**LC-MS condition**

LC-ESI-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 (2.1100 mm, 1.8 μm, Waters, Milford, USA) at 30 °C. The mobile phase consisting of water with 2.5 mM [ammonium](javascript:;) [formate](javascript:;) 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 Fig. 1

Supplement Table 1 MS/MS conditions and retention time of IS and AAs in positive ion mode.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Analytes | Precursor Ion (*m/z*) | Product Ion (*m/z*) | 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 |
| Tau | 126.0 | 108.0 | 98 | 6 | 7 | 2.76 |
| 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 |

**C:\厦门大学附属第一医院\在开展的课题\运动干预NAFLD的定量分析\AA\20191111\paper\Supplement Fig.1.tif**

Supplement Fig. 1 Chromatogram of eighteen AAs in human plasma on positive ion mode.

**Validation of LC-MS/MS Method**

Calibration curve showed good linearity (r2 > 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 2. Data of accuracy, intra-day/inter-day precision, recovery and matrix effect were summarized in Supplement Table 3.

Supplement Table 2 Linearity range, LLOD and LLOQ of AAs in human plasma.

|  |  |  |  |
| --- | --- | --- | --- |
| Analytes | Range (μg/mL) | LLOD (μg/mL) | LLOQ (μg/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 |

Supplement Table 3 Accuracy, intra-day/inter-day precision, recovery and matrix effect of AAs in human plasma (data were represented as mean ± standard deviation).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Analytes | Nominal Cone. | Intra-day precision (n=6) | | Inter-day precision (n=18) | | Recovery  (%，n=6) | Matrix Effect  (%，n=6) |
| (μg/mL) | Accuracy (%) | RSD (%) | Accuracy (%) | RSD (%) |
| Ala | 0.5 | 100.4 ± 9.7 | 9.7 | 103.0 ± 7.9 | 7.6 | 103.1 ± 11.3 | 104.5 ± 8.1 |
| 5.0 | 101.6 ± 9.7 | 9.6 | 97.8 ± 9.3 | 9.5 | 100.2 ± 7.8 | 98.3 ± 12.8 |
| 40.0 | 103.8 ± 7.2 | 6.9 | 99.0 ± 11.8 | 11.9 | 103.1 ± 7.8 | 100.6 ± 13.1 |
| Arg | 0.2 | 106.6 ± 5.7 | 5.3 | 103.1 ± 10.4 | 10.1 | 97.4 ± 7.6 | 103.3 ± 10.7 |
| 2.0 | 104.3 ± 8.2 | 7.8 | 99.6 ± 8.7 | 8.8 | 97.4 ± 11.1 | 100.5 ± 8.5 |
| 15.0 | 104.7 ± 8.3 | 8.0 | 103.2 ± 8.8 | 8.5 | 93.5 ± 9.0 | 103.4 ± 7.9 |
| Gln | 0.5 | 101.3 ± 7.0 | 6.9 | 103.6 ± 6.1 | 5.9 | 97.0 ± 11.2 | 104.9 ± 6.0 |
| 5.0 | 101.1 ± 8.3 | 8.2 | 109.5 ± 2.9 | 2.6 | 97.7 ± 7.2 | 103.1 ± 8.6 |
| 80.0 | 101.9 ± 8.4 | 8.2 | 99.8 ± 11.1 | 11.1 | 96.9 ± 11.5 | 98.6 ± 6.7 |
| Gly | 0.5 | 101.6 ± 10.3 | 10.1 | 104.7 ± 6.9 | 6.6 | 95.7 ± 5.3 | 102.5 ± 5.9 |
| 5.0 | 99.8 ± 10.5 | 10.5 | 100.9 ± 7.9 | 7.8 | 104.2 ± 8.6 | 100.8 ± 7.6 |
| 40.0 | 98.7 ± 9.8 | 9.9 | 94.3 ± 6.3 | 6.7 | 103.6 ± 8.7 | 97.6 ± 5.3 |
| His | 0.2 | 101.0 ± 8.8 | 8.7 | 102.4 ± 6.9 | 6.7 | 100.0 ± 7.3 | 98.8 ± 7.7 |
| 2.0 | 104.3 ± 7.5 | 7.2 | 101.0 ± 8.8 | 8.7 | 104.3 ± 10.0 | 101.5 ± 9.7 |
| 15.0 | 104.7 ± 7.0 | 6.7 | 101.7 ± 7.7 | 7.5 | 99.4 ± 10.5 | 104.4 ± 9.1 |
| Ile | 0.2 | 102.6 ± 6.8 | 6.6 | 100.3 ± 9.0 | 9.0 | 93.0 ± 6.8 | 104.2 ± 7.3 |
| 5.0 | 101.4 ± 6.2 | 6.1 | 101.8 ± 4.8 | 4.7 | 102.8 ± 8.4 | 104.6 ± 6.8 |
| 40.0 | 101.8 ± 7.8 | 7.7 | 101.4 ± 11.2 | 11.1 | 98.6 ± 9.2 | 102.4 ± 10.3 |
| Leu | 0.2 | 100.0 ± 7.5 | 7.5 | 102.5 ± 9.4 | 9.2 | 101.5 ± 8.3 | 103.3 ± 11.7 |
| 5.0 | 98.3 ± 8.6 | 8.7 | 100.8 ± 12.0 | 11.9 | 102.9 ± 6.3 | 98.2 ± 12.3 |
| 40.0 | 97.7 ± 9.5 | 9.7 | 96.0 ± 10.6 | 11.0 | 95.8 ± 11.0 | 95.5 ± 8.9 |
| Lys | 0.2 | 100.9 ± 11.0 | 10.9 | 95.5 ± 8.9 | 9.3 | 99.2 ± 9.6 | 101.1 ± 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 |
| Met | 0.2 | 102.6 ± 8.7 | 8.5 | 105.4 ± 7.7 | 7.3 | 96.6 ± 9.3 | 104.7 ± 7.8 |
| 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 |
| Orn | 0.5 | 98.4 ± 8.2 | 8.4 | 102.8 ± 7.6 | 7.4 | 100.8 ± 7.8 | 99.7 ± 7.7 |
| 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 |
| Phe | 0.2 | 99.4 ± 9.3 | 9.4 | 97.5 ± 5.7 | 5.9 | 100.3 ± 6.6 | 101.9 ± 7.6 |
| 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 |
| Pro | 0.2 | 96.8 ± 8.8 | 9.1 | 107.0 ± 8.4 | 7.9 | 103.8 ± 7.6 | 105.8 ± 7.8 |
| 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 |
| Ser | 0.5 | 100.1 ± 8.8 | 8.8 | 101.3 ± 10.0 | 9.9 | 101.4 ± 11.5 | 94.7 ± 8.2 |
| 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 |
| Tau | 0.2 | 99.5 ± 9.0 | 9.0 | 100.9 ± 8.6 | 8.5 | 102.6 ± 8.2 | 104.9 ± 5.8 |
| 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 |
| Thr | 0.2 | 99.0 ± 9.1 | 9.2 | 98.7 ± 9.1 | 9.3 | 106.3 ± 3.9 | 97.4 ± 9.6 |
| 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 |
| Trp | 0.2 | 102.7 ± 8.4 | 8.2 | 93.5 ± 8.9 | 9.5 | 101.6 ± 9.3 | 97.4 ± 9.7 |
| 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 |

Supplement Table 4 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, cm2 | 133.8 (43.2) | 140.9 （41.9） | 135.5 (42.6) |
| Subcutaneous fat, cm2 | 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.

Supplement Table 5 Clinical characteristics changes after 6 month exercise training on NAFLD participants.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Outcomes | Change (95% CIs) | | |  | P Value |  |
| Control | Vigorous exercise | Moderate exercise | Vigorous *vs* Control | Moderate *vs* Control | Moderate *vs* Vigorous |
| 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, cm2 | -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, cm2 | 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 |