

Characterization of Urinary N-Acetyltaurine as a Biomarker of Hyperacetatemia in Mice

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SUPPLEMENTARY DATA

Table S1. Reagents and chemicals used in sample preparation and LC-MS analysis.

| Chemical/Reagents | Vendor |
|--|--|
| Acetic acid, Acetone (LC-MS grade), Acetonitrile (LC-MS grade, ACN), Ammonium formate, Formic acid (LC-MS grade), Water (LC-MS grade) | Fisher Scientific (Houston, TX) |
| 2-Hydrazinoquinoline (HQ), Triphenylphosphine (TPP) | Alfa Aesar (Ward Hill, MA) |
| N-acetyl-L-glutamine, Ammonium carbonate ((NH ₄) ₂ CO ₃), 2-2'-Dipyridyl disulfide (DPDS) | MP Biomedicals, LLC (Irvine, CA) |
| Creatinine, D ₄ -acetic acid, Dansyl chloride (DC), Leucine enkephalin, Sodium carbonate (Na ₂ CO ₃), D ₅ -Tryptophan | Sigma-Aldrich (St. Louis, MO) |
| | Cambridge Isotope Laboratories (Tewksbury, MA) |
| Taurine | Thermo Fisher Scientific (Waltham, MA) |
| Acetyl-L-glutamic acid, Acetyl-L-isoleucine, Acetyl-L-leucine, Acetyl-L-phenylalanine, Acetyl-L-tyrosine | Chem-Impex International (Wood Dale, IL) |
| Ethanol (reagent alcohol) | Ricca Chemicals (Arlington, TX) |

Table S2. LC-MS instrumental settings for data acquisition.

| Target compounds | LC column and temperature | LC mobile phase | Lock mass and MS detection mode | Capillary and cone voltage | Source and desolvation temperature | Cone and desolvation gas flow | *Collision gas and energy ramp |
|--|---------------------------------------|---|--|----------------------------|------------------------------------|-------------------------------------|--------------------------------|
| Taurine (DC derivatization), and N-acetyl amino acids (except for NAT) | Waters BEH C18 (reverse phase), 40 °C | A: 0.1% formic acid in water B: 0.1% formic acid in ACN | Leucine enkephalin ([M + H] ⁺ = m/z 556.2771), Positive | 0.2 kV, 40 V | 120 °C, 350 °C | 50 L/hr, 600 L/hr (N ₂) | Argon, 10-50 eV |
| Acetic acid (HQ derivatization) | Waters BEH C18 (reverse phase), 40 °C | A: 2 mM NH ₄ OAc in water with 0.05% acetic acid B: 2 mM NH ₄ OAc in 95% ACN and 5% water with | Leucine enkephalin ([M + H] ⁺ = m/z 556.2771), Positive | 0.2 kV, 40 V | 120 °C, 350 °C | 50 L/hr, 600 L/hr (N ₂) | NA |

| | | | | | | | |
|------------|---------------------------------------|--|--|----------------|----------------|-------------------------------------|----|
| | | 0.05% acetic acid | | | | | |
| NAT | Waters BEH C18 (reverse phase), 40 °C | A: 0.1% formic acid in water B: 0.1% formic acid in ACN | Leucine enkephalin ([M - H] ⁻ = m/z 554.2615), Negative | -0.2 kV, -40 V | 120 °C, 350 °C | 50 L/hr, 600 L/hr (N ₂) | NA |
| Creatinine | Waters Amide (HILIC), 40 °C | A: 0.1% formic acid in water B: 0.1% formic acid in ACN | Leucine enkephalin ([M + H] ⁺ = m/z 556.2771), Positive | 0.2 kV, 40 V | 120 °C, 350 °C | 50 L/hr, 600 L/hr (N ₂) | NA |

*Settings for MSMS fragmentation analysis. NA: not applicable.

Table S3. Information on selected triacetin-responsive urinary metabolites. Enlisted metabolites are selected from the volcano plot of the mouse urine metabolome with greater fold change (FC) and smaller *p*-values than NAT (Figure 1M). Accurate mass-based elemental composition analysis was performed to determine potential molecular formula.

| Retention time (min) | Detected M/Z | Ion adduct | FC [#] | <i>p</i> -value | Potential molecular formula | Monoisotopic M/Z | Mass deviation (Δppm) |
|----------------------|--------------|--------------------|-----------------|-----------------|---|------------------|-----------------------|
| 5.9152 | 240.232 | [M+H] ⁺ | 7.7838 | 1.36E-06 | C ₁₅ H ₂₉ NO [^] | 239.2249 | 3 |
| 2.11 | 391.0629 | [M+H] ⁺ | 7.763 | 2.73E-06 | C ₁₄ H ₁₀ N ₆ O ₈ [^] | 390.056 | 2 |
| 6.1748 | 459.4871 | [M+H] ⁺ | 8.0529 | 5.46E-06 | C ₂₈ H ₆₂ N ₂ O ₂ [^] | 458.4811 | 4 |
| 2.1065 | 177.0741 | [M+H] ⁺ | 8.188 | 5.58E-06 | C ₇ H ₁₂ O ₅ [^] | 176.0685 | 9 |
| 6.2401 | 485.1465 | [M-H] ⁻ | 8.5178 | 5.75E-06 | C ₂₆ H ₂₂ N ₄ O ₆ [^] | 486.1539 | 2 |
| 2.1269 | 353.1073 | [M+H] ⁺ | 8.4198 | 6.22E-06 | C ₂₃ H ₁₄ NO ₃ [^] | 352.0974 | 7 |
| 6.6641 | 515.5503 | [M+H] ⁺ | 7.7516 | 7.91E-06 | C ₃₂ H ₇₀ N ₂ O ₂ [^] | 514.5437 | 3 |
| 2.1197 | 370.1341 | [M+H] ⁺ | 9.7848 | 1.22E-05 | C ₁₄ H ₂₇ NO ₆ S ₂ [^] | 370.1353 | 3 |
| 1.0254 | 309.0815 | [M-H] ⁻ | 8.6626 | 1.31E-05 | C ₁₁ H ₁₈ O ₁₀ [^] | 310.0907 | 5 |
| 3.1667 | 229.0163 | [M-H] ⁻ | 8.7126 | 2.46E-05 | C ₉ H ₁₀ O ₅ S [^] | 230.0249 | 6 |
| 0.3157 | 166.0169 | [M-H] ⁻ | 7.6126 | 9.07E-05 | C ₄ H ₉ NO ₄ S [*] | 167.0252 | 6 |

[#]FC, fold change, calculated by the relative abundance of metabolites in the triacetin group divided by that of the glycerol group;

[^] Database search yielded no match with known urinary metabolites

^{*}N-acetyltaurine, identity confirmed by comparison with authentic standard

Figure S1. Extracted ion chromatograms (EICs) of targeted analytes. The retention time (RT), and monoisotopic mass-over-charge ratio (M/Z) of the detected ion adducts of analytes are enlisted. A) Creatinine, RT = 2.56, $[M+H]^+ = 114.0667$. B) Acetic acid, RT = 2.32 min, $[M+HQ]^+ = 202.0980$. C) NAT, RT = 0.33 min, $[M-H]^- = 166.0174$. D) Taurine, RT = 3.4 min, $[M+DC]^+ = 359.0736$. E) *N*-acetyl-glutamine (NA-Gln), RT = 0.35 min, $[M+H]^+ = 189.0875$. F) *N*-acetyl-glutamic acid (NA-Glu), RT = 0.6 min, $[M+H]^+ = 190.0715$. G) *N*-acetyl-tyrosine (NA-Tyr), RT = 2.1 min, $[M+H]^+ = 224.0923$. H) *N*-acetyl-phenylalanine (NA-Phe), RT = 3.44 min, $[M+H]^+ = 208.0974$. I) *N*-acetyl-leucine (NA-Leu), RT = 2.97 min; *N*-acetyl-isoleucine (NA-Ile), RT = 3.13 min, $[M+H]^+ = 174.1130$. J) *d*₄-Acetic acid, RT = 2.32 min, $[M+HQ]^+ = 205.1169$. K) *d*₅-Tryptophan, RT = 5.71 min, $[M+DC]^+ = 443.1801$. L) Sulfadimethoxine (SDM), RT = 4.68 min in a BEH C18 column, $[M-H]^- = 309.0658$. M) Sulfadimethoxine (SDM), RT = 0.68 min in an Amide column, $[M+H]^+ = 311.0814$.

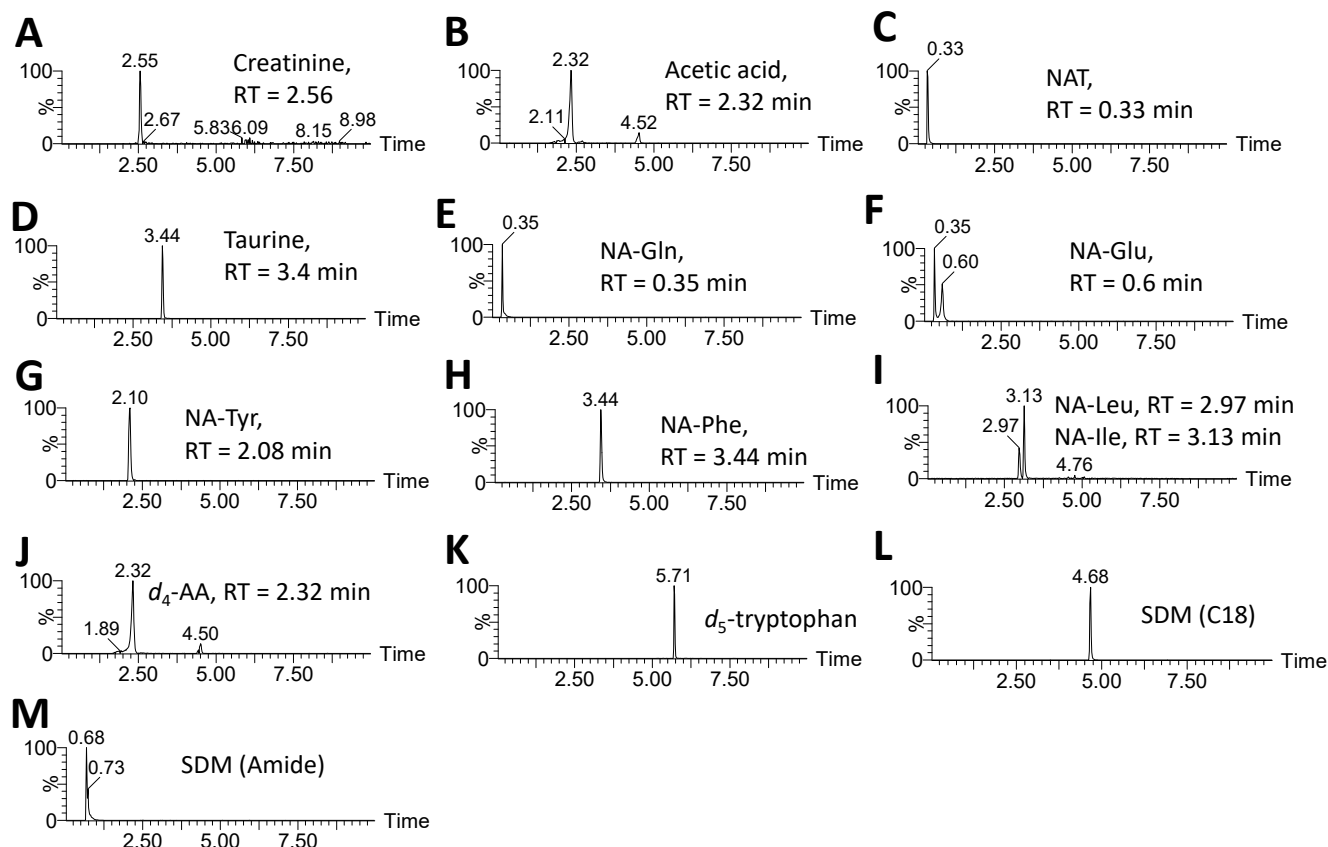


Figure S2. Correlations of urinary NAT and serum acetate levels in the animal models of hyperacetatemia. The correlations were examined by linear regression. A) The correlation in all three animal models, including triacetin-dosing, EtOH-dosing, and STZ-dosing with $R^2 = 0.55$ and p -value of the slope < 0.0001 . B) The correlation in EtOH-dosing and STZ-dosing animal models, excluding triacetin-dosing, with $R^2 = 0.02$ and p -value of the slope $= 0.4$.

