

Supplementary Materials

UHPLC-MS condition

UHPLC-MS/MS was run on an Agilent 6460 triple stage quadrupole mass spectrometer equipped with an ESI ion source and an Agilent 1290 HPLC system with auto-sampler (Agilent Technologies, Santa Clara, CA, USA). AAs were separated on an HSS T3 column (2.1×100 mm, 1.8 μm, Waters, Milford, USA) at 30 °C. The mobile phase consisting of water with 2.5 mM ammonium formate and 0.1% formic acid (solvent A) and acetonitrile (Solvent B) was used with a gradient elution at a flow rate of 0.1 ml/min: 0-4 min, 3-5 % B; 4-8 min, 5-8 % B; 8-9 min, 8-95%B, 9-13 min, 95 % B; and then back to initial conditions with 2 min for equilibration, the injection volume was set at 5 μL. ESI-MS/MS conditions were as follows: Gas temperature 300 °C, Gas flow 8 L/min, Nebulizer 45 psi, Sheath gas temperature 375 °C, Sheath gas flow 8 L/min, Capillary 3000 V, Nozzle voltage 500 V, Positive. Multiple reaction monitoring (MRM) mode was used for AAs quantitation, the MS/MS conditions of AAs and L-phenyl-d5-alanine (internal standard, IS) were summarized in Supplement Table 1, chromatogram of eighteen AAs in human plasma was showed in Supplement Figure S1

Table S1. MS/MS conditions and retention time of IS and AAs in positive ion mode.

Analytes	Precursor Ion (<i>m/z</i>)	Product Ion (<i>m/z</i>)	Fragmentor (V)	Collision Energy (V)	Cell Accelerator Voltage (V)	Retention time (min)
IS	171.1	125.1	83	10	0	9.88
Ala	90.0	44.2	36	6	0	2.76
Arg	175.2	116.3	101	10	4	2.69
Gln	147.0	130.0	78	4	7	2.74
Gly	76.1	30.2	38	3	7	2.72
His	156.0	110.0	87	11	7	2.70
Ile	132.2	86.3	58	5	7	5.68
Leu	132.3	86.3	62	5	7	6.21
Lys	147.0	130.0	79	5	7	2.49
Met	150.0	104.0	77	4	7	4.44
Orn	133.2	116.2	78	4	4	2.46
Phe	166.0	120.0	81	8	7	10.06
Pro	116.0	70.1	77	12	0	2.99
Ser	106.0	60.0	67	7	0	2.73
Thr	120.0	74.1	75	6	7	2.77
Trp	205.0	188.0	86	4	0	12.16
Tyr	182.0	165.0	78	4	7	5.92
Val	118.2	72.3	71	5	7	3.54

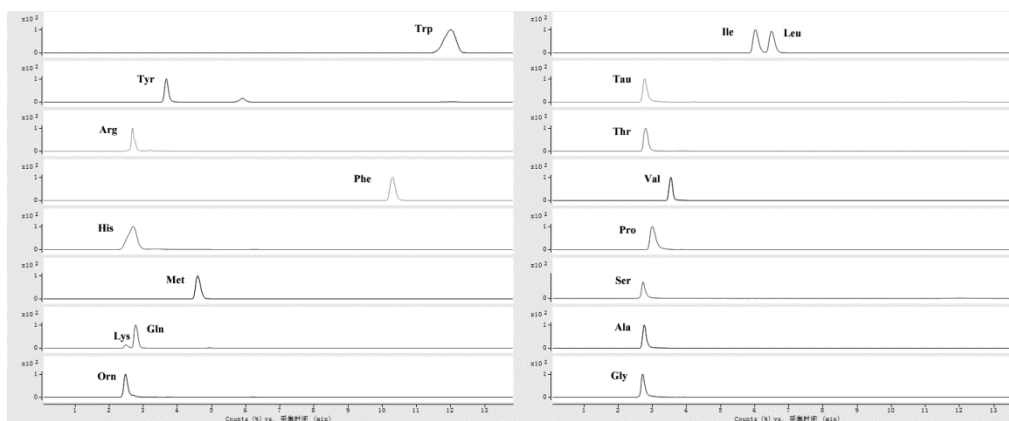


Figure S1 Chromatogram of eighteen AAs in human plasma on positive ion mode.

Validation of UHPLC-MS/MS Method

Calibration curve showed good linearity ($r^2 > 0.995$) over the concentration range. Linearity range, the lower limit of detection (LLOD) and lower limit of quantitation (LLOQ) of AAs in human plasma were showed in Supplement Table S2. Data of accuracy, intra-day/inter-day precision, recovery and matrix effect were summarized in Supplement Table S3.

Table S2 Linearity range, LLOD and LLOQ of AAs in human plasma.

Analytes	Range ($\mu\text{g/mL}$)	LLOD ($\mu\text{g/mL}$)	LLOQ ($\mu\text{g/mL}$)
Ala	0.5-50	0.1	0.5
Arg	0.1-20	0.05	0.1
Gln	0.2-100	0.05	0.2
Gly	0.2-50	0.1	0.2
His	0.1-20	0.05	0.1
Ile	0.1-50	0.05	0.1
Leu	0.1-50	0.02	0.1
Lys	0.1-50	0.05	0.1
Met	0.1-50	0.05	0.1
Orn	0.2-50	0.05	0.2
Phe	0.1-50	0.05	0.1
Pro	0.1-20	0.05	0.1
Ser	0.2-50	0.1	0.2
Tau	0.1-50	0.05	0.1
Thr	0.1-50	0.05	0.1
Trp	0.1-50	0.02	0.1
Tyr	0.1-50	0.05	0.1
Val	0.1-50	0.05	0.1

Table S3 Accuracy, intra-day/inter-day precision, recovery and matrix effect of AAs in human plasma (data were represented as mean $\pm$ standard deviation).

Analytes	Nominal Cone. ($\mu\text{g/mL}$)	Intra-day precision (n=6)		Inter-day precision (n=18)		Recovery (%, n=6)	Matrix Effect (%, n=6)
		Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)		
Ala	0.5	100.4 $\pm$ 9.7	9.7	103.0 $\pm$ 7.9	7.6	103.1 $\pm$ 11.3	104.5 $\pm$ 8.1
	5.0	101.6 $\pm$ 9.7	9.6	97.8 $\pm$ 9.3	9.5	100.2 $\pm$ 7.8	98.3 $\pm$ 12.8
	40.0	103.8 $\pm$ 7.2	6.9	99.0 $\pm$ 11.8	11.9	103.1 $\pm$ 7.8	100.6 $\pm$ 13.1
Arg	0.2	106.6 $\pm$ 5.7	5.3	103.1 $\pm$ 10.4	10.1	97.4 $\pm$ 7.6	103.3 $\pm$ 10.7
	2.0	104.3 $\pm$ 8.2	7.8	99.6 $\pm$ 8.7	8.8	97.4 $\pm$ 11.1	100.5 $\pm$ 8.5
	15.0	104.7 $\pm$ 8.3	8.0	103.2 $\pm$ 8.8	8.5	93.5 $\pm$ 9.0	103.4 $\pm$ 7.9
Gln	0.5	101.3 $\pm$ 7.0	6.9	103.6 $\pm$ 6.1	5.9	97.0 $\pm$ 11.2	104.9 $\pm$ 6.0
	5.0	101.1 $\pm$ 8.3	8.2	109.5 $\pm$ 2.9	2.6	97.7 $\pm$ 7.2	103.1 $\pm$ 8.6
	80.0	101.9 $\pm$ 8.4	8.2	99.8 $\pm$ 11.1	11.1	96.9 $\pm$ 11.5	98.6 $\pm$ 6.7
Gly	0.5	101.6 $\pm$ 10.3	10.1	104.7 $\pm$ 6.9	6.6	95.7 $\pm$ 5.3	102.5 $\pm$ 5.9
	5.0	99.8 $\pm$ 10.5	10.5	100.9 $\pm$ 7.9	7.8	104.2 $\pm$ 8.6	100.8 $\pm$ 7.6
	40.0	98.7 $\pm$ 9.8	9.9	94.3 $\pm$ 6.3	6.7	103.6 $\pm$ 8.7	97.6 $\pm$ 5.3
His	0.2	101.0 $\pm$ 8.8	8.7	102.4 $\pm$ 6.9	6.7	100.0 $\pm$ 7.3	98.8 $\pm$ 7.7
	2.0	104.3 $\pm$ 7.5	7.2	101.0 $\pm$ 8.8	8.7	104.3 $\pm$ 10.0	101.5 $\pm$ 9.7
	15.0	104.7 $\pm$ 7.0	6.7	101.7 $\pm$ 7.7	7.5	99.4 $\pm$ 10.5	104.4 $\pm$ 9.1
Ile	0.2	102.6 $\pm$ 6.8	6.6	100.3 $\pm$ 9.0	9.0	93.0 $\pm$ 6.8	104.2 $\pm$ 7.3
	5.0	101.4 $\pm$ 6.2	6.1	101.8 $\pm$ 4.8	4.7	102.8 $\pm$ 8.4	104.6 $\pm$ 6.8
	40.0	101.8 $\pm$ 7.8	7.7	101.4 $\pm$ 11.2	11.1	98.6 $\pm$ 9.2	102.4 $\pm$ 10.3
Leu	0.2	100.0 $\pm$ 7.5	7.5	102.5 $\pm$ 9.4	9.2	101.5 $\pm$ 8.3	103.3 $\pm$ 11.7
	5.0	98.3 $\pm$ 8.6	8.7	100.8 $\pm$ 12.0	11.9	102.9 $\pm$ 6.3	98.2 $\pm$ 12.3
	40.0	97.7 $\pm$ 9.5	9.7	96.0 $\pm$ 10.6	11.0	95.8 $\pm$ 11.0	95.5 $\pm$ 8.9
Lys	0.2	100.9 $\pm$ 11.0	10.9	95.5 $\pm$ 8.9	9.3	99.2 $\pm$ 9.6	101.1 $\pm$ 8.5

	5.0	102.6 ± 10.0	9.8	104.6 ± 8.4	8.0	101.4 ± 8.0	102.2 ± 9.0
	40.0	104.6 ± 8.4	8.0	103.0 ± 7.7	7.5	96.5 ± 8.6	102.5 ± 8.1
	0.2	102.6 ± 8.7	8.5	105.4 ± 7.7	7.3	96.6 ± 9.3	104.7 ± 7.8
Met	5.0	102.1 ± 9.2	9.0	105.7 ± 6.5	6.1	95.1 ± 8.5	102.4 ± 8.8
	40.0	98.8 ± 8.4	8.5	99.4 ± 5.6	5.7	97.6 ± 9.8	98.2 ± 7.3
	0.5	98.4 ± 8.2	8.4	102.8 ± 7.6	7.4	100.8 ± 7.8	99.7 ± 7.7
Orn	5.0	100.9 ± 8.9	8.9	102.2 ± 5.9	5.7	96.1 ± 9.2	99.2 ± 10.2
	40.0	100.1 ± 9.2	9.2	100.4 ± 10.6	10.5	98.0 ± 9.4	100.2 ± 9.4
	0.2	99.4 ± 9.3	9.4	97.5 ± 5.7	5.9	100.3 ± 6.6	101.9 ± 7.6
Phe	5.0	97.9 ± 8.5	8.7	97.1 ± 9.9	10.2	98.4 ± 7.5	103.7 ± 12.5
	40.0	96.2 ± 8.6	9.0	98.5 ± 13.3	13.5	98.6 ± 5.5	106.1 ± 12.2
	0.2	96.8 ± 8.8	9.1	107.0 ± 8.4	7.9	103.8 ± 7.6	105.8 ± 7.8
Pro	2.0	100.3 ± 9.9	9.9	102.4 ± 7.2	7.1	106.4 ± 6.7	104.9 ± 8.0
	15.0	102.1 ± 8.5	8.3	104.2 ± 10.1	9.7	102.8 ± 8.7	99.9 ± 11.4
	0.5	100.1 ± 8.8	8.8	101.3 ± 10.0	9.9	101.4 ± 11.5	94.7 ± 8.2
Ser	5.0	99.1 ± 8.4	8.5	100.9 ± 8.8	8.8	97.1 ± 12.0	95.9 ± 7.5
	40.0	98.9 ± 9.2	9.3	94.5 ± 7.4	7.8	98.6 ± 9.9	101.0 ± 7.5
	0.2	99.5 ± 9.0	9.0	100.9 ± 8.6	8.5	102.6 ± 8.2	104.9 ± 5.8
Tau	5.0	97.4 ± 7.9	8.1	99.7 ± 8.6	8.6	99.3 ± 9.7	101.7 ± 10.6
	40.0	99.1 ± 9.1	9.2	102.0 ± 11.0	10.8	102.3 ± 8.1	99.7 ± 11.2
	0.2	99.0 ± 9.1	9.2	98.7 ± 9.1	9.3	106.3 ± 3.9	97.4 ± 9.6
Thr	5.0	101.2 ± 8.2	8.1	97.5 ± 8.7	8.9	97.3 ± 8.6	96.0 ± 7.0
	40.0	102.6 ± 7.9	7.7	97.7 ± 9.2	9.4	98.5 ± 7.9	94.6 ± 6.1
	0.2	102.7 ± 8.4	8.2	93.5 ± 8.9	9.5	101.6 ± 9.3	97.4 ± 9.7
Trp	5.0	101.2 ± 9.1	9.0	99.2 ± 9.0	9.1	99.4 ± 8.2	106.6 ± 8.9
	40.0	98.9 ± 9.9	10.0	108.1 ± 6.6	6.1	102.3 ± 9.3	105.7 ± 8.3

Tyr	0.2	98.4 ± 9.2	9.3	99.0 ± 7.3	7.3	96.8 ± 12.6	97.8 ± 6.7
	5.0	98.2 ± 8.7	8.9	93.2 ± 5.4	5.8	94.4 ± 9.7	97.4 ± 6.8
	40.0	101.0 ± 8.3	8.2	105.2 ± 8.6	8.2	105.0 ± 9.6	99.3 ± 6.9
Val	0.2	102.2 ± 7.8	7.7	98.1 ± 9.9	10.1	107.2 ± 6.5	94.4 ± 5.9
	5.0	102.2 ± 7.1	6.9	95.2 ± 6.1	6.4	108.2 ± 4.9	97.8 ± 9.0
	40.0	102.9 ± 6.3	6.1	99.0 ± 8.3	8.4	106.2 ± 10.5	100.9 ± 11.0

Table S4 Baseline characteristics of study participants with NAFLD (n=220).

Characteristics	Control (n=74)	Vigorous exercise (n=73)	Moderate exercise (n=73)
Female, No. (%)	46 (62.2)	52 (71.2)	51 (69.9)
Age, y	54.0 (6.8)	53.2 (7.1)	54.4 (7.4)
High school education, No. (%)	15 (20.3)	23 (31.5)	21 (28.8)
Current cigarette smoking, No. (%)	18 (24.3)	10 (13.7)	17 (23.3)
Current alcohol drinking, No. (%)	26 (35.1)	20 (27.4)	21 (28.8)
Physical activity, METs/wk	29.1 (9.3)	31.7 (8.5)	30.9 (11.1)
Total energy intake, kcal/d	2140.7 (458.0)	2124.8 (454.4)	2125.6 (454.4)
Fat intake, %	33.3 (6.9)	31.9 (7.3)	32.7 (6.3)
Waist circumference, cm	96.1 (6.9)	95.2 (7.4)	95.7 (6.7)
Weight, kg	72.1 (8.5)	71.7 (10.1)	71.1 (10.1)
BMI	28.0 (2.7)	27.9 (2.7)	28.1 (3.3)
Heart rate, /min	81.7 (12.1)	80.8 (11.1)	80.9 (9.7)
Systolic blood pressure, mm Hg	134.7 (16.7)	132.1 (15.6)	131.7 (12.8)
Diastolic blood pressure, mm Hg	80.8 (10.6)	79.7 (9.9)	81.0 (8.5)
Plasma glucose, mg/dL	103.5 (9.1)	102.6 (10.9)	104.0 (9.3)
Serum triglycerides, median (IQR), mg/dL	161.1 (126.5-225.7)	165.5 (112.4-212.4)	161.1 (123.0-202.7)

Serum total cholesterol, mg/dL	232.3 (35.0)	225.6 (44.8)	239.2 (43.0)
HDL-C, mg/dL	49.2 (10.8)	48.3 (9.2)	50.3 (9.4)
LDL-C, mg/dL	144.4 (34.5)	137.9 (44.3)	146.7 (36.9)
Visceral fat, cm ²	133.8 (43.2)	140.9 (41.9)	135.5 (42.6)
Subcutaneous fat, cm ²	240.8 (80.8)	241.4 (72.6)	225.7 (70.2)
Body fat, %	33.7 (7.1)	34.8 (5.3)	33.6 (5.5)
Intrahepatic triglyceride content, %	17.5 (11.0)	18.4 (9.9)	18.0 (9.9)

Data are presented as mean (SD) unless otherwise indicated.

$P > 0.05$ for all difference among the characters.

Abbreviations: BMI, body mass index (calculated as the weight in kilograms divided by height in meters squared); HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; METs, metabolic equivalents.

Table S5 Clinical characteristics changes after 6 month exercise training on NAFLD participants.

Outcomes	Change (95% CIs)			P Value	Moderate vs Control	Moderate vs Vigorous
	Control	Vigorous exercise	Moderate exercise			
Weight, kg	-1.49 (-2.1 to -0.88)	-4.33 (-4.96 to -3.7)	-2.02 (-2.65 to -1.40)	<0.001	0.23	<0.001
Waist circumference, cm	-1.35 (-2.18 to -0.52)	-4.87 (-5.74 to -4.01)	-2.26 (-3.12 to -1.40)	<0.001	<0.001	0.21
Body fat mass, kg	-0.83 (-1.26 to -0.41)	-2.48 (-2.92 to -2.04)	-1.16 (-1.60 to -0.73)	<0.001	0.29	<0.001
Body fat, %	-0.82 (-1.25 to -0.4)	-2.29 (-2.74 to -1.84)	-0.85 (-1.29 to -0.41)	<0.001	0.93	<0.001
Visceral fat, cm ²	-17.0 (-22.7 to -11.2)	-27.5 (-33.5 to -21.6)	-13.1 (-19.1 to -7.2)	0.01	0.36	<0.001
Subcutaneous fat, cm ²	0.7 (-5.4 to 6.7)	-13.4 (-19.7 to -7.1)	-10.5 (-16.8 to -4.2)	0.002	0.01	0.52
Systolic blood pressure, mm Hg	-3.9 (-5.9 to -2.0)	-10.1 (-12.2 to -8.1)	-5.0 (-7.0 to -3.0)	<0.001	0.46	<0.001

Diastolic blood pressure, mm Hg	-1.7 (-3.2 to -0.2)	-5.9 (-7.4 to -4.3)	-2.8 (-4.3 to -1.3)	<0.001	0.32	0.06
Plasma glucose, mg/dL	-7.4 (-9.2 to -5.6)	-5.9 (-7.8 to -4.1)	-4.4 (-6.2 to -2.6)	0.25	0.02	0.25
Serum triglycerides, mg/dL	-18.0 (-32.5 to -3.5)	-26.3 (-41.5 to -11.1)	-19.8 (-34.9 to -4.8)	0.44	0.87	0.55
serum total cholesterol, mg/dL	-15.3 (-21.0 to -9.5)	-13.0 (-19.0 to -7.0)	-14.0 (-19.9 to -8.0)	0.59	0.75	0.82
HDL-C, mg/dL	-0.7 (-2.0 to 0.6)	0.5 (-0.9 to 1.9)	1.0 (-0.3 to 2.4)	0.22	0.07	0.59
LDL-C, mg/dL	-9.8 (-15.2 to -4.5)	-7.4 (-13.0 to 1.8)	-6.2 (-11.7 to -0.6)	0.53	0.35	0.76
ALT, U/L	-3.3 (-5.9 to -0.8)	-4.1 (-6.8 to -1.5)	-2.7 (-5.3 to -0.1)	0.67	0.73	0.45
AST, U/L	0.2 (-1.1 to 1.5)	1.8 (0.4 to 3.2)	-1.3 (-2.7 to 0.1)	0.11	0.12	0.002
γ-Glutamyltransferase, U/L	-1.3 (-4.8 to 2.2)	-0.4 (-4.0 to 3.2)	-3.8 (-7.4 to -0.2)	0.73	0.32	0.19
Intrahepatic triglyceride content, %	-2.2 (-3.7 to -0.7)	-7.2 (-8.7 to -5.6)	-6.3 (-7.9 to -4.8)	<0.001	<0.001	0.45