 (14.9)	73.2 (16.5)	70.8 (17.1)	68.0 (17.4)	<0.001
Median (IRQ)	75.7 (64.9, 87.5)	73.8 (60.6, 87.3)	70.8 (59.2, 83.9)	67.4 (56.7, 81.4)	

Characteristics	Phenylalanine (mmol/L)				p-value for trend
	1st(<=0.056) (N = 885)	2nd(0.056 to <=0.061) (N = 886)	3rd(0.061 to <=0.066) (N = 884)	4th(>0.066) (N = 884)	
CRP (nmol/l)					
N	871	856	861	867	
Mean (SD)	21.9 (31.4)	29.5 (49.5)	36.2 (70.5)	51.4 (93.3)	<0.001
Median (IRQ)	12.4 (6.7, 24.8)	15.2 (8.6, 37.1)	20.0 (9.5, 41.9)	24.8 (10.5, 51.4)	
hs-cTnT (ng/l)					
N	870	856	860	866	
Mean (SD)	7.8 (12.9)	7.5 (8.7)	8.9 (17.8)	11.4 (32.0)	<0.001
Median (IRQ)	4.0 (1.5, 9.0)	5.0 (1.5, 10.0)	6.0 (1.5, 11.0)	6.0 (1.5, 13.0)	
NT-proBNP (pg/mL)					
N	870	856	860	866	
Mean (SD)	177.4 (556.8)	206.3 (418.9)	259.8 (557.4)	366.4 (1487.4)	<0.001
Median (IRQ)	67.0 (29.0, 159.0)	83.0 (31.0, 202.5)	97.0 (39.5, 247.5)	113.0 (40.0, 281.0)	

Values are n (%), mean (standard deviation), or median (interquartile range)

ESM Table 3 Baseline characteristics classified by quarters of the isoleucine distribution

Characteristics	Isoleucine (mmol/L)				p-value for trend
	1st(≤ 0.051) (N = 897)	2nd(0.051 to ≤ 0.060) (N = 900)	3rd(0.060 to ≤ 0.071) (N = 894)	4th(> 0.071) (N = 896)	
Sex					
Male	426/ 897 (47.5%)	522/ 900 (58.0%)	578/ 894 (64.7%)	644/ 896 (71.9%)	<0.001
Female	471/ 897 (52.5%)	378/ 900 (42.0%)	316/ 894 (35.3%)	252/ 896 (28.1%)	
Current smokers					
No	788/ 897 (87.8%)	778/ 900 (86.4%)	738/ 894 (82.6%)	750/ 896 (83.7%)	0.0022
Yes	109/ 897 (12.2%)	122/ 900 (13.6%)	156/ 894 (17.4%)	146/ 896 (16.3%)	
History of macrovascular event					
No	640/ 897 (71.3%)	592/ 900 (65.8%)	554/ 894 (62.0%)	549/ 896 (61.3%)	<0.001
Yes	257/ 897 (28.7%)	308/ 900 (34.2%)	340/ 894 (38.0%)	347/ 896 (38.7%)	
Age (years)					
N	897	900	894	896	<0.001
Mean (SD)	67.9 (6.8)	67.6 (6.3)	66.7 (6.4)	65.2 (6.5)	
Median (IRQ)	68.0 (63.0, 72.0)	68.0 (63.0, 72.0)	67.0 (61.0, 71.0)	65.0 (60.0, 70.0)	
Duration of diabetes (years)					
N	897	900	893	896	0.7730
Mean (SD)	8.1 (7.0)	7.8 (6.3)	7.8 (6.4)	8.0 (6.1)	
Median (IRQ)	6.0 (3.0, 11.0)	6.0 (3.0, 11.0)	6.0 (3.0, 11.0)	6.5 (3.0, 12.0)	
BMI (Kg/m ²)					
N	897	900	892	896	<0.001
Mean (SD)	28.8 (5.3)	29.9 (5.2)	30.5 (5.4)	30.8 (4.9)	

Characteristics	Isoleucine (mmol/L)				p-value for trend
	1st(<=0.051) (N = 897)	2nd(0.051 to <=0.060) (N = 900)	3rd(0.060 to <=0.071) (N = 894)	4th(>0.071) (N = 896)	
Median (IRQ)	28.0 (25.2, 31.6)	29.3 (26.5, 32.9)	29.8 (27.0, 33.2)	30.1 (27.4, 33.3)	
History of heart failure					
No	858/ 897 (95.7%)	850/ 900 (94.4%)	858/ 894 (96.0%)	855/ 896 (95.4%)	0.7875
Yes	39/ 897 (4.3%)	50/ 900 (5.6%)	36/ 894 (4.0%)	41/ 896 (4.6%)	
Participation in moderate or vigorous activity					
No	473/ 897 (52.7%)	440/ 900 (48.9%)	474/ 894 (53.0%)	464/ 896 (51.8%)	0.8619
Yes	424/ 897 (47.3%)	460/ 900 (51.1%)	420/ 894 (47.0%)	432/ 896 (48.2%)	
SBP (mmHg)					
N	897	900	893	896	
Mean (SD)	146.0 (22.1)	147.3 (21.8)	148.0 (21.5)	147.4 (20.6)	0.1452
Median (IRQ)	144.0 (130.5, 158.0)	145.0 (133.0, 159.5)	145.0 (133.5, 160.0)	146.5 (133.0, 160.0)	
DBP (mmHg)					
N	897	900	893	896	
Mean (SD)	80.0 (10.7)	81.6 (10.7)	82.3 (11.1)	82.5 (10.8)	<0.001
Median (IRQ)	80.0 (72.5, 87.0)	81.0 (74.5, 88.0)	81.5 (75.0, 89.0)	82.0 (75.0, 90.0)	
Total chol (mmol/l)					
N	897	900	893	895	
Mean (SD)	5.1 (1.1)	5.1 (1.1)	5.1 (1.1)	5.3 (1.3)	0.0092
Median (IRQ)	5.0 (4.3, 5.8)	5.0 (4.3, 5.8)	5.0 (4.3, 5.8)	5.2 (4.4, 6.0)	
HDL Chol (mmol/l)					
N	895	900	893	894	

Characteristics	Isoleucine (mmol/L)				p-value for trend
	1st(<=0.051) (N = 897)	2nd(0.051 to <=0.060) (N = 900)	3rd(0.060 to <=0.071) (N = 894)	4th(>0.071) (N = 896)	
Mean (SD)	1.3 (0.4)	1.3 (0.3)	1.2 (0.3)	1.1 (0.3)	<0.001
Median (IRQ)	1.3 (1.1, 1.6)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.1 (0.9, 1.3)	
Triacylglycerol (mmol/l)					
N	897	900	893	892	
Mean (SD)	1.4 (0.6)	1.7 (0.7)	2.0 (1.0)	2.8 (1.7)	<0.001
Median (IRQ)	1.2 (0.9, 1.6)	1.5 (1.2, 2.0)	1.8 (1.4, 2.4)	2.5 (1.8, 3.4)	
HbA1c (%)					
N	894	896	889	892	
Mean % (SD) [mmol/mol]	7.2 (1.3) [55]	7.3 (1.4) [56]	7.5 (1.4) [59]	7.7 (1.5) [61]	<0.001
Urinary albumin:creatinine ratio (mg/mmol)					
N	842	853	844	833	
Median (IRQ)	1.7 (0.8, 4.5)	1.8 (0.8, 4.9)	2.1 (0.9, 5.4)	2.1 (0.9, 6.5)	0.2396
Glucose (mmol/l)					
N	897	900	893	895	
Mean (SD)	7.9 (2.5)	8.1 (2.5)	8.6 (2.7)	9.2 (3.0)	<0.001
Median (IRQ)	7.5 (6.4, 8.9)	7.7 (6.4, 9.2)	8.0 (6.7, 9.8)	8.8 (7.0, 10.7)	
eGFR (mL/min/1.73 m ²)					
N	894	898	889	889	
Mean (SD)	70.8 (16.4)	71.7 (16.8)	72.8 (16.3)	71.8 (17.2)	0.0930
Median (IRQ)	71.2 (59.2, 83.8)	72.3 (60.6, 85.5)	72.9 (61.8, 85.0)	71.5 (59.7, 85.7)	

Characteristics	Isoleucine (mmol/L)				p-value for trend
	1st(<=0.051) (N = 897)	2nd(0.051 to <=0.060) (N = 900)	3rd(0.060 to <=0.071) (N = 894)	4th(>0.071) (N = 896)	
CRP (nmol/l)					
N	874	877	866	874	
Mean (SD)	41.9 (97.1)	30.5 (57.1)	36.2 (59.0)	31.4 (41.9)	0.0091
Median (IRQ)	15.2 (7.6, 40.0)	17.1 (8.6, 37.1)	18.1 (8.6, 41.0)	19.0 (9.5, 39.0)	
hs-cTnT (ng/l)					
N	873	877	864	874	
Mean (SD)	8.4 (12.2)	8.2 (10.2)	9.9 (31.8)	9.1 (18.0)	0.2203
Median (IRQ)	5.0 (1.5, 11.0)	5.0 (1.5, 11.0)	5.0 (1.5, 11.0)	6.0 (1.5, 11.0)	
NT-proBNP (pg/mL)					
N	873	877	864	874	
Mean (SD)	324.9 (1392.9)	268.0 (609.5)	223.3 (469.3)	197.6 (703.4)	0.0012
Median (IRQ)	103.0 (46.0, 252.0)	89.0 (35.0, 222.0)	85.0 (30.5, 212.0)	70.0 (27.0, 179.0)	

Values are n (%), mean (standard deviation), or median (interquartile range)

ESM Table 4 Baseline characteristics classified by quarters of the glutamine distribution

Characteristics	Glutamine (mmol/L)				p-value for trend
	1st(≤ 0.310) (N = 557)	2nd(0.310 to ≤ 0.396) (N = 557)	3rd(0.396 to ≤ 0.456) (N = 557)	4th(> 0.456) (N = 557)	
Sex					
Male	307/ 557 (55.1%)	347/ 557 (62.3%)	363/ 557 (65.2%)	365/ 557 (65.5%)	<0.001
Female	250/ 557 (44.9%)	210/ 557 (37.7%)	194/ 557 (34.8%)	192/ 557 (34.5%)	
Current smokers					
No	482/ 557 (86.5%)	467/ 557 (83.8%)	473/ 557 (84.9%)	471/ 557 (84.6%)	0.4742
Yes	75/ 557 (13.5%)	90/ 557 (16.2%)	84/ 557 (15.1%)	86/ 557 (15.4%)	
History of macrovascular event					
No	378/ 557 (67.9%)	364/ 557 (65.4%)	347/ 557 (62.3%)	338/ 557 (60.7%)	0.0069
Yes	179/ 557 (32.1%)	193/ 557 (34.6%)	210/ 557 (37.7%)	219/ 557 (39.3%)	
Age (years)					
N	557	557	557	557	0.3772
Mean (SD)	66.7 (6.6)	66.5 (6.5)	66.7 (6.4)	67.0 (6.6)	
Median (IRQ)	67.0 (62.0, 72.0)	67.0 (61.0, 71.0)	67.0 (62.0, 71.0)	67.0 (62.0, 72.0)	
Duration of diabetes (years)					
N	557	557	557	557	<0.001
Mean (SD)	8.5 (6.8)	8.0 (6.5)	7.1 (5.9)	7.4 (6.3)	
Median (IRQ)	7.0 (3.0, 12.0)	7.0 (3.0, 11.0)	6.0 (2.0, 10.0)	6.0 (2.0, 11.0)	
BMI (Kg/m ²)					
N	557	555	557	557	<0.001
Mean (SD)	30.1 (4.9)	30.5 (5.5)	29.9 (5.2)	28.8 (5.1)	

Characteristics	Glutamine (mmol/L)				p-value for trend
	1st(<=0.310) (N = 557)	2nd(0.310 to <=0.396) (N = 557)	3rd(0.396 to <=0.456) (N = 557)	4th(>0.456) (N = 557)	
Median (IRQ)	29.7 (26.7, 32.9)	29.7 (26.6, 33.5)	29.2 (26.5, 32.5)	27.9 (25.5, 31.1)	
History of heart failure					
No	529/ 557 (95.0%)	536/ 557 (96.2%)	539/ 557 (96.8%)	544/ 557 (97.7%)	0.0154
Yes	28/ 557 (5.0%)	21/ 557 (3.8%)	18/ 557 (3.2%)	13/ 557 (2.3%)	
Participation in moderate or vigorous activity					
No	273/ 557 (49.0%)	310/ 557 (55.7%)	278/ 557 (49.9%)	284/ 557 (51.0%)	0.9849
Yes	284/ 557 (51.0%)	247/ 557 (44.3%)	279/ 557 (50.1%)	273/ 557 (49.0%)	
SBP (mmHg)					
N	557	557	557	557	0.2289
Mean (SD)	146.7 (21.6)	147.1 (20.9)	145.9 (21.3)	145.5 (22.3)	
Median (IRQ)	145.5 (131.0, 160.0)	145.5 (133.5, 158.5)	143.5 (131.5, 158.0)	143.5 (130.0, 159.0)	
DBP (mmHg)					
N	557	557	557	557	0.1532
Mean (SD)	81.3 (10.8)	81.4 (10.8)	81.1 (10.3)	80.5 (10.8)	
Median (IRQ)	81.0 (74.0, 88.5)	81.5 (74.0, 88.5)	81.0 (74.0, 88.0)	80.0 (73.0, 87.5)	
Total chol (mmol/l)					
N	556	557	556	557	0.0449
Mean (SD)	5.1 (1.1)	5.1 (1.0)	5.0 (1.1)	5.0 (1.1)	
Median (IRQ)	5.1 (4.3, 5.9)	5.0 (4.4, 5.8)	4.9 (4.3, 5.7)	4.9 (4.3, 5.7)	
HDL Chol (mmol/l)					
N	556	557	555	557	

Characteristics	Glutamine (mmol\L)				p-value for trend
	1st(<=0.310) (N = 557)	2nd(0.310 to <=0.396) (N = 557)	3rd(0.396 to <=0.456) (N = 557)	4th(>0.456) (N = 557)	
Mean (SD)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	1.3 (0.4)	0.0011
Median (IRQ)	1.1 (1.0, 1.4)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.2 (1.0, 1.5)	
Triacylglycerol (mmol/l)					
N	555	557	556	557	
Mean (SD)	2.0 (1.3)	2.0 (1.3)	1.9 (0.9)	1.8 (1.0)	<0.001
Median (IRQ)	1.7 (1.2, 2.4)	1.7 (1.3, 2.4)	1.7 (1.3, 2.3)	1.5 (1.1, 2.1)	
HbA1c (%)					
N	556	556	555	555	
Mean % (SD) [mmol/mol]	7.6 (1.6) [60]	7.4 (1.4) [57]	7.3 (1.2) [56]	6.9 (1.0) [52]	<0.001
Urinary albumin:creatinine ratio (mg/mmol)					
N	522	520	527	529	
Median (IRQ)	1.9 (0.9, 5.5)	1.6 (0.7, 5.8)	1.5 (0.7, 4)	1.5 (0.7, 4.4)	0.0977
Glucose (mmol/l)					
N	556	556	557	557	
Mean (SD)	8.9 (3.1)	8.6 (2.8)	8.4 (2.4)	7.6 (1.9)	<0.001
Median (IRQ)	8.2 (6.8, 10.3)	8.1 (6.7, 10.2)	8.0 (6.7, 9.7)	7.4 (6.3, 8.5)	
eGFR (mL/min/1.73 m ²)					
N	554	556	554	554	
Mean (SD)	70.9 (16.6)	71.7 (16.9)	72.0 (15.5)	71.7 (16.6)	0.3863
Median (IRQ)	69.9 (59.4, 83.6)	70.1 (59.6, 86.1)	72.2 (60.9, 84.3)	72.5 (61.4, 83.9)	

Characteristics	Glutamine (mmol\L)				p-value for trend
	1st(<=0.310) (N = 557)	2nd(0.310 to <=0.396) (N = 557)	3rd(0.396 to <=0.456) (N = 557)	4th(>0.456) (N = 557)	
CRP (nmol/l)					
N	533	544	545	543	
Mean (SD)	41.9 (97.1)	42.9 (68.6)	28.6 (37.1)	21.9 (30.5)	<0.001
Median (IRQ)	19.0 (8.6, 42.9)	21.0 (9.5, 45.7)	17.1 (9.5, 36.2)	12.4 (6.7, 24.8)	
hs-cTnT (ng/l)					
N	533	544	544	542	
Mean (SD)	11.2 (43.0)	8.9 (14.7)	7.9 (9.5)	8.5 (10.3)	0.0433
Median (IRQ)	5.0 (1.5, 11.0)	5.0 (1.5, 11.0)	5.0 (1.5, 10.0)	6.0 (1.5, 11.0)	
NT-proBNP (pg/mL)					
N	533	544	544	542	
Mean (SD)	252.8 (662.7)	267.3 (1528.5)	181.1 (301.6)	266.2 (952.0)	0.8065
Median (IRQ)	78.0 (29.0, 216.0)	91.5 (36.5, 225.5)	78.0 (31.0, 187.0)	93.0 (34.0, 200.0)	

Values are n (%), mean (standard deviation), or median (interquartile range)

ESM Table 5 Baseline characteristics classified by quarters of the leucine distribution

Characteristics	Leucine (mmol/L)				p-value for trend
	1st(<=0.069) (N = 896)	2nd(0.069 to <=0.080) (N = 897)	3rd(0.080 to <=0.092) (N = 895)	4th(>0.092) (N = 895)	
Sex					
Male	434/ 896 (48.4%)	502/ 897 (56.0%)	579/ 895 (64.7%)	653/ 895 (73.0%)	<0.001
Female	462/ 896 (51.6%)	395/ 897 (44.0%)	316/ 895 (35.3%)	242/ 895 (27.0%)	
Current smokers					
No	794/ 896 (88.6%)	767/ 897 (85.5%)	745/ 895 (83.2%)	746/ 895 (83.4%)	<0.001
Yes	102/ 896 (11.4%)	130/ 897 (14.5%)	150/ 895 (16.8%)	149/ 895 (16.6%)	
History of macrovascular event					
No	612/ 896 (68.3%)	576/ 897 (64.2%)	589/ 895 (65.8%)	555/ 895 (62.0%)	0.0153
Yes	284/ 896 (31.7%)	321/ 897 (35.8%)	306/ 895 (34.2%)	340/ 895 (38.0%)	
Age (years)					
N	896	897	895	895	
Mean (SD)	68.0 (6.8)	67.5 (6.5)	66.8 (6.4)	65.0 (6.3)	<0.001
Median (IRQ)	69.0 (63.5, 73.0)	68.0 (63.0, 72.0)	67.0 (62.0, 71.0)	65.0 (60.0, 70.0)	
Duration of diabetes (years)					
N	896	897	894	895	
Mean (SD)	8.2 (7.2)	7.9 (6.4)	7.5 (6.2)	8.0 (6.0)	0.2179
Median (IRQ)	6.0 (3.0, 11.0)	7.0 (3.0, 11.0)	6.0 (2.0, 11.0)	7.0 (3.0, 12.0)	
BMI (Kg/m ²)					
N	896	897	893	895	
Mean (SD)	28.9 (5.3)	30.1 (5.5)	30.3 (5.2)	30.7 (4.8)	<0.001

Characteristics	Leucine (mmol/L)				p-value for trend
	1st(<=0.069) (N = 896)	2nd(0.069 to <=0.080) (N = 897)	3rd(0.080 to <=0.092) (N = 895)	4th(>0.092) (N = 895)	
Median (IRQ)	28.2 (25.4, 31.6)	29.4 (26.3, 33.2)	29.7 (26.7, 33.0)	30.1 (27.4, 33.3)	
History of heart failure					
No	854/ 896 (95.3%)	850/ 897 (94.8%)	861/ 895 (96.2%)	852/ 895 (95.2%)	0.7284
Yes	42/ 896 (4.7%)	47/ 897 (5.2%)	34/ 895 (3.8%)	43/ 895 (4.8%)	
Participation in moderate or vigorous activity					
No	483/ 896 (53.9%)	438/ 897 (48.8%)	480/ 895 (53.6%)	447/ 895 (49.9%)	0.3431
Yes	413/ 896 (46.1%)	459/ 897 (51.2%)	415/ 895 (46.4%)	448/ 895 (50.1%)	
SBP (mmHg)					
N	896	896	895	895	0.6098
Mean (SD)	146.6 (22.6)	147.8 (21.6)	146.8 (21.0)	147.5 (20.7)	
Median (IRQ)	144.5 (130.8, 159.0)	146.0 (133.0, 160.0)	144.5 (133.0, 158.0)	146.5 (133.0, 160.0)	
DBP (mmHg)					
N	896	896	895	895	<0.001
Mean (SD)	80.1 (10.7)	81.8 (11.1)	81.7 (10.7)	82.8 (10.7)	
Median (IRQ)	80.0 (72.5, 87.0)	81.5 (74.8, 88.5)	81.0 (74.5, 88.5)	82.5 (75.0, 90.0)	
Total chol (mmol/l)					
N	896	897	894	894	0.0765
Mean (SD)	5.1 (1.1)	5.1 (1.1)	5.1 (1.1)	5.2 (1.3)	
Median (IRQ)	5.0 (4.3, 5.9)	5.0 (4.3, 5.8)	5.0 (4.3, 5.8)	5.1 (4.3, 5.9)	
HDL Chol (mmol/l)					
N	895	897	893	893	

Characteristics	Leucine (mmol/L)				p-value for trend
	1st(<=0.069) (N = 896)	2nd(0.069 to <=0.080) (N = 897)	3rd(0.080 to <=0.092) (N = 895)	4th(>0.092) (N = 895)	
Mean (SD)	1.3 (0.4)	1.3 (0.3)	1.2 (0.3)	1.1 (0.3)	<0.001
Median (IRQ)	1.3 (1.0, 1.5)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.1 (0.9, 1.3)	
Triacylglycerol (mmol/l)					
N	896	897	894	891	
Mean (SD)	1.4 (0.7)	1.7 (0.7)	2.0 (1.0)	2.7 (1.8)	<0.001
Median (IRQ)	1.3 (1.0, 1.7)	1.5 (1.2, 2.0)	1.8 (1.4, 2.4)	2.3 (1.6, 3.2)	
HbA1c (%)					
N	894	892	892	889	
Mean % (SD) [mmol/mol]	7.2 (1.4) [55]	7.3 (1.4) [56]	7.4 (1.4) [57]	7.7 (1.5) [61]	<0.001
Urinary albumin:creatinine ratio (mg/mmol)					
N	843	850	839	836	
Median (IRQ)	1.8 (0.8, 4.9)	1.9 (0.8, 5.1)	2 (0.9, 5.3)	2 (0.9, 6.3)	0.7714
Glucose (mmol/l)					
N	896	897	894	894	
Mean (SD)	7.9 (2.5)	8.1 (2.5)	8.5 (2.6)	9.3 (3.0)	<0.001
Median (IRQ)	7.5 (6.3, 8.9)	7.7 (6.4, 9.2)	8.0 (6.7, 9.8)	8.8 (7.2, 10.8)	
eGFR (mL/min/1.73 m ²)					
N	894	892	892	888	
Mean (SD)	70.2 (17.2)	71.1 (16.7)	72.6 (16.5)	73.4 (16.3)	<0.001
Median (IRQ)	70.3 (57.9, 83.6)	71.8 (59.7, 84.7)	72.5 (62.0, 86.0)	73.7 (62.3, 86.2)	

Characteristics	Leucine (mmol/L)				p-value for trend
	1st(<=0.069) (N = 896)	2nd(0.069 to <=0.080) (N = 897)	3rd(0.080 to <=0.092) (N = 895)	4th(>0.092) (N = 895)	
CRP (nmol/l)					
N	872	871	876	872	
Mean (SD)	37.1 (84.8)	33.3 (61.0)	32.4 (48.6)	36.2 (69.5)	0.6817
Median (IRQ)	15.2 (7.6, 38.1)	18.1 (8.6, 39.0)	17.1 (8.6, 39.0)	19.0 (8.6, 41.0)	
hs-cTnT (ng/l)					
N	871	871	874	872	
Mean (SD)	9.0 (12.7)	8.8 (13.0)	8.4 (26.9)	9.3 (23.0)	0.9453
Median (IRQ)	5.0 (1.5, 11.0)	5.0 (1.5, 12.0)	5.0 (1.5, 10.0)	5.0 (1.5, 11.0)	
NT-proBNP (pg/mL)					
N	871	871	874	872	
Mean (SD)	391.2 (1564.3)	268.2 (554.7)	190.1 (402.4)	164.9 (307.3)	<0.001
Median (IRQ)	117.0 (51.0, 286.0)	100.0 (38.0, 229.0)	74.0 (31.0, 186.0)	65.0 (22.0, 164.0)	

Values are n (%), mean (standard deviation), or median (interquartile range)

ESM Table 6 Baseline characteristics classified by quarters of the alanine distribution

Characteristics	Alanine (mmol/L)				p-value for trend
	1st(≤ 0.326) (N = 897)	2nd(0.326 to ≤ 0.365) (N = 897)	3rd(0.365 to ≤ 0.407) (N = 897)	4th(> 0.407) (N = 895)	
Sex					
Male	591/ 897 (65.9%)	565/ 897 (63.0%)	511/ 897 (57.0%)	503/ 895 (56.2%)	<0.001
Female	306/ 897 (34.1%)	332/ 897 (37.0%)	386/ 897 (43.0%)	392/ 895 (43.8%)	
Current smokers					
No	760/ 897 (84.7%)	773/ 897 (86.2%)	738/ 897 (82.3%)	783/ 895 (87.5%)	0.4112
Yes	137/ 897 (15.3%)	124/ 897 (13.8%)	159/ 897 (17.7%)	112/ 895 (12.5%)	
History of macrovascular event					
No	613/ 897 (68.3%)	589/ 897 (65.7%)	600/ 897 (66.9%)	532/ 895 (59.4%)	<0.001
Yes	284/ 897 (31.7%)	308/ 897 (34.3%)	297/ 897 (33.1%)	363/ 895 (40.6%)	
Age (years)					
N	897	897	897	895	<0.001
Mean (SD)	67.5 (6.8)	67.4 (6.3)	66.2 (6.5)	66.2 (6.5)	
Median (IRQ)	68.0 (63.0, 72.0)	67.0 (63.0, 72.0)	66.0 (61.0, 71.0)	67.0 (61.0, 71.0)	
Duration of diabetes (years)					
N	896	897	897	895	0.2860
Mean (SD)	8.4 (6.9)	7.7 (6.4)	7.4 (6.3)	8.2 (6.3)	
Median (IRQ)	7.0 (3.0, 12.0)	6.0 (3.0, 11.0)	6.0 (2.0, 11.0)	7.0 (3.0, 12.0)	
BMI (Kg/m ²)					
N	897	896	896	895	<0.001
Mean (SD)	29.2 (5.1)	29.9 (5.3)	30.6 (5.6)	30.3 (4.9)	

Characteristics	Alanine (mmol/L)				p-value for trend
	1st(<=0.326) (N = 897)	2nd(0.326 to <=0.365) (N = 897)	3rd(0.365 to <=0.407) (N = 897)	4th(>0.407) (N = 895)	
Median (IRQ)	28.6 (25.7, 32.2)	29.1 (26.3, 32.8)	29.7 (26.8, 33.3)	29.7 (27.0, 32.8)	
History of heart failure					
No	857/ 897 (95.5%)	856/ 897 (95.4%)	853/ 897 (95.1%)	854/ 895 (95.4%)	0.8234
Yes	40/ 897 (4.5%)	41/ 897 (4.6%)	44/ 897 (4.9%)	41/ 895 (4.6%)	
Participation in moderate or vigorous activity					
No	479/ 897 (53.4%)	457/ 897 (50.9%)	452/ 897 (50.4%)	462/ 895 (51.6%)	0.4291
Yes	418/ 897 (46.6%)	440/ 897 (49.1%)	445/ 897 (49.6%)	433/ 895 (48.4%)	
SBP (mmHg)					
N	897	896	897	895	
Mean (SD)	146.7 (21.4)	147.5 (21.6)	147.1 (21.0)	147.4 (22.0)	0.5705
Median (IRQ)	145.0 (131.5, 159.5)	145.0 (133.0, 158.5)	145.0 (133.0, 160.0)	145.0 (132.0, 160.0)	
DBP (mmHg)					
N	897	896	897	895	
Mean (SD)	80.8 (10.8)	81.4 (10.6)	81.7 (10.6)	82.5 (11.4)	0.0014
Median (IRQ)	80.0 (73.5, 88.0)	80.8 (73.8, 88.5)	81.5 (75.0, 88.5)	82.0 (74.5, 90.0)	
Total chol (mmol/l)					
N	896	897	897	894	
Mean (SD)	5.0 (1.1)	5.1 (1.1)	5.2 (1.3)	5.2 (1.2)	0.0012
Median (IRQ)	4.9 (4.2, 5.7)	5.1 (4.3, 5.9)	5.1 (4.4, 5.9)	5.1 (4.4, 5.9)	
HDL Chol (mmol/l)					
N	895	897	896	893	

Characteristics	Alanine (mmol/L)				p-value for trend
	1st(<=0.326) (N = 897)	2nd(0.326 to <=0.365) (N = 897)	3rd(0.365 to <=0.407) (N = 897)	4th(>0.407) (N = 895)	
Mean (SD)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	0.8507
Median (IRQ)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	
Triacylglycerol (mmol/l)					
N	896	896	896	893	
Mean (SD)	1.6 (1.0)	1.9 (1.1)	2.1 (1.3)	2.3 (1.4)	<0.001
Median (IRQ)	1.4 (1.0, 1.8)	1.6 (1.2, 2.3)	1.8 (1.4, 2.5)	1.9 (1.5, 2.7)	
HbA1c (%)					
N	893	892	893	892	
Mean % (SD) [mmol/mol]	7.3 (1.5) [56]	7.4 (1.5) [57]	7.4 (1.4) [57]	7.5 (1.4) [59]	0.0175
Urinary albumin:creatinine ratio (mg/mmol)					
N	842	851	833	845	
Median (IRQ)	1.8 (0.8, 5.5)	1.9 (0.8, 4.9)	1.9 (0.8, 4.8)	2.1 (0.9, 6)	0.1522
Glucose (mmol/l)					
N	897	897	896	894	
Mean (SD)	8.1 (2.7)	8.4 (2.7)	8.5 (2.7)	8.9 (2.7)	<0.001
Median (IRQ)	7.6 (6.4, 9.3)	7.9 (6.6, 9.6)	8.0 (6.7, 9.7)	8.3 (7.0, 10.3)	
eGFR (mL/min/1.73 m ²)					
N	893	894	891	891	
Mean (SD)	71.7 (16.8)	71.3 (17.0)	72.1 (16.9)	72.1 (16.3)	0.4152
Median (IRQ)	72.2 (59.4, 85.0)	71.5 (60.4, 84.2)	72.9 (61.1, 85.3)	71.7 (60.1, 85.4)	

Characteristics	Alanine (mmol/L)				p-value for trend
	1st(<=0.326) (N = 897)	2nd(0.326 to <=0.365) (N = 897)	3rd(0.365 to <=0.407) (N = 897)	4th(>0.407) (N = 895)	
CRP (nmol/l)					
N	877	871	874	869	
Mean (SD)	49.5 (109.5)	32.4 (43.8)	32.4 (48.6)	25.7 (39.0)	<0.001
Median (IRQ)	19.0 (8.6, 49.5)	16.2 (8.6, 39.0)	19.0 (8.6, 41.0)	15.2 (7.6, 30.5)	
hs-cTnT (ng/l)					
N	876	871	872	869	
Mean (SD)	10.4 (29.0)	9.3 (18.4)	8.0 (17.0)	7.8 (10.3)	0.0025
Median (IRQ)	5.0 (1.5, 12.0)	5.0 (1.5, 12.0)	5.0 (1.5, 10.0)	5.0 (1.5, 10.0)	
NT-proBNP (pg/mL)					
N	876	871	872	869	
Mean (SD)	306.6 (789.9)	320.6 (1458.9)	186.7 (367.0)	199.9 (371.1)	<0.001
Median (IRQ)	105.0 (42.0, 259.0)	90.0 (37.0, 246.0)	73.5 (32.0, 168.5)	85.0 (28.0, 203.0)	

Values are n (%), mean (standard deviation), or median (interquartile range)

ESM Table 7 Baseline characteristics classified by quarters of the tyrosine distribution

Characteristics	Tyrosine (mmol/L)				p-value for trend
	1st(<=0.045) (N = 897)	2nd(0.045 to <=0.052) (N = 893)	3rd(0.052 to <=0.059) (N = 895)	4th(>0.059) (N = 894)	
Sex					
Male	521/ 897 (58.1%)	547/ 893 (61.3%)	512/ 895 (57.2%)	586/ 894 (65.5%)	0.0121
Female	376/ 897 (41.9%)	346/ 893 (38.7%)	383/ 895 (42.8%)	308/ 894 (34.5%)	
Current smokers					
No	763/ 897 (85.1%)	761/ 893 (85.2%)	764/ 895 (85.4%)	760/ 894 (85.0%)	0.99
Yes	134/ 897 (14.9%)	132/ 893 (14.8%)	131/ 895 (14.6%)	134/ 894 (15.0%)	
History of macrovascular event					
No	616/ 897 (68.7%)	574/ 893 (64.3%)	582/ 895 (65.0%)	557/ 894 (62.3%)	0.0100
Yes	281/ 897 (31.3%)	319/ 893 (35.7%)	313/ 895 (35.0%)	337/ 894 (37.7%)	
Age (years)					
N	897	893	895	894	0.0042
Mean (SD)	67.3 (6.7)	67.1 (6.6)	66.4 (6.5)	66.6 (6.6)	
Median (IRQ)	67.0 (62.0, 72.0)	67.0 (62.0, 72.0)	67.0 (61.0, 71.0)	66.0 (61.0, 71.0)	
Duration of diabetes (years)					
N	897	892	895	894	<0.001
Mean (SD)	9.4 (6.9)	8.2 (6.6)	7.1 (6.0)	6.9 (5.9)	
Median (IRQ)	9.0 (4.0, 14.0)	7.0 (3.0, 12.0)	5.0 (2.0, 10.0)	5.0 (2.0, 10.0)	
BMI (Kg/m ²)					
N	897	891	895	894	<0.001
Mean (SD)	29.0 (5.3)	29.7 (5.1)	30.5 (5.3)	30.8 (5.2)	

Characteristics	Tyrosine (mmol/L)				p-value for trend
	1st(<=0.045) (N = 897)	2nd(0.045 to <=0.052) (N = 893)	3rd(0.052 to <=0.059) (N = 895)	4th(>0.059) (N = 894)	
Median (IRQ)	28.4 (25.3, 31.8)	29.0 (26.3, 32.3)	29.7 (27.0, 33.2)	30.1 (27.2, 33.7)	
History of heart failure					
No	866/ 897 (96.5%)	855/ 893 (95.7%)	850/ 895 (95.0%)	842/ 894 (94.2%)	0.0128
Yes	31/ 897 (3.5%)	38/ 893 (4.3%)	45/ 895 (5.0%)	52/ 894 (5.8%)	
Participation in moderate or vigorous activity					
No	441/ 897 (49.2%)	477/ 893 (53.4%)	465/ 895 (52.0%)	467/ 894 (52.2%)	0.2980
Yes	456/ 897 (50.8%)	416/ 893 (46.6%)	430/ 895 (48.0%)	427/ 894 (47.8%)	
SBP (mmHg)					
N	897	893	894	894	0.0221
Mean (SD)	148.2 (22.8)	147.8 (21.4)	146.4 (21.0)	146.2 (20.7)	
Median (IRQ)	145.5 (132.0, 161.5)	146.0 (132.5, 160.0)	145.0 (132.0, 158.0)	145.0 (132.5, 157.5)	
DBP (mmHg)					
N	897	893	894	894	0.0160
Mean (SD)	80.9 (11.0)	81.4 (10.6)	82.2 (11.0)	81.9 (10.8)	
Median (IRQ)	80.0 (73.5, 88.0)	81.0 (74.0, 88.5)	82.0 (75.0, 89.0)	81.5 (74.5, 89.0)	
Total chol (mmol/l)					
N	897	892	895	893	0.1502
Mean (SD)	5.2 (1.1)	5.1 (1.1)	5.3 (1.3)	5.0 (1.1)	
Median (IRQ)	5.1 (4.3, 5.9)	5.0 (4.3, 5.9)	5.1 (4.4, 5.9)	4.9 (4.3, 5.7)	
HDL Chol (mmol/l)					
N	897	891	894	892	

Characteristics	Tyrosine (mmol/L)				p-value for trend
	1st(<=0.045) (N = 897)	2nd(0.045 to <=0.052) (N = 893)	3rd(0.052 to <=0.059) (N = 895)	4th(>0.059) (N = 894)	
Mean (SD)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	0.0072
Median (IRQ)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	
Triacylglycerol (mmol/l)					
N	896	892	894	892	0.0446
Mean (SD)	1.9 (1.1)	1.9 (1.2)	2.0 (1.4)	2.0 (1.2)	
Median (IRQ)	1.6 (1.2, 2.3)	1.6 (1.2, 2.3)	1.8 (1.3, 2.4)	1.7 (1.3, 2.4)	
HbA1c (%)					
N	894	891	890	888	<0.001
Mean % (SD) [mmol/mol]	7.6 (1.6) [60]	7.4 (1.4) [57]	7.3 (1.4) [56]	7.3 (1.3) [56]	
Urinary albumin:creatinine ratio (mg/mmol)					
N	844	839	841	840	<0.001
Median (IRQ)	2.4 (1.1, 7.1)	2 (0.9, 5.5)	1.6 (0.8, 4.3)	1.6 (0.8, 4.4)	
Glucose (mmol/l)					
N	896	893	895	893	0.0152
Mean (SD)	8.6 (2.9)	8.5 (2.6)	8.4 (2.8)	8.3 (2.4)	
Median (IRQ)	8.0 (6.6, 10.0)	8.0 (6.6, 9.7)	7.8 (6.6, 9.7)	7.9 (6.6, 9.6)	
eGFR (mL/min/1.73 m ²)					
N	894	889	890	889	0.0011
Mean (SD)	70.9 (17.7)	70.9 (17.1)	72.4 (16.2)	73.1 (15.6)	
Median (IRQ)	71.9 (58.1, 85.1)	71.2 (59.1, 84.4)	72.8 (61.6, 84.9)	72.2 (62.3, 86.0)	

Characteristics	Tyrosine (mmol/L)				p-value for trend
	1st(<=0.045) (N = 897)	2nd(0.045 to <=0.052) (N = 893)	3rd(0.052 to <=0.059) (N = 895)	4th(>0.059) (N = 894)	
CRP (nmol/l)					
N	881	867	868	867	
Mean (SD)	35.2 (81.0)	32.4 (54.3)	34.3 (56.2)	37.1 (73.3)	0.5176
Median (IRQ)	15.2 (7.6, 34.3)	16.2 (8.6, 37.1)	19.0 (9.5, 41.9)	19.0 (8.6, 41.9)	
hs-cTnT (ng/l)					
N	880	866	868	866	
Mean (SD)	9.4 (12.9)	8.9 (26.9)	8.1 (13.1)	9.2 (23.0)	0.6502
Median (IRQ)	5.0 (1.5, 12.0)	5.0 (1.5, 11.0)	5.0 (1.5, 10.0)	5.0 (1.5, 11.0)	
NT-proBNP (pg/mL)					
N	880	866	868	866	
Mean (SD)	318.3 (1443.2)	256.6 (562.7)	217.2 (627.6)	220.9 (474.8)	0.0119
Median (IRQ)	103.0 (42.0, 246.0)	90.0 (36.0, 222.0)	76.0 (28.0, 191.0)	79.0 (31.0, 200.0)	

Values are n (%), mean (standard deviation), or median (interquartile range)

ESM Table 8 Baseline characteristics classified by quarters of the histidine distribution

Characteristics	Histidine (mmol/L)				p-value for trend
	1st(<=0.044) (N = 892)	2nd(0.044 to <=0.050) (N = 891)	3rd(0.050 to <=0.056) (N = 893)	4th(>0.056) (N = 890)	
Sex					
Male	444/ 892 (49.8%)	534/ 891 (59.9%)	565/ 893 (63.3%)	617/ 890 (69.3%)	<0.001
Female	448/ 892 (50.2%)	357/ 891 (40.1%)	328/ 893 (36.7%)	273/ 890 (30.7%)	
Current smokers					
No	756/ 892 (84.8%)	753/ 891 (84.5%)	764/ 893 (85.6%)	765/ 890 (86.0%)	0.3826
Yes	136/ 892 (15.2%)	138/ 891 (15.5%)	129/ 893 (14.4%)	125/ 890 (14.0%)	
History of macrovascular event					
No	581/ 892 (65.1%)	599/ 891 (67.2%)	585/ 893 (65.5%)	556/ 890 (62.5%)	0.1747
Yes	311/ 892 (34.9%)	292/ 891 (32.8%)	308/ 893 (34.5%)	334/ 890 (37.5%)	
Age (years)					
N	892	891	893	890	0.0019
Mean (SD)	67.2 (6.6)	66.9 (6.6)	67.0 (6.6)	66.2 (6.5)	
Median (IRQ)	68.0 (62.0, 72.0)	67.0 (62.0, 72.0)	67.0 (62.0, 71.0)	66.0 (61.0, 71.0)	
Duration of diabetes (years)					
N	892	891	892	890	0.1189
Mean (SD)	8.1 (6.6)	7.9 (6.5)	8.1 (6.6)	7.6 (6.2)	
Median (IRQ)	7.0 (3.0, 11.0)	6.0 (3.0, 11.0)	6.0 (3.0, 12.0)	6.0 (3.0, 11.0)	
BMI (Kg/m ²)					
N	892	889	893	890	0.0066
Mean (SD)	30.2 (5.4)	30.3 (5.4)	29.8 (4.9)	29.7 (5.3)	

Characteristics	Histidine (mmol/L)				p-value for trend
	1st(<=0.044) (N = 892)	2nd(0.044 to <=0.050) (N = 891)	3rd(0.050 to <=0.056) (N = 893)	4th(>0.056) (N = 890)	
Median (IRQ)	29.6 (26.6, 33.3)	29.7 (26.6, 33.2)	29.1 (26.5, 32.4)	29.0 (26.3, 32.1)	
History of heart failure					
No	829/ 892 (92.9%)	854/ 891 (95.8%)	857/ 893 (96.0%)	860/ 890 (96.6%)	<0.001
Yes	63/ 892 (7.1%)	37/ 891 (4.2%)	36/ 893 (4.0%)	30/ 890 (3.4%)	
Participation in moderate or vigorous activity					
No	514/ 892 (57.6%)	472/ 891 (53.0%)	443/ 893 (49.6%)	415/ 890 (46.6%)	<0.001
Yes	378/ 892 (42.4%)	419/ 891 (47.0%)	450/ 893 (50.4%)	475/ 890 (53.4%)	
SBP (mmHg)					
N	892	890	893	890	0.0645
Mean (SD)	146.5 (21.7)	146.3 (20.9)	147.6 (21.7)	148.0 (21.3)	
Median (IRQ)	144.5 (131.3, 159.5)	144.5 (132.0, 158.5)	146.0 (133.5, 159.0)	145.5 (132.5, 160.5)	
DBP (mmHg)					
N	892	890	893	890	0.0163
Mean (SD)	80.9 (10.8)	81.4 (10.9)	81.8 (10.9)	82.1 (10.6)	
Median (IRQ)	80.0 (73.5, 88.3)	81.0 (74.0, 89.0)	81.5 (75.0, 88.5)	82.0 (74.5, 89.0)	
Total chol (mmol/l)					
N	891	891	893	889	0.4220
Mean (SD)	5.2 (1.1)	5.2 (1.1)	5.1 (1.1)	5.1 (1.3)	
Median (IRQ)	5.1 (4.3, 5.9)	5.1 (4.3, 5.9)	5.0 (4.3, 5.9)	5.0 (4.3, 5.8)	
HDL Chol (mmol/l)					
N	889	891	893	888	

Characteristics	Histidine (mmol/L)				p-value for trend
	1st(<=0.044) (N = 892)	2nd(0.044 to <=0.050) (N = 891)	3rd(0.050 to <=0.056) (N = 893)	4th(>0.056) (N = 890)	
Mean (SD)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	0.8696
Median (IRQ)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	
Triacylglycerol (mmol/l)					
N	889	890	893	889	
Mean (SD)	2.0 (1.1)	2.0 (1.2)	2.0 (1.3)	1.9 (1.2)	0.8676
Median (IRQ)	1.7 (1.2, 2.4)	1.7 (1.2, 2.4)	1.7 (1.3, 2.3)	1.6 (1.2, 2.3)	
HbA1c (%)					
N	888	885	889	888	
Mean % (SD) [mmol/mol]	7.7 (1.7) [61]	7.5 (1.4) [59]	7.3 (1.3) [56]	7.1 (1.3) [54]	<0.001
Urinary albumin:creatinine ratio (mg/mmol)					
N	844	831	844	833	
Median (IRQ)	2.2 (1.1, 7)	2 (0.8, 5.3)	1.8 (0.8, 4.7)	1.6 (0.7, 4.5)	0.0161
Glucose (mmol/l)					
N	892	891	892	889	
Mean (SD)	8.8 (3.0)	8.6 (2.8)	8.3 (2.6)	8.1 (2.4)	<0.001
Median (IRQ)	8.2 (6.7, 10.3)	8.0 (6.7, 9.8)	7.9 (6.5, 9.4)	7.6 (6.5, 9.2)	
eGFR (mL/min/1.73 m ²)					
N	890	887	888	884	
Mean (SD)	70.6 (16.9)	71.7 (16.7)	72.4 (16.0)	72.5 (17.1)	0.0106
Median (IRQ)	70.1 (57.6, 83.7)	71.8 (60.0, 84.8)	72.9 (61.6, 85.3)	73.3 (61.0, 85.7)	

Characteristics	Histidine (mmol/L)				p-value for trend
	1st(<=0.044) (N = 892)	2nd(0.044 to <=0.050) (N = 891)	3rd(0.050 to <=0.056) (N = 893)	4th(>0.056) (N = 890)	
CRP (nmol/l)					
N	870	872	872	866	
Mean (SD)	51.4 (98.1)	34.3 (51.4)	30.5 (51.4)	24.8 (53.3)	<0.001
Median (IRQ)	23.8 (10.5, 56.2)	19.0 (9.5, 40.0)	16.2 (7.6, 34.3)	12.4 (6.7, 27.6)	
hs-cTnT (ng/l)					
N	870	870	872	865	
Mean (SD)	9.6 (31.4)	8.8 (11.3)	8.4 (12.6)	8.8 (17.6)	0.3204
Median (IRQ)	4.0 (1.5, 11.0)	5.0 (1.5, 11.0)	5.0 (1.5, 11.0)	6.0 (1.5, 11.0)	
NT-proBNP (pg/mL)					
N	870	870	872	865	
Mean (SD)	312.6 (785.2)	281.4 (1302.1)	203.6 (426.7)	216.8 (739.2)	0.0058
Median (IRQ)	100.0 (37.0, 268.0)	96.0 (40.0, 223.0)	81.5 (30.5, 192.5)	75.0 (31.0, 193.0)	

Values are n (%), mean (standard deviation), or median (interquartile range)

ESM Table 9 Baseline characteristics classified by quarters of the valine distribution

Characteristics	Valine (mmol/L)				p-value for trend
	1st(<=0.150) (N = 897)	2nd(0.150 to <=0.173) (N = 901)	3rd(0.173 to <=0.196) (N = 895)	4th(>0.196) (N = 894)	
Sex					
Male	432/ 897 (48.2%)	514/ 901 (57.0%)	593/ 895 (66.3%)	631/ 894 (70.6%)	<0.001
Female	465/ 897 (51.8%)	387/ 901 (43.0%)	302/ 895 (33.7%)	263/ 894 (29.4%)	
Current smokers					
No	755/ 897 (84.2%)	780/ 901 (86.6%)	761/ 895 (85.0%)	758/ 894 (84.8%)	0.9536
Yes	142/ 897 (15.8%)	121/ 901 (13.4%)	134/ 895 (15.0%)	136/ 894 (15.2%)	
History of macrovascular event					
No	612/ 897 (68.2%)	582/ 901 (64.6%)	590/ 895 (65.9%)	551/ 894 (61.6%)	0.0096
Yes	285/ 897 (31.8%)	319/ 901 (35.4%)	305/ 895 (34.1%)	343/ 894 (38.4%)	
Age (years)					
N	897	901	895	894	<0.001
Mean (SD)	67.9 (6.9)	67.1 (6.6)	66.9 (6.3)	65.4 (6.3)	
Median (IRQ)	69.0 (63.0, 73.0)	67.0 (62.0, 72.0)	67.0 (62.0, 71.0)	66.0 (60.0, 70.0)	
Duration of diabetes (years)					
N	897	901	894	894	0.1826
Mean (SD)	8.1 (7.0)	8.1 (6.7)	7.5 (6.0)	7.9 (6.1)	
Median (IRQ)	6.0 (3.0, 12.0)	7.0 (3.0, 11.0)	6.0 (2.0, 11.0)	6.0 (3.0, 12.0)	
BMI (Kg/m ²)					
N	897	899	895	894	<0.001
Mean (SD)	29.0 (5.2)	29.9 (5.2)	30.3 (5.4)	30.8 (5.1)	
Median (IRQ)	28.4 (25.5, 31.6)	29.1 (26.6, 32.7)	29.4 (26.7, 33.0)	30.3 (27.3, 33.6)	

Characteristics	Valine (mmol/L)				p-value for trend
	1st(≤ 0.150) (N = 897)	2nd(0.150 to ≤ 0.173) (N = 901)	3rd(0.173 to ≤ 0.196) (N = 895)	4th(> 0.196) (N = 894)	
History of heart failure					
No	851/ 897 (94.9%)	852/ 901 (94.6%)	861/ 895 (96.2%)	857/ 894 (95.9%)	0.1424
Yes	46/ 897 (5.1%)	49/ 901 (5.4%)	34/ 895 (3.8%)	37/ 894 (4.1%)	
Participation in moderate or vigorous activity					
No	484/ 897 (54.0%)	472/ 901 (52.4%)	446/ 895 (49.8%)	449/ 894 (50.2%)	0.0656
Yes	413/ 897 (46.0%)	429/ 901 (47.6%)	449/ 895 (50.2%)	445/ 894 (49.8%)	
SBP (mmHg)					
N	897	900	895	894	0.9113
Mean (SD)	147.1 (22.4)	147.3 (21.7)	147.6 (21.1)	146.8 (20.8)	
Median (IRQ)	145.5 (131.5, 160.0)	145.0 (132.5, 159.0)	145.0 (133.0, 160.0)	145.0 (133.0, 159.0)	
DBP (mmHg)					
N	897	900	895	894	0.0011
Mean (SD)	80.9 (11.2)	81.2 (10.8)	82.1 (10.5)	82.3 (10.9)	
Median (IRQ)	80.0 (72.5, 88.5)	80.3 (74.0, 87.8)	81.5 (75.0, 88.5)	82.0 (75.0, 89.5)	
Total chol (mmol/l)					
N	897	901	895	892	<0.001
Mean (SD)	5.3 (1.2)	5.2 (1.1)	5.0 (1.1)	5.1 (1.3)	
Median (IRQ)	5.2 (4.5, 6.1)	5.1 (4.4, 5.9)	5.0 (4.3, 5.8)	4.9 (4.3, 5.7)	
HDL Chol (mmol/l)					
N	896	900	894	892	<0.001
Mean (SD)	1.3 (0.4)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	

Characteristics	Valine (mmol/L)				p-value for trend
	1st(<=0.150) (N = 897)	2nd(0.150 to <=0.173) (N = 901)	3rd(0.173 to <=0.196) (N = 895)	4th(>0.196) (N = 894)	
Median (IRQ)	1.3 (1.1, 1.5)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.1 (1.0, 1.3)	
Triacylglycerol (mmol/l)					
N	896	901	895	890	
Mean (SD)	1.8 (1.3)	1.9 (1.0)	2.0 (1.2)	2.2 (1.3)	<0.001
Median (IRQ)	1.5 (1.1, 2.1)	1.6 (1.2, 2.3)	1.7 (1.2, 2.3)	1.9 (1.4, 2.6)	
HbA1c (%)					
N	893	898	891	889	
Mean % (SD) [mmol/mol]	7.2 (1.5) [55]	7.3 (1.4) [56]	7.4 (1.3) [57]	7.7 (1.5) [61]	<0.001
Urinary albumin:creatinine ratio (mg/mmol)					
N	836	844	854	838	
Median (IRQ)	1.9 (0.8, 5.3)	2.1 (0.9, 5.9)	1.8 (0.8, 4.5)	1.9 (0.8, 5.9)	0.0028
Glucose (mmol/l)					
N	897	901	894	893	
Mean (SD)	8.0 (2.8)	8.2 (2.5)	8.5 (2.5)	9.2 (2.9)	<0.001
Median (IRQ)	7.4 (6.2, 9.1)	7.7 (6.5, 9.3)	8.0 (6.8, 9.6)	8.6 (7.1, 10.6)	
eGFR (mL/min/1.73 m ²)					
N	894	895	895	886	
Mean (SD)	68.8 (17.8)	71.7 (16.7)	72.4 (16.0)	74.3 (15.8)	<0.001
Median (IRQ)	69.0 (56.7, 82.7)	71.7 (60.4, 84.4)	72.5 (61.4, 85.0)	74.3 (63.2, 87.3)	
CRP (nmol/l)					

Characteristics	Valine (mmol\L)				p-value for trend
	1st(<=0.150) (N = 897)	2nd(0.150 to <=0.173) (N = 901)	3rd(0.173 to <=0.196) (N = 895)	4th(>0.196) (N = 894)	
N	877	872	878	864	
Mean (SD)	40.0 (88.6)	35.2 (61.0)	29.5 (48.6)	35.2 (64.8)	0.0619
Median (IRQ)	17.1 (7.6, 41.0)	18.1 (9.4, 40.0)	17.1 (8.6, 35.2)	18.1 (8.6, 41.0)	
hs-cTnT (ng/l)					
N	877	871	876	864	
Mean (SD)	9.7 (13.4)	9.7 (28.3)	7.5 (9.3)	8.7 (22.8)	0.0902
Median (IRQ)	5.0 (1.5, 12.0)	5.0 (1.5, 11.0)	5.0 (1.5, 10.0)	5.0 (1.5, 10.0)	
NT-proBNP (pg/mL)					
N	877	871	876	864	
Mean (SD)	377.0 (1443.8)	274.2 (835.6)	190.1 (328.3)	171.6 (334.4)	<0.001
Median (IRQ)	115.0 (49.0, 279.0)	97.0 (37.0, 221.0)	75.0 (28.0, 203.0)	66.0 (27.0, 167.0)	

Values are n (%), mean (standard deviation), or median (interquartile range)

ESM Table 10 Associations of the amino acids with risk of all 3 endpoints (per 1 standard deviation increase)

	Macrovascular events			Microvascular events			All Deaths		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
Model 1									
Phenylalanine (1SD=0.0088)	1.22	1.12,1.32	<0.001	1.09	0.98,1.22	0.110	1.21	1.12,1.32	<0.001
Isoleucine (1SD=0.0170)	1.06	0.98,1.15	0.152	1.09	0.99,1.2	0.064	1.00	0.91,1.1	0.950
Glutamine (1SD=0.1096)	0.88	0.79,0.98	0.020	0.87	0.76,1.01	0.062	0.85	0.76,0.94	0.002
Leucine (1SD=0.0197)	0.96	0.87,1.05	0.325	1.04	0.93,1.16	0.508	0.89	0.8,0.99	0.031
Alanine (1SD=0.0645)	0.96	0.88,1.05	0.385	0.95	0.85,1.07	0.425	0.91	0.83,1	0.040
Tyrosine (1SD=0.0112)	1.01	0.92,1.1	0.874	0.74	0.64,0.86	<0.001	0.96	0.87,1.06	0.418
Histidine (1SD=0.0095)	0.86	0.79,0.94	0.001	0.93	0.83,1.05	0.237	0.83	0.76,0.91	<0.001
Valine (1SD=0.0352)	0.94	0.86,1.03	0.167	1.00	0.89,1.13	0.970	0.81	0.73,0.89	<0.001
Model 2									
Phenylalanine (1SD=0.0088)	1.09	0.98,1.21	0.105	1.02	0.89,1.17	0.767	1.04	0.94,1.16	0.440
Isoleucine (1SD=0.0170)	1.01	0.90,1.13	0.905	0.93	0.80,1.08	0.321	0.90	0.79,1.02	0.112
Glutamine (1SD=0.1096)	0.93	0.83,1.05	0.261	0.91	0.78,1.06	0.229	0.93	0.83,1.05	0.262
Leucine (1SD=0.0197)	0.91	0.81,1.02	0.101	0.89	0.77,1.04	0.140	0.79	0.69,0.90	0.001
Alanine (1SD=0.0645)	0.98	0.89,1.08	0.704	0.86	0.76,0.98	0.022	0.91	0.83,1.01	0.075
Tyrosine (1SD=0.0112)	1.01	0.91,1.12	0.868	0.78	0.67,0.91	0.002	0.96	0.86,1.07	0.456
Histidine (1SD=0.0095)	0.92	0.84,1.01	0.089	0.92	0.82,1.03	0.147	0.89	0.81,0.99	0.024
Valine (1SD=0.0352)	0.94	0.84,1.04	0.247	0.91	0.80,1.04	0.150	0.79	0.70,0.88	<0.001

Model 1 adjusted for Age, sex, regions and randomised treatment

Model 2 additionally adjusted for previous macrovascular event, duration of diabetes, current smoking, systolic blood pressure, BMI (Kg/m²), albumin/creatinine ratio, estimated glomerular filtration rate, HbA1c, plasma Glucose (mmol/l), total and HDL Chol (mmol/l) cholesterol, Triacylglycerol (mmol/l), aspirin or other antiplatelet agent, statin or other lipid-lowering agent, β -

blocker, ACE inhibitor or angiotensin receptor blocker, metformin use, history of heart failure, participation in moderate and/or vigorous exercise for >15 min at least once weekly, and C-reactive protein.

ESM Table 11: Sensitivity analysis - Associations of the amino acids with risk of all 3 endpoints (per 1 standard deviation increase) in those with no missing values in model 2 of table s10

	Macrovascular events			Microvascular events			All Deaths		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
Model 1 * (sensitivity)									
Phenylalanine (1SD=0.0088)	1.19	1.09,1.30	<0.001	1.05	0.98,1.22	0.381	1.17	1.07,1.28	<0.001
Isoleucine (1SD=0.0170)	1.05	0.96,1.14	0.300	1.05	0.99,1.2	0.312	0.98	0.88,1.08	0.635
Glutamine (1SD=0.1096)	0.87	0.78,0.98	0.017	0.84	0.76,1.01	0.017	0.86	0.77,0.96	0.009
Leucine (1SD=0.0197)	0.95	0.86,1.05	0.321	1.01	0.93,1.16	0.868	0.87	0.78,0.98	0.017
Alanine (1SD=0.0645)	0.97	0.89,1.06	0.499	0.90	0.85,1.07	0.091	0.90	0.82,0.99	0.033
Tyrosine (1SD=0.0112)	0.99	0.89,1.09	0.766	0.70	0.64,0.86	<0.001	0.95	0.86,1.06	0.364
Histidine (1SD=0.0095)	0.86	0.79,0.95	0.002	0.89	0.83,1.05	0.044	0.83	0.76,0.92	<0.001
Valine (1SD=0.0352)	0.95	0.86,1.04	0.282	0.97	0.89,1.13	0.609	0.81	0.73,0.90	<0.001

Model 1 adjusted for Age, sex, regions and randomised treatment

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