Supplementary Data

Characterization and quantification of endogenous fatty acid nitroalkene metabolites in human urine

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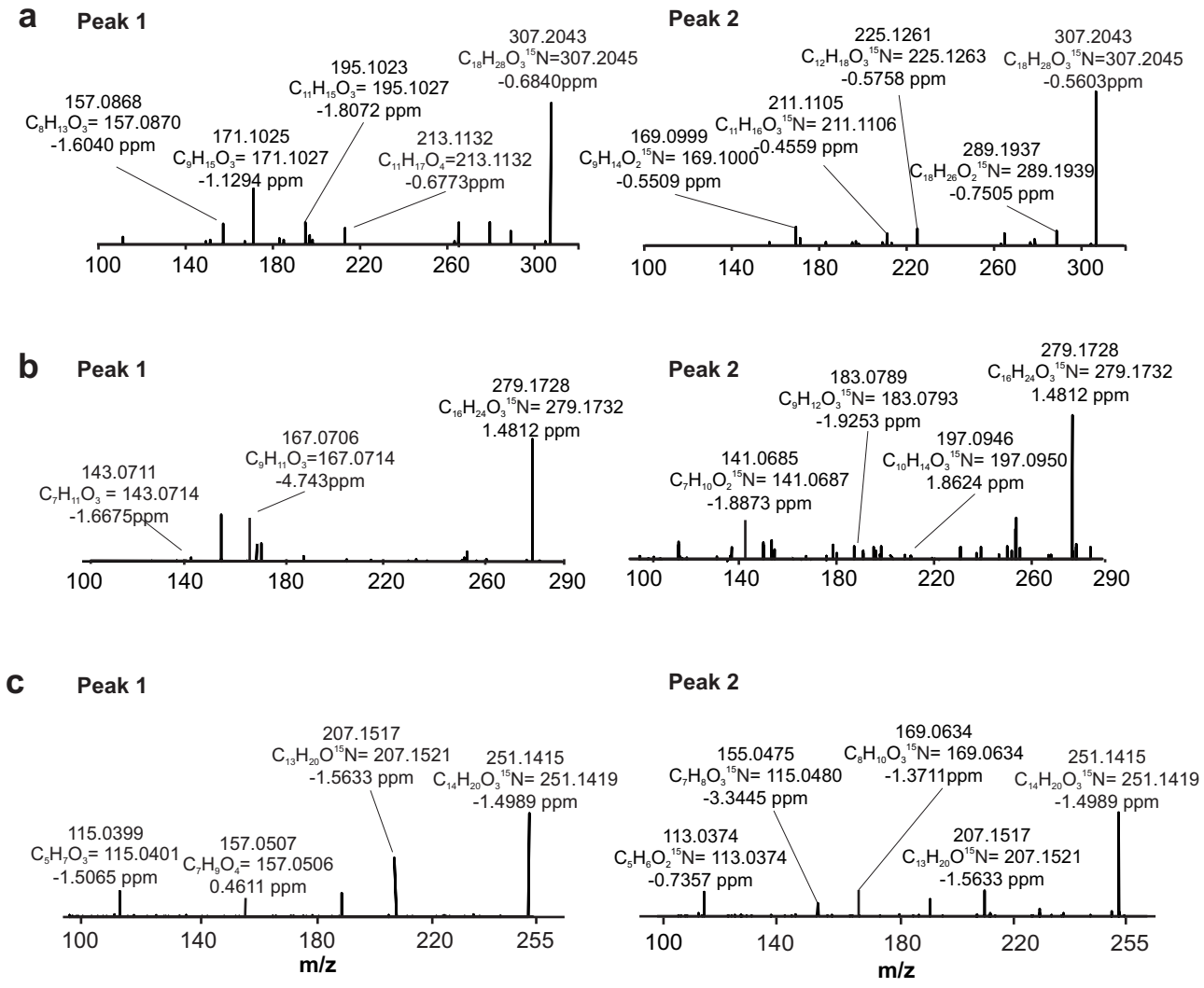
Suppl. Fig 1 High resolution MS/MS data obtained upon fragmentation of the different $^{15}NO_2$-CLA metabolites from the effluent of $^{15}NO_2$-CLA Langendorff-perfused isolated rat hearts. a) MS/MS data on peaks 1 and 2 indicate 12-$^{15}NO_2$-CLA and 9-$^{15}NO_2$-CLA respectively. **b)** Peaks 1 and 2 indicate dinor-10-$^{15}NO_2$-CLA and dinor-7-$^{15}NO_2$-CLA respectively. **(c)** Peaks 1 and 2 indicate tetranor-8-$^{15}NO_2$-CLA and tetranor-5-$^{15}NO_2$-CLA respectively.

Suppl. Fig 2 Detection and confirmation of cysteine-conjugated nitro-fatty acids in urine. **a)** Chromatographic profiles of Cys-NO_2-CLA and its β-oxidation metabolites followed in the positive ion mode. The neutral loss of 47 (HNO_2) is indicative of the presence of an organic nitro group. **b)** Chromatographic profiles of Cys-NO_2-CLA and its β-oxidation metabolites followed as the neutral loss of 325.2 (NO_2-CLA), 297.2 (dinor-NO_2-CLA), 269.2 (tetranor-NO_2-CLA) and 241.2 (hexanor-NO_2-CLA) in the negative ion mode. **c)** Specific product ions and accurate mass determinations for urine- and heart-derived Cys-NO_2-CLA metabolites in the positive ion mode.

Suppl. Scheme 1 Proposed structures for product ions obtained upon fragmentation of Cys-NO_2-CLA

Suppl Table 1 NO_2-CLA concentration in urine samples obtained from 14 healthy human volunteers. Samples were measured by quadruplicate in 2 consecutive days.



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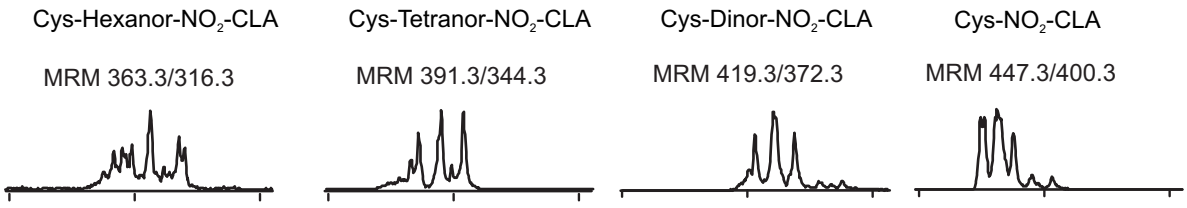
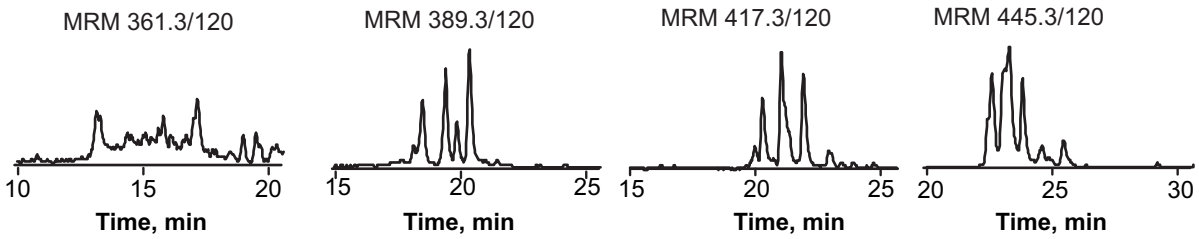
a



b



c

Cys-NO_2-FA	Fragment Exp $[M+H]^+$	Fragment Theoretical $[M+H]^+$	Fragment Composition
Cys-NO_2-CLA	447.2519	447.2523	$C_{21}H_{39}N_2O_6S^+$
Cys-Dinor-NO_2-CLA	419.2206	419.2210	$C_{19}H_{35}N_2O_6S^+$
Cys-Tetranor-NO_2-CLA	391.1885	391.1897	$C_{17}H_{31}N_2O_6S^+$
Cys-Hexanor-NO_2-CLA	363.1577	363.1584	$C_{15}H_{27}N_2O_6S^+$
Cys-$^{15}NO_2$-CLA	448.2482	448.2492	$C_{21}H_{39}N_1{}^{15}N_1O_6S^+$
Cys-Dinor-$^{15}NO_2$-CLA	420.2289	420.2181	$C_{19}H_{35}N_1{}^{15}N_1O_6S^+$
Cys-Tetranor-$^{15}NO_2$-CLA	392.185	392.1868	$C_{17}H_{31}N_1{}^{15}N_1O_6S^+$
Cys-Hexanor-$^{15}NO_2$-CLA	364.1537	364.1555	$C_{15}H_{27}N^{15}N_1O_6S^+$

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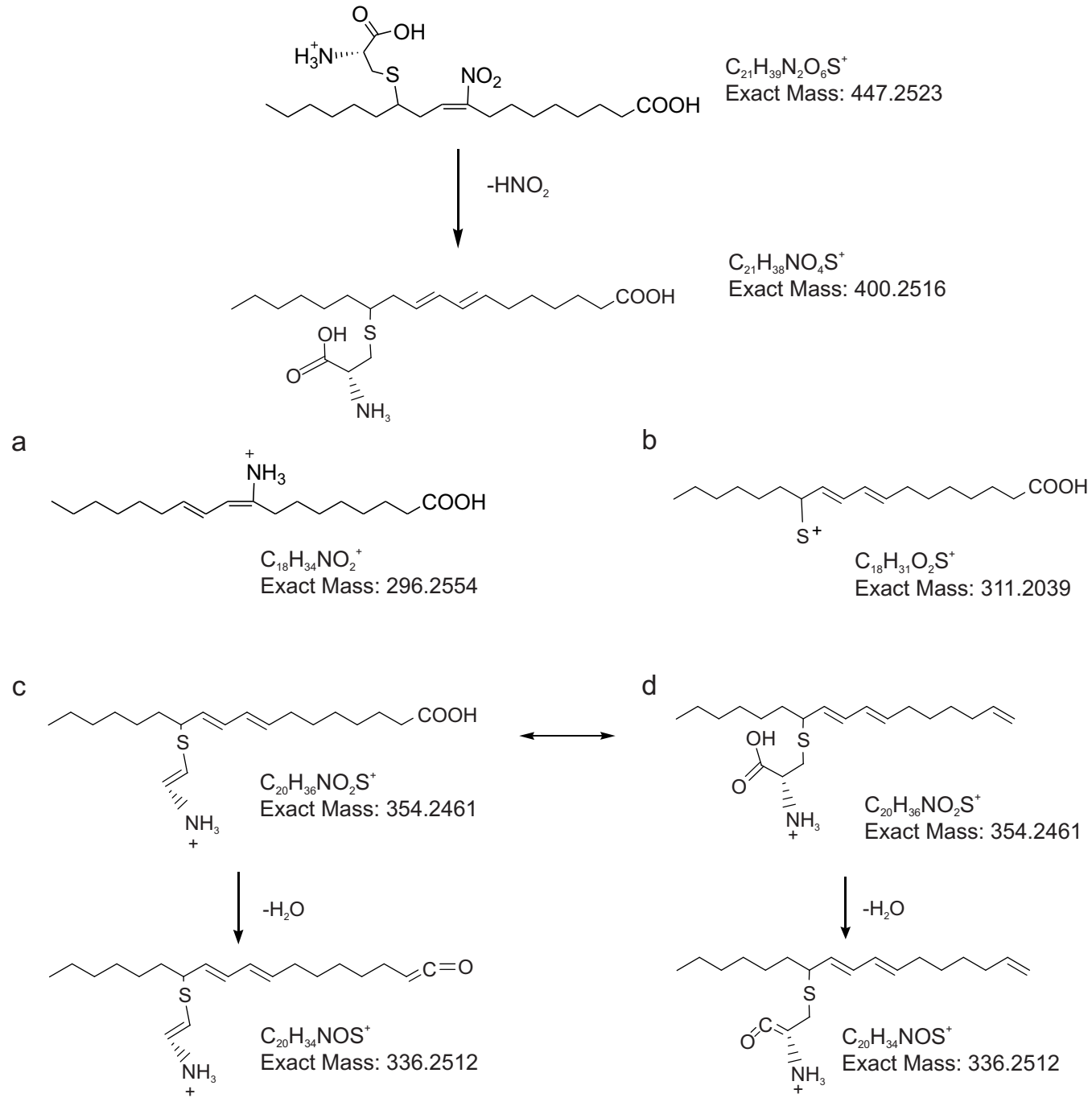

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Urine Sample #	NO_2-CLA (pmol/mg creatinine)	Std. Dev.
1	0.49	0.05
2	3.86	0.82
3	2.11	0.64
4	3.19	0.45
5	0.8	0.19
6	4.43	1.05
7	3.37	1.02
8	41.95	5.64
9	42.58	2.24
10	1.81	0.03
11	1.21	0.27
12	24	4.17
13	7.26	4.39
14	2.56	0.78

Urinary levels of NO_2-CLA:

9.97 +/-3.98 (pmol/mg creatinine)

9.22 +/- 4.31 (nM)

Range:

0.49-42.58 (pmol/mg creatinine)

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