

**Supplementary Table S1: Data acquisition parameters of MRM method for analysis of amino acids and acylcarnitines.**

| <b>Analytes</b> | <b>MRM (Analyte)</b> | <b>Internal Standard</b>   | <b>MRM (Internal Standard)</b> | <b>Target Dwell Time (msec)</b> | <b>Target Q1 Pre Bias (V)</b> | <b>Target Collision Energy (V)</b> | <b>Target Q3 Pre Bias (V)</b> |
|-----------------|----------------------|--|--------------------------------|---------------------------------|-------------------------------|------------------------------------|-------------------------------|
| <b>Ala</b>      | 90.1→44.2            | <sup>2</sup> H <sub>4</sub> -Ala                                 | 94.1→48.2                      | 5                               | -11                           | -13                                | -19                           |
| <b>Arg</b>      | 175.1→70.1           | <sup>2</sup> H <sub>4</sub> - <sup>13</sup> C-Arg                | 180.1→75.1                     | 15                              | -13                           | -24                                | -29                           |
| <b>Cit</b>      | 176.1→70.1           | <sup>2</sup> H <sub>2</sub> -Cit                                 | 178.1→72.1                     | 5                               | -13                           | -25                                | -28                           |
| <b>Xle</b>      | 132.1→86.1           | <sup>2</sup> H <sub>3</sub> -Leu                                 | 135.1→89.1                     | 5                               | -10                           | -12                                | -16                           |
| <b>Lys</b>      | 147.1→84.1           | <sup>13</sup> C <sub>6</sub> - <sup>15</sup> N <sub>2</sub> -Lys | 155.1→90.1                     | 10                              | -11                           | -18                                | -16                           |
| <b>Met</b>      | 150.2→61.2           | <sup>2</sup> H <sub>3</sub> -Met                                 | 153.2→64.2                     | 5                               | -11                           | -25                                | -26                           |
| <b>Orn</b>      | 133.2→70.2           | <sup>2</sup> H <sub>6</sub> -Orn                                 | 139.2→76.2                     | 10                              | -10                           | -23                                | -29                           |
| <b>Phe</b>      | 166.1→120.1          | <sup>13</sup> C <sub>6</sub> -Phe                                | 172.1→126.1                    | 5                               | -12                           | -16                                | -12                           |
| <b>Pro</b>      | 116.2→70.2           | <sup>2</sup> H <sub>5</sub> -Pro                                 | 121.2→74.2                     | 5                               | -14                           | -18                                | -28                           |
| <b>Ser</b>      | 106.1→60.1           | <sup>13</sup> C <sub>3</sub> -Ser                                | 109.1→62.1                     | 5                               | -12                           | -14                                | -24                           |
| <b>Tyr</b>      | 182.1→91.1           | <sup>13</sup> C <sub>6</sub> -Tyr                                | 188.1→142.1                    | 5                               | -13                           | -15                                | -27                           |
| <b>Val</b>      | 118.1→72.2           | <sup>2</sup> H <sub>8</sub> -Val                                 | 126.1→80.2                     | 5                               | -14                           | -14                                | -14                           |
| <b>C0</b>       | 162.1→85             | <sup>2</sup> H <sub>9</sub> -C0                                  | 171.1→85                       | 5                               | -12                           | -23                                | -15                           |
| <b>C2</b>       | 204.1→85             | <sup>2</sup> H <sub>3</sub> -C2                                  | 207.1→85                       | 5                               | -10                           | -22                                | -14                           |
| <b>C3</b>       | 218.1→85             | <sup>2</sup> H <sub>3</sub> -C3                                  | 221.1→85                       | 5                               | -11                           | -23                                | -15                           |
| <b>C4</b>       | 232.2→85             | <sup>2</sup> H <sub>3</sub> -C4                                  | 235.2→85                       | 5                               | -12                           | -23                                | -15                           |
| <b>C5</b>       | 246.2→85             | <sup>2</sup> H <sub>9</sub> -C5                                  | 255.2→85                       | 5                               | -13                           | -23                                | -15                           |
| <b>C5:1</b>     | 244.2→85             | <sup>2</sup> H <sub>9</sub> -C5                                  | 255.2→85                       | 5                               | -13                           | -23                                | -15                           |
| <b>C5DC</b>     | 276.2→85             | <sup>2</sup> H <sub>3</sub> -C5DC                                | 279.2→85                       | 10                              | -13                           | -25                                | -16                           |

|                |          |                                  |          |   |     |     |     |
|----------------|----------|----------------------------------|----------|---|-----|-----|-----|
| <b>C5OH</b>    | 262.2→85 | <sup>2</sup> H <sub>9</sub> -C5  | 255.2→85 | 5 | -13 | -23 | -15 |
| <b>C6</b>      | 260.2→85 | <sup>2</sup> H <sub>3</sub> -C6  | 263.2→85 | 5 | -10 | -24 | -15 |
| <b>C8</b>      | 288.2→85 | <sup>2</sup> H <sub>3</sub> -C8  | 291.2→85 | 5 | -11 | -23 | -15 |
| <b>C10</b>     | 316.2→85 | <sup>2</sup> H <sub>3</sub> -C10 | 319.2→85 | 5 | -12 | -25 | -15 |
| <b>C10:1</b>   | 314.2→85 | <sup>2</sup> H <sub>3</sub> -C10 | 319.2→85 | 5 | -12 | -25 | -15 |
| <b>C10:2</b>   | 312.2→85 | <sup>2</sup> H <sub>3</sub> -C10 | 319.2→85 | 5 | -12 | -25 | -15 |
| <b>C12</b>     | 344.2→85 | <sup>2</sup> H <sub>3</sub> -C12 | 347.2→85 | 5 | -13 | -27 | -14 |
| <b>C14</b>     | 372.2→85 | <sup>2</sup> H <sub>9</sub> -C14 | 381.2→85 | 5 | -14 | -28 | -15 |
| <b>C14:1</b>   | 370.2→85 | <sup>2</sup> H <sub>9</sub> -C14 | 381.2→85 | 5 | -14 | -28 | -15 |
| <b>C14:2</b>   | 368.2→85 | <sup>2</sup> H <sub>9</sub> -C14 | 381.2→85 | 5 | -14 | -28 | -15 |
| <b>C16</b>     | 400.3→85 | <sup>2</sup> H <sub>3</sub> -C16 | 403.3→85 | 5 | -15 | -28 | -15 |
| <b>C16OH</b>   | 416.3→85 | <sup>2</sup> H <sub>3</sub> -C16 | 403.3→85 | 5 | -15 | -28 | -15 |
| <b>C16:1OH</b> | 414.3→85 | <sup>2</sup> H <sub>3</sub> -C16 | 403.3→85 | 5 | -15 | -28 | -15 |
| <b>C18</b>     | 428.3→85 | <sup>2</sup> H <sub>3</sub> -C18 | 431.3→85 | 5 | -16 | -30 | -15 |
| <b>C18:1</b>   | 426.3→85 | <sup>2</sup> H <sub>3</sub> -C18 | 431.3→85 | 5 | -16 | -30 | -15 |
| <b>C18OH</b>   | 444.3→85 | <sup>2</sup> H <sub>3</sub> -C18 | 431.3→85 | 5 | -16 | -30 | -15 |
| <b>C18:1OH</b> | 442.3→85 | <sup>2</sup> H <sub>3</sub> -C18 | 431.3→85 | 5 | -16 | -30 | -15 |

Abbreviations: MRM, Multiple Reaction Monitoring; MRM transitions: precursor ion → target ion; Ala, alanine; Arg, arginine; Cit, citrulline; Xle, (Leucine and isoleucine ); Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Tyr, tyrosine; Val, valine; C0, free carnitine; C2, acetylcarnitine; C3, propionylcarnitine; C4 butyryl-/isobutyrylcarnitine; C5, isovaleryl-/2-methylbutyrylcarnitine, C5:1, tiglylcarnitine; C5DC, glutarylcarnitine; C5OH, hydroxy isovalerylcarnitine; C6, hexanoylcarnitine; C8, octanoylcarnitine; C10, decanoylcarnitine; C10:1, decenoylcarnitine; C10:2, decadienoylcarnitine; C12, dodecanoylcarnitine; C14, tetradecanoylcarnitine; C14:1, tetradecenoylcarnitine; C14:2, tetradecadienoylcarnitine; C16, palmitoylcarnitine; C16OH, hydroxy palmitoylcarnitine; C16:1OH, hydroxy hexadecenoylcarnitine; C18, stearyl carnitine; C18:1, oleylcarnitine; C18OH, hydroxy stearyl carnitine; C18:1OH, hydroxy oleylcarnitine.

**Supplementary Table S2: Intra-assay precision and accuracy analysis of LC-MS/MS method for amino acids and acylcarnitines using low, medium and high control specimens.**

| Analytes | Low Control     |                           |                    | Medium Control  |                           |                    | High Control    |                           |                    | Average intra-assay CV (%) | Average RE (%) |
|----------|-----------------|---------------------------|--------------------|-----------------|---------------------------|--------------------|-----------------|---------------------------|--------------------|----------------------------|----------------|
|          | Target (µmol/L) | Intra assay mean (µmol/L) | Intra assay CV (%) | Target (µmol/L) | Intra assay mean (µmol/L) | Intra assay CV (%) | Target (µmol/L) | Intra assay mean (µmol/L) | Intra assay CV (%) |                            |                |
| Ala      | 149.6           | 128.54                    | 16.72              | 465.1           | 357.41                    | 20.34              | 767             | 595.87                    | 13.25              | 16.77                      | -19.85         |
| Arg      | 38.7            | 32.2                      | 5.46               | 100.6           | 102.87                    | 2.43               | 374.6           | 467.33                    | 0.95               | 2.95                       | 3.41           |
| Cit      | 34              | 30.78                     | 14.82              | 77.5            | 75.669                    | 9.84               | 282.6           | 322.15                    | 12.01              | 12.22                      | 0.73           |
| Xle      | 160             | 162.61                    | 7.34               | 483.4           | 493.84                    | 7.17               | 767.2           | 814.62                    | 4.75               | 6.42                       | 3.32           |
| Lys      | 80.8            | 71                        | 4.63               | 123.9           | 121.37                    | 3.89               | 332.9           | 353.93                    | 2.66               | 3.73                       | -2.62          |
| Met      | 35.7            | 38.54                     | 12.37              | 117             | 110.95                    | 9.08               | 506.6           | 475.56                    | 5.47               | 8.97                       | -1.11          |
| Orn      | 57              | 40.4                      | 10.1               | 110             | 99.68                     | 6.99               | 351.5           | 389.29                    | 3.77               | 6.95                       | 9.33           |
| Phe      | 55.9            | 57.74                     | 7.08               | 121.3           | 123.17                    | 4.89               | 425             | 441                       | 3.97               | 5.31                       | 2.87           |
| Pro      | 123.7           | 124.87                    | 3.14               | 401.5           | 357.17                    | 5.13               | 684.6           | 656.88                    | 3.26               | 3.84                       | -4.72          |
| Ser      | 168.6           | 153.2                     | 23.68              | 533.1           | 566.67                    | 16.07              | 892.8           | 897.62                    | 9.03               | 16.26                      | -0.77          |
| Tyr      | 106.4           | 115.37                    | 33.8               | 283.2           | 280.34                    | 12.7               | 501.5           | 440.17                    | 22.94              | 23.15                      | -1.6           |
| Val      | 88.1            | 88.37                     | 11.69              | 182.4           | 173.55                    | 9.22               | 589.5           | 557.49                    | 4.44               | 8.45                       | -3.33          |
| C0       | 20.57           | 20.96                     | 12.82              | 49.5            | 51.39                     | 6.8                | 90.6            | 94.45                     | 5.24               | 8.29                       | 3.32           |
| C2       | 24.97           | 23.63                     | 2.92               | 67.5            | 69.66                     | 4.42               | 127.7           | 137.27                    | 4.86               | 4.07                       | 1.77           |
| C3       | 3.6             | 3.65                      | 3.79               | 11.2            | 11.62                     | 6.4                | 21.8            | 22.43                     | 3.82               | 4.67                       | 2.72           |
| C4       | 0.16            | 0.15                      | 9.91               | 0.76            | 0.79                      | 4.18               | 1.5             | 1.67                      | 5.21               | 6.43                       | 2.21           |
| C5       | 0.25            | 0.23                      | 15.29              | 1.49            | 1.64                      | 8.26               | 3.06            | 3.57                      | 5.1                | 9.55                       | 6.93           |
| C6       | 0.08            | 0.08                      | 37.06              | 0.65            | 0.67                      | 3.61               | 1.35            | 1.46                      | 5.73               | 15.47                      | 3.93           |
| C8       | 0.13            | 0.1                       | 90.58              | 1.01            | 1.18                      | 10.47              | 2.12            | 2.55                      | 6.15               | 35.73                      | 5.03           |
| C10      | 0.06            | 0.08                      | 25.58              | 0.46            | 0.46                      | 5.31               | 0.96            | 0.94                      | 5.7                | 12.20                      | 9.00           |
| C12      | 0.06            | 0.07                      | 21.11              | 0.43            | 0.42                      | 6.71               | 0.91            | 0.83                      | 5.03               | 10.95                      | 1.81           |
| C14      | 0.12            | 0.11                      | 8.43               | 0.45            | 0.43                      | 7.25               | 0.87            | 0.82                      | 4.44               | 6.71                       | -6.46          |
| C16      | 3.85            | 3.78                      | 1.93               | 9.24            | 9.76                      | 2.63               | 16.67           | 17.76                     | 1.49               | 2.02                       | 3.45           |
| C18      | 0.97            | 0.98                      | 3.42               | 1.93            | 2.06                      | 2.8                | 3.32            | 3.49                      | 4.06               | 3.43                       | 4.29           |

Abbreviations: RE, relative error; Ala, alanine; Arg, arginine; Cit, citrulline; Xle, (Leucine and isoleucine ); Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanin; Pro, proline; Ser, serine; Tyr, tyrosine; Val, valine; C0, free carnitine; C2, acetylcarnitine; C3, propionylcarnitine; C4 butyryl-/isobutyrylcarnitine; C5, isovaleryl-/2-methylbutyrylcarnitine; C6, hexanoylcarnitine; C8, octanoylcarnitine; C10, decanoylcarnitine; C12, dodecanoylcarnitine; C14, tetradecanoylcarnitine; C16, palmitoylcarnitine and C18, stearyl carnitine.

**Supplementary Table S3: Inter-assay precision and accuracy analysis of LC-MS/MS method for amino acids, free carnitine and acylcarnitines using low, medium and high control specimens.**

| Analytes | Low Control     |                           |                    | Medium Control  |                           |                    | High Control    |                           |                    | Average intra-assay CV (%) | Average RE (%) |
|----------|-----------------|---------------------------|--------------------|-----------------|---------------------------|--------------------|-----------------|---------------------------|--------------------|----------------------------|----------------|
|          | Target (µmol/L) | Inter assay mean (µmol/L) | Inter assay CV (%) | Target (µmol/L) | Inter assay mean (µmol/L) | Inter assay CV (%) | Target (µmol/L) | Inter assay mean (µmol/L) | Inter assay CV (%) |                            |                |
| Ala      | 149.6           | 129.56                    | 6.85               | 465.1           | 361.5                     | 14.4               | 767             | 596.23                    | 2.3                | 7.85                       | -19.31         |
| Arg      | 38.7            | 32.37                     | 4.64               | 100.6           | 102.86                    | 0.85               | 374.6           | 467.37                    | 0.42               | 1.97                       | 3.55           |
| Cit      | 34              | 30.93                     | 7.31               | 77.5            | 76.15                     | 5.74               | 282.6           | 322.81                    | 1.78               | 4.94                       | 1.15           |
| Xle      | 160             | 163.23                    | 4.63               | 483.4           | 492.23                    | 3.71               | 767.2           | 812.81                    | 1.98               | 3.44                       | 3.26           |
| Lys      | 80.8            | 71.12                     | 1.58               | 123.9           | 121.1                     | 1.94               | 332.9           | 353.81                    | 0.45               | 1.32                       | -2.65          |
| Met      | 35.7            | 38.51                     | 3.38               | 117             | 111.28                    | 2.57               | 506.6           | 475.17                    | 3.97               | 3.31                       | -1.07          |
| Orn      | 57              | 40.67                     | 6.2                | 110             | 99.88                     | 2.16               | 351.5           | 390.43                    | 2.52               | 3.63                       | -9             |
| Phe      | 55.9            | 57.53                     | 4.31               | 121.3           | 122.54                    | 5.17               | 425             | 442.09                    | 2.35               | 3.94                       | 2.65           |
| Pro      | 123.7           | 124.71                    | 1.59               | 401.5           | 357.15                    | 4                  | 684.6           | 657.23                    | 1.53               | 2.37                       | -4.74          |
| Ser      | 168.6           | 156.31                    | 18.21              | 533.1           | 571.77                    | 10.1               | 892.8           | 894.98                    | 6.48               | 11.60                      | 0.07           |
| Tyr      | 106.4           | 117.31                    | 15.05              | 283.2           | 281.14                    | 7.13               | 501.5           | 436.47                    | 8                  | 10.06                      | -1.15          |
| Val      | 88.1            | 88.29                     | 2.36               | 182.4           | 173.89                    | 7.49               | 589.5           | 556.5                     | 2.71               | 4.19                       | -3.56          |
| C0       | 20.57           | 21.04                     | 6.36               | 49.5            | 51.6                      | 3.93               | 90.6            | 94.36                     | 1.06               | 3.78                       | 3.56           |
| C2       | 24.97           | 23.64                     | 1.19               | 67.5            | 69.58                     | 1.75               | 127.7           | 137.58                    | 1.97               | 1.64                       | 1.83           |
| C3       | 3.6             | 3.65                      | 1.08               | 11.2            | 11.62                     | 5.14               | 21.8            | 22.42                     | 0.88               | 2.37                       | 2.67           |
| C4       | 0.16            | 0.14                      | 9.17               | 0.76            | 0.79                      | 2.17               | 1.5             | 1.67                      | 2.05               | 4.46                       | 1.83           |
| C5       | 0.25            | 0.23                      | 8.64               | 1.49            | 1.64                      | 1.74               | 3.06            | 3.58                      | 2.98               | 4.45                       | 6.66           |
| C6       | 0.08            | 0.08                      | 25.33              | 0.65            | 0.67                      | 1                  | 1.35            | 1.46                      | 0.41               | 8.91                       | 3.45           |
| C8       | 0.13            | 0.1                       | 27.9               | 1.01            | 1.17                      | 11.05              | 2.12            | 2.56                      | 3.47               | 14.14                      | 4.82           |
| C10      | 0.06            | 0.08                      | 24.51              | 0.46            | 0.46                      | 3.7                | 0.96            | 0.94                      | 4.21               | 10.81                      | 8.36           |
| C12      | 0.06            | 0.07                      | 12.59              | 0.43            | 0.42                      | 1.74               | 0.91            | 0.83                      | 4.15               | 6.16                       | 2.19           |
| C14      | 0.12            | 0.11                      | 2.93               | 0.45            | 0.43                      | 5.02               | 0.87            | 0.82                      | 2.53               | 3.49                       | -6.61          |
| C16      | 3.85            | 3.78                      | 1.25               | 9.24            | 9.74                      | 1.69               | 16.67           | 17.7                      | 0.53               | 1.16                       | 3.38           |
| C18      | 0.97            | 0.98                      | 2.53               | 1.93            | 2.06                      | 0.51               | 3.32            | 3.48                      | 3.24               | 1.88                       | 4.08           |

Abbreviations: RE, relative error; Ala, alanine; Arg, arginine; Cit, citrulline; Xle, (Leucine and isoleucine ); Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanin; Pro, proline; Ser, serine; Tyr, tyrosine; Val, valine; C0, free carnitine; C2, acetylcarnitine; C3, propionylcarnitine; C4 butyryl-/isobutyrylcarnitine; C5, isovaleryl-/2-methylbutyrylcarnitine; C6, hexanoylcarnitine; C8, octanoylcarnitine; C10, decanoylcarnitine; C12, dodecanoylcarnitine; C14, tetradecanoylcarnitine; C16, palmitoylcarnitine and C18, stearylcarnitine.

**Supplementary Table S4: Linearity, Limit of detection (LOD), Limit of quantitation (LOQ) and recovery analysis of LC-MS/MS method for amino acids and acylcarnitines using low, medium and high control specimens.**

| Analytes | R <sup>2</sup> | y-Intercept | Slope | LOD    | LOQ    | Recovery (%) |
|----------|----------------|-------------|-------|--------|--------|--------------|
| Ala      | 0.9997         | 14.38       | 0.76  | 48.53  | 147.05 | 80.68        |
| Arg      | 0.9995         | -22.80      | 1.31  | 28.91  | 87.62  | 103.54       |
| Cit      | 0.9996         | -12.12      | 1.18  | 19.61  | 59.42  | 101.15       |
| Leu      | 0.9991         | -13.02      | 1.07  | 91.65  | 277.72 | 103.26       |
| Lys      | 1.0000         | -18.48      | 1.12  | 7.10   | 21.51  | 97.34        |
| Met      | 1.0000         | 4.07        | 0.93  | 10.40  | 31.50  | 98.92        |
| Orn      | 0.9999         | -29.09      | 1.19  | 13.29  | 40.27  | 90.99        |
| Phe      | 0.9999         | -2.40       | 1.05  | 12.09  | 36.64  | 102.65       |
| Pro      | 0.9954         | -3.31       | 0.95  | 179.85 | 545.01 | 95.25        |
| Ser      | 0.9954         | -3.31       | 0.95  | 179.85 | 545.01 | 100.06       |
| Tyr      | 0.9942         | 39.47       | 0.80  | 147.06 | 445.65 | 98.85        |
| Val      | 1.0000         | 4.69        | 0.94  | 11.02  | 33.38  | 96.65        |
| C0       | 1.0000         | -0.38       | 1.05  | 1.47   | 4.45   | 103.55       |
| C2       | 0.9998         | -4.56       | 1.11  | 5.97   | 18.08  | 101.83       |
| C3       | 0.9999         | -0.01       | 1.03  | 0.66   | 1.99   | 102.66       |
| C4       | 0.9993         | -0.06       | 1.14  | 0.15   | 0.44   | 101.82       |
| C5       | 0.9995         | -0.09       | 1.19  | 0.26   | 0.80   | 106.65       |
| C6       | 0.9995         | -0.02       | 1.09  | 0.12   | 0.37   | 103.44       |
| C8       | 0.9999         | -0.07       | 1.24  | 0.06   | 0.20   | 104.82       |
| C10      | 1.0000         | 0.02        | 0.96  | 0.01   | 0.03   | 108.35       |
| C12      | 0.9994         | 0.02        | 0.89  | 0.10   | 0.29   | 102.18       |
| C14      | 0.9997         | 0.00        | 0.95  | 0.04   | 0.12   | 93.37        |
| C16      | 0.9999         | -0.36       | 1.08  | 0.57   | 1.72   | 103.37       |
| C18      | 0.9992         | -0.03       | 1.06  | 0.35   | 1.07   | 104.08       |

Abbreviations: Ala, alanine; Arg, arginine; Cit, citrulline; Xle, (Leucine and isoleucine ); Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanin; Pro, proline; Ser, serine; Tyr, tyrosine; Val, valine; C0, free carnitine; C2, acetylcarnitine; C3, propionylcarnitine; C4 butyryl-/isobutyrylcarnitine; C5, isovaleryl-/2-methylbutyrylcarnitine; C6, hexanoylcarnitine; C8, octanoylcarnitine; C10, decanoylcarnitine; C12, dodecanoylcarnitine; C14, tetradecanoylcarnitine; C16, palmitoylcarnitine and C18, stearyl carnitine; R<sup>2</sup>, Coefficient of determination; LOD, Limit of detection and LOQ, Limit of quantitation

**Supplementary Table S5: Percentile distribution of amino acids, acylcarnitines and related ratios in different age groups of healthy participants.**

|                         | Group A     |        |        | Group B     |        |        | Group C     |        |        |
|-------------------------|-------------|--------|--------|-------------|--------|--------|-------------|--------|--------|
|                         | Percentiles |        |        | Percentiles |        |        | Percentiles |        |        |
|                         | 2.50        | 50.00  | 97.50  | 2.50        | 50.00  | 97.50  | 2.50        | 50.00  | 97.50  |
| <b>Amino acids</b>      |             |        |        |             |        |        |             |        |        |
| <b>Ala</b>              | 74.36       | 136.60 | 282.85 | 88.18       | 190.95 | 339.86 | 111.76      | 205.15 | 405.78 |
| <b>Arg</b>              | 0.50        | 4.57   | 21.39  | 3.26        | 19.51  | 74.98  | 4.26        | 29.78  | 95.36  |
| <b>Cit</b>              | 4.68        | 9.78   | 17.17  | 11.51       | 20.83  | 35.91  | 13.67       | 24.27  | 39.74  |
| <b>Xle</b>              | 70.02       | 114.62 | 200.85 | 75.68       | 128.43 | 201.74 | 102.02      | 155.75 | 220.69 |
| <b>Lys</b>              | 57.34       | 166.53 | 416.22 | 57.39       | 201.54 | 295.92 | 67.03       | 237.56 | 379.11 |
| <b>Met</b>              | 7.51        | 17.96  | 42.38  | 10.61       | 22.45  | 40.65  | 11.69       | 22.03  | 42.17  |
| <b>Orn</b>              | 21.29       | 55.87  | 112.77 | 34.98       | 86.88  | 145.61 | 51.48       | 94.93  | 148.49 |
| <b>Phe</b>              | 27.17       | 42.54  | 85.45  | 32.34       | 52.40  | 88.12  | 33.54       | 53.72  | 82.08  |
| <b>Pro</b>              | 84.25       | 145.36 | 320.14 | 76.18       | 136.11 | 256.02 | 92.59       | 158.86 | 324.41 |
| <b>Ser</b>              | 186.92      | 339.78 | 581.31 | 111.44      | 198.15 | 374.39 | 123.99      | 209.80 | 370.87 |
| <b>Tyr</b>              | 7.11        | 82.40  | 199.96 | 9.19        | 44.16  | 128.12 | 0.37        | 47.95  | 136.63 |
| <b>Val</b>              | 51.55       | 101.89 | 188.83 | 52.78       | 117.78 | 187.45 | 59.58       | 138.84 | 210.34 |
| <b>Amino acid ratio</b> |             |        |        |             |        |        |             |        |        |
| <b>Phe/Tyr</b>          | 0.16        | 0.54   | 3.47   | 0.39        | 1.20   | 5.11   | 0.06        | 1.13   | 8.03   |
| <b>Val/Phe</b>          | 1.13        | 2.37   | 5.37   | 1.12        | 2.11   | 3.35   | 1.24        | 2.52   | 4.05   |
| <b>Leu/Ala</b>          | 0.37        | 0.80   | 1.37   | 0.34        | 0.66   | 1.44   | 0.35        | 0.74   | 1.23   |
| <b>Leu/Phe</b>          | 1.43        | 2.51   | 5.62   | 1.53        | 2.48   | 3.37   | 1.83        | 2.75   | 4.14   |
| <b>Met/Phe</b>          | 0.21        | 0.43   | 0.74   | 0.21        | 0.43   | 0.77   | 0.22        | 0.41   | 0.78   |
| <b>Met/Leu</b>          | 0.05        | 0.17   | 0.39   | 0.09        | 0.18   | 0.29   | 0.08        | 0.15   | 0.26   |
| <b>Met/Cit</b>          | 0.72        | 1.86   | 4.77   | 0.47        | 1.09   | 2.16   | 0.47        | 0.91   | 1.83   |
| <b>Met/Tyr</b>          | 0.06        | 0.23   | 1.15   | 0.18        | 0.48   | 2.05   | 0.03        | 0.47   | 2.55   |
| <b>Cit/Phe</b>          | 0.10        | 0.23   | 0.42   | 0.21        | 0.39   | 0.75   | 0.24        | 0.46   | 0.80   |
| <b>Cit/Arg</b>          | 0.53        | 2.25   | 16.26  | 0.31        | 1.05   | 5.68   | 0.30        | 0.86   | 4.89   |
| <b>Tyr/Phe</b>          | 0.13        | 1.82   | 5.90   | 0.19        | 0.83   | 2.50   | 0.01        | 0.86   | 2.56   |
| <b>Arg/Orn</b>          | 0.01        | 0.08   | 0.26   | 0.05        | 0.21   | 1.00   | 0.05        | 0.36   | 1.04   |
| <b>Acylcarnitines</b>   |             |        |        |             |        |        |             |        |        |
| <b>C0</b>               | 15.60       | 32.00  | 73.73  | 12.34       | 39.18  | 69.76  | 13.38       | 43.53  | 75.67  |
| <b>C2</b>               | 6.88        | 20.39  | 64.68  | 8.71        | 16.57  | 34.90  | 9.05        | 15.63  | 29.31  |
| <b>C3</b>               | 0.61        | 1.86   | 6.88   | 0.63        | 1.38   | 2.69   | 0.64        | 1.40   | 2.70   |
| <b>C4</b>               | 0.08        | 0.18   | 0.63   | 0.06        | 0.13   | 0.33   | 0.05        | 0.15   | 0.38   |
| <b>C5</b>               | 0.09        | 0.19   | 0.55   | 0.04        | 0.11   | 0.26   | 0.05        | 0.13   | 0.29   |
| <b>C5:1</b>             | 0.00        | 0.00   | 0.02   | 0.00        | 0.00   | 0.02   | 0.00        | 0.01   | 0.02   |
| <b>C5DC</b>             | 0.04        | 0.11   | 0.29   | 0.00        | 0.06   | 0.17   | 0.00        | 0.07   | 0.20   |
| <b>C5OH</b>             | 0.08        | 0.14   | 0.25   | 0.12        | 0.25   | 0.45   | 0.13        | 0.27   | 0.53   |
| <b>C6</b>               | 0.00        | 0.03   | 0.09   | 0.00        | 0.02   | 0.09   | 0.00        | 0.02   | 0.12   |

|                             |      |      |        |      |       |       |      |       |       |
|-----------------------------|------|------|--------|------|-------|-------|------|-------|-------|
| <b>C8</b>                   | 0.00 | 0.02 | 0.14   | 0.00 | 0.03  | 0.14  | 0.00 | 0.04  | 0.30  |
| <b>C10</b>                  | 0.03 | 0.08 | 0.18   | 0.00 | 0.06  | 0.23  | 0.01 | 0.08  | 0.58  |
| <b>C10:1</b>                | 0.02 | 0.05 | 0.11   | 0.00 | 0.05  | 0.16  | 0.00 | 0.07  | 0.21  |
| <b>C10:2</b>                | 0.00 | 0.01 | 0.02   | 0.00 | 0.02  | 0.08  | 0.00 | 0.02  | 0.08  |
| <b>C12</b>                  | 0.03 | 0.08 | 0.23   | 0.00 | 0.05  | 0.14  | 0.00 | 0.04  | 0.15  |
| <b>C14</b>                  | 0.11 | 0.21 | 0.51   | 0.04 | 0.08  | 0.27  | 0.03 | 0.07  | 0.13  |
| <b>C14:1</b>                | 0.04 | 0.11 | 0.34   | 0.03 | 0.07  | 0.16  | 0.03 | 0.08  | 0.26  |
| <b>C14:2</b>                | 0.01 | 0.02 | 0.05   | 0.00 | 0.02  | 0.07  | 0.00 | 0.03  | 0.12  |
| <b>C16</b>                  | 0.88 | 3.07 | 7.23   | 0.61 | 1.19  | 2.47  | 0.55 | 1.15  | 2.20  |
| <b>C16OH</b>                | 0.00 | 0.01 | 0.05   | 0.00 | 0.01  | 0.01  | 0.00 | 0.00  | 0.01  |
| <b>C16:1OH</b>              | 0.01 | 0.03 | 0.07   | 0.00 | 0.02  | 0.08  | 0.00 | 0.02  | 0.05  |
| <b>C18</b>                  | 0.32 | 0.69 | 1.69   | 0.25 | 0.47  | 0.91  | 0.22 | 0.40  | 0.88  |
| <b>C18:1</b>                | 0.45 | 0.94 | 1.98   | 0.46 | 0.82  | 1.57  | 0.42 | 0.79  | 1.67  |
| <b>C18OH</b>                | 0.00 | 0.01 | 0.05   | 0.00 | 0.00  | 0.01  | 0.00 | 0.00  | 0.01  |
| <b>C18:1OH</b>              | 0.01 | 0.02 | 0.03   | 0.00 | 0.01  | 0.02  | 0.00 | 0.01  | 0.02  |
| <b>Acylcarnitines ratio</b> |      |      |        |      |       |       |      |       |       |
| <b>C0/(C16+C18)</b>         | 3.75 | 8.45 | 21.67  | 7.12 | 23.57 | 43.54 | 7.96 | 27.07 | 56.08 |
| <b>C3/C2</b>                | 0.04 | 0.09 | 0.19   | 0.04 | 0.08  | 0.15  | 0.05 | 0.08  | 0.14  |
| <b>C3/C16</b>               | 0.22 | 0.64 | 1.42   | 0.50 | 1.12  | 2.37  | 0.49 | 1.23  | 2.36  |
| <b>C3/Met</b>               | 0.03 | 0.10 | 0.33   | 0.02 | 0.06  | 0.17  | 0.03 | 0.06  | 0.15  |
| <b>C4/C2</b>                | 0.00 | 0.01 | 0.03   | 0.00 | 0.01  | 0.02  | 0.00 | 0.01  | 0.02  |
| <b>C4/C3</b>                | 0.04 | 0.11 | 0.28   | 0.04 | 0.09  | 0.27  | 0.05 | 0.10  | 0.27  |
| <b>C5/C3</b>                | 0.04 | 0.10 | 0.42   | 0.03 | 0.08  | 0.16  | 0.04 | 0.09  | 0.19  |
| <b>C5/C0</b>                | 0.00 | 0.01 | 0.01   | 0.00 | 0.00  | 0.01  | 0.00 | 0.00  | 0.01  |
| <b>C5OH/C8</b>              | 0.00 | 2.18 | 165.14 | 0.00 | 4.38  | 72.55 | 0.00 | 4.04  | 87.13 |
| <b>C5OH/C3</b>              | 0.02 | 0.08 | 0.24   | 0.07 | 0.18  | 0.40  | 0.08 | 0.20  | 0.43  |
| <b>C5OH/C0</b>              | 0.00 | 0.00 | 0.01   | 0.00 | 0.01  | 0.02  | 0.00 | 0.01  | 0.02  |
| <b>C8/C10</b>               | 0.00 | 0.27 | 1.67   | 0.00 | 0.53  | 4.43  | 0.00 | 0.48  | 2.45  |
| <b>C8/C2</b>                | 0.00 | 0.00 | 0.01   | 0.00 | 0.00  | 0.01  | 0.00 | 0.00  | 0.02  |
| <b>C5DC/C5OH</b>            | 0.28 | 0.72 | 1.66   | 0.00 | 0.22  | 0.80  | 0.00 | 0.24  | 1.06  |
| <b>C14:1/C2</b>             | 0.00 | 0.01 | 0.02   | 0.00 | 0.00  | 0.01  | 0.00 | 0.01  | 0.01  |
| <b>C14:1/C16</b>            | 0.02 | 0.04 | 0.09   | 0.02 | 0.06  | 0.16  | 0.02 | 0.07  | 0.22  |
| <b>C16OH/C16</b>            | 0.00 | 0.00 | 0.01   | 0.00 | 0.00  | 0.01  | 0.00 | 0.00  | 0.01  |
| <b>C18OH/C18</b>            | 0.00 | 0.02 | 0.04   | 0.00 | 0.01  | 0.03  | 0.00 | 0.01  | 0.04  |

Abbreviations: Ala, alanine; Arg, arginine; Cit, citrulline; Xle, (Leucine and isoleucine ); Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Tyr, tyrosine; Val, valine; C0, free carnitine; C2, acetylcarnitine; C3, propionylcarnitine; C4 butyryl-/isobutyrylcarnitine; C5, isovaleryl-/2-methylbutyrylcarnitine, C5:1, tiglylcarnitine; C5DC,

glutaryl carnitine; C5OH, hydroxy isovalerylcarnitine; C6, hexanoylcarnitine; C8, octanoylcarnitine; C10, decanoylcarnitine; C10:1, decenoylcarnitine; C10:2, decadienoylcarnitine; C12, dodecanoylcarnitine; C14, tetradecanoylcarnitine; C14:1, tetradecenoylcarnitine; C14:2, tetradecadienoylcarnitine; C16, palmitoylcarnitine; C16OH, hydroxy palmitoylcarnitine; C16:1OH, hydroxy hexadecenoylcarnitine; C18, stearyl carnitine; C18:1, oleylcarnitine; C18OH, hydroxy stearyl carnitine; C18:1OH, hydroxy oleylcarnitine.

**Supplementary Table S6: GC-MS-based urinary metabolic profiling for confirmatory diagnosis of the screening-positive patients with IEMs.**

| Name of disorder | Case No.      | Metabolites detected       | Concentration ( $\mu\text{mol}/\text{mmol}$ of creatinine) | Biological reference interval ( $\mu\text{mol}/\text{mmol}$ of creatinine) <sup>a</sup> |         |
|------------------|---------------|----------------------------|--|---|---------|
| <b>PKU</b>       | Case 1        | 4-Hydroxyphenylacetic acid | 392  | 3.2 – 180.2   |         |
|                  |               | 4-Hydroxyphenyllactic acid | 31.6   | 0 – 8.7   |         |
|                  | Case 2        | 2-Hydroxyphenylacetic acid | 52.3   | 0 – 20  |         |
|                  |               | Phenyllactic acid          | 11.9   | 0 – 4   |         |
|                  |               | 4-Hydroxyphenylacetic acid | 343  | 3.2 – 180.2   |         |
|                  |               | 4-Hydroxyphenyllactic acid | 24.9   | 0 – 8.7   |         |
|                  |               | Mandelic acid              | 117  | 0 - 64  |         |
|                  | Case 3        | 2-Hydroxyphenylacetic acid | 94.7   | 0 – 20  |         |
|                  |               | Phenyllactic acid          | 38.6   | 0- 4  |         |
|                  |               | 4-Hydroxyphenylacetic acid | 278  | 3.2 – 180.2   |         |
|                  |               | 4-Hydroxyphenyllactic acid | 32.4   | 0 – 8.7   |         |
|                  |               | Mandelic acid              | 95   | 0 - 64  |         |
|                  | <b>CIT-II</b> | Case 4                     | Citrulline   | 18803   | 10 - 99 |
|                  | <b>MMA</b>    | Case 5                     | Methylmalonic acid   | 10.4  | 0 - 6   |
|                  | <b>IVA</b>    | Case 6                     | Isovalerylglycine  | 91.4  | 0 - 10  |
| 3-OH-isovalerate |               |                            | 65.3   | 3.1 23.1  |         |
| <b>CUD</b>       | Case 7        | 3-Hydroxybutyric acid      | 108  | 0 -11.1   |         |
|                  |               | 3-Hydroxyisovaleric Acid   | 64.7   | 3.1 – 23.1  |         |
|                  |               | Adipic acid                | 63.2   | 0 – 35  |         |
|                  |               | p-Cresol                   | 57.8   | 0 - 11  |         |
| <b>MCAD</b>      | Case 8        | Hexanoyl glycine           | 0.0  | 0 – 2.9   |         |
|                  |               | Octanoyl glycine           | 0.0  | 0 – 0.2   |         |
|                  |               | Phenylpropionyl glycine    | 0.0  | 0 – 0.1   |         |
|                  |               | Adipic acid                | 0.1  | 0 - 35  |         |
|                  |               | Octanoic acid              | 0.0  | 0 – 7.7   |         |
|                  |               | Sebacic acid               | 0.0  | 0 – 12.3  |         |
|                  | Case 9        | Hexanoyl glycine,          | 0.0  | 0 – 2.9   |         |



|  |  |                          |     |          |
|--|--|--------------------------|-----|----------|
|  |  | Octanoyl glycine,        | 0.0 | 0 – 0.2  |
|  |  | Phenylpropionyl glycine, | 0.0 | 0 – 0.1  |
|  |  | Adipic acid              | 0.1 | 0 - 35   |
|  |  | Octanoic acid            | 0.0 | 0 – 7.7  |
|  |  | Sebacic acid             | 0.0 | 0 – 12.3 |

Abbreviations: PKU, phenylketonuria; CIT-II, citrullinemia type II; MMA, methylmalonic acidemia; IVA, isovaleric acidemia; CUD, carnitine uptake defect; and MCAD, medium-chain acyl-CoA dehydrogenase deficiency. <sup>a</sup>Reference ranges were obtained from Human Metabolome Database (Version 4.0)[1].

## References

1. Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R et al. HMDB 4.0: the human metabolome database for 2018. *Nucleic acids research*. 2017;46(D1):D608-D17.