

Supporting Information

Profiling of Branched-Chain Fatty Acids via Nitroxide Radical-Directed Dissociation Integrated on an LC-MS/MS Workflow

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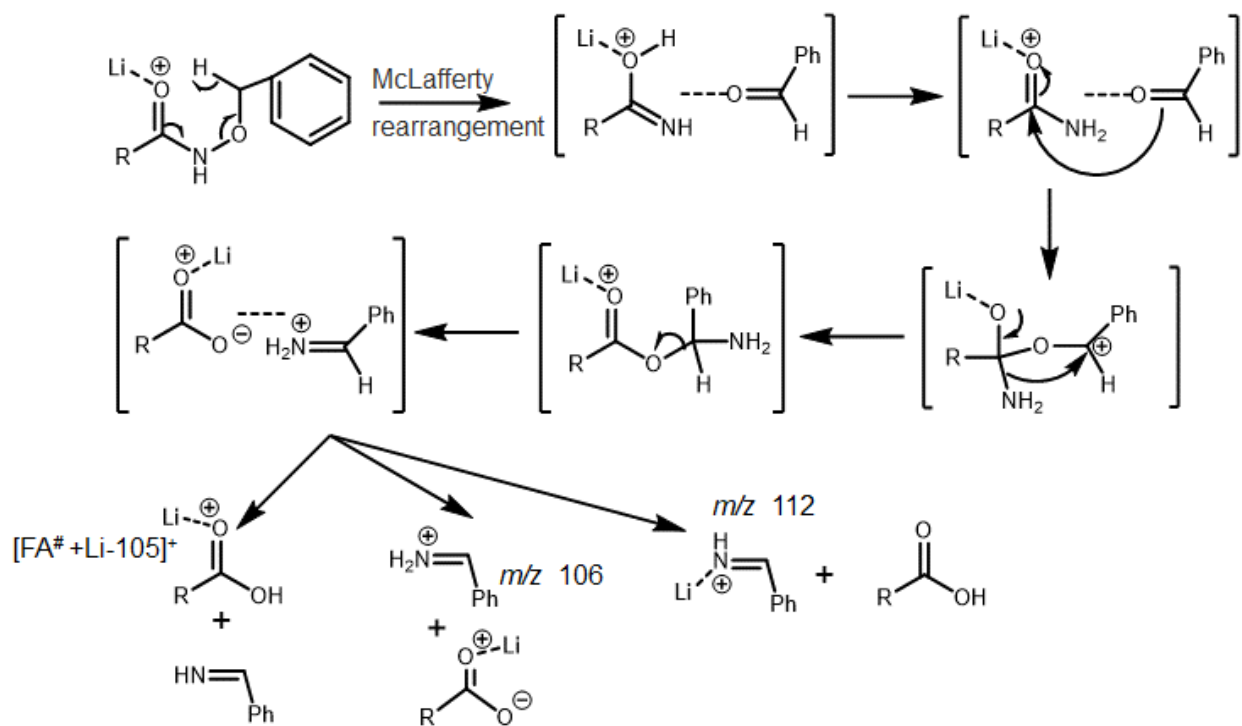
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Scheme S1. Proposed fragmentation pathways for N-O rearrangement during CID of $[M^{\#} + Li]^+$.

“#” stands for O-BHA modification of FA.

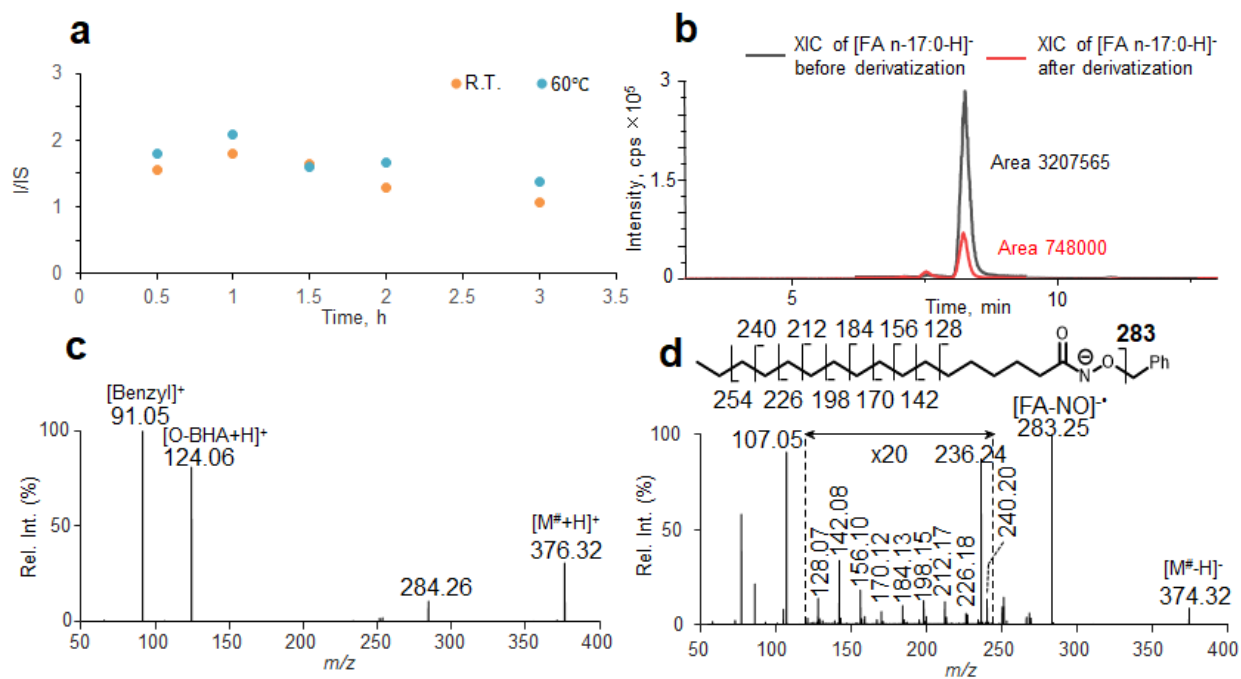


Figure S1. (a) Intensity ratio of FA n-17:0 ($[M^{\#} + H]^+$, m/z 376, 10 μ M) over the internal standard (m/z 394, 5 μ M) as a function of reaction time. FA 18:0-d₄ derivatized by O-BHA at 60°C for 1h was added as the internal standard at the end of FA n-17:0 derivatization. (b) XICs of $[M-H]^-$ (m/z 269) of FA n-17:0 before and after 1h reaction at 60°C. (c) MS² CID of FA n-17:0 ($[M^{\#} + H]^+$, m/z 376). (d) MS² CID of FA n-17:0 ($[M^{\#} - H]^-$, m/z 374) collected on QTOF mass spectrometer.

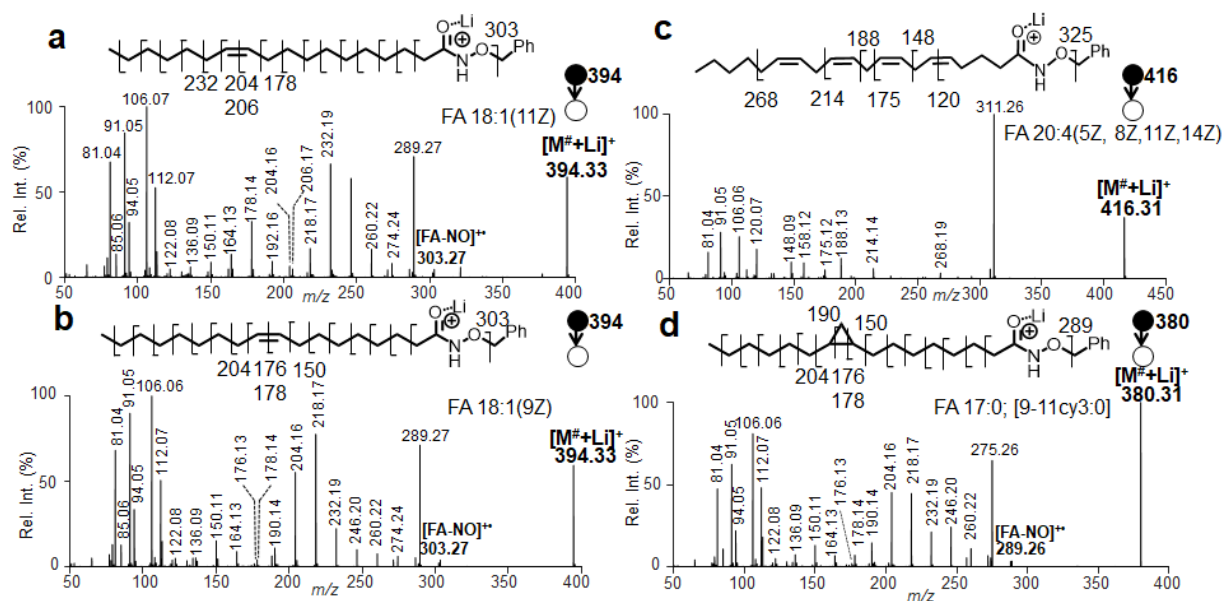


Figure S2. Analysis of monounsaturated and cyclopropane FAs via nitroxide-RDD. MS² CID of $[M^{\#} + Li]^+$, the lithium adduct ions of O-BHA derivatized (a) FA 18:1 (11Z) (m/z 394), (b) FA 18:1 (9Z) (m/z 394), (c) FA 18:1 (5Z, 8Z, 11Z, 14Z) (m/z 416) and (d) FA 17:0; [9-11cy3:0] (m/z 380) collected on a QTOF mass spectrometer.

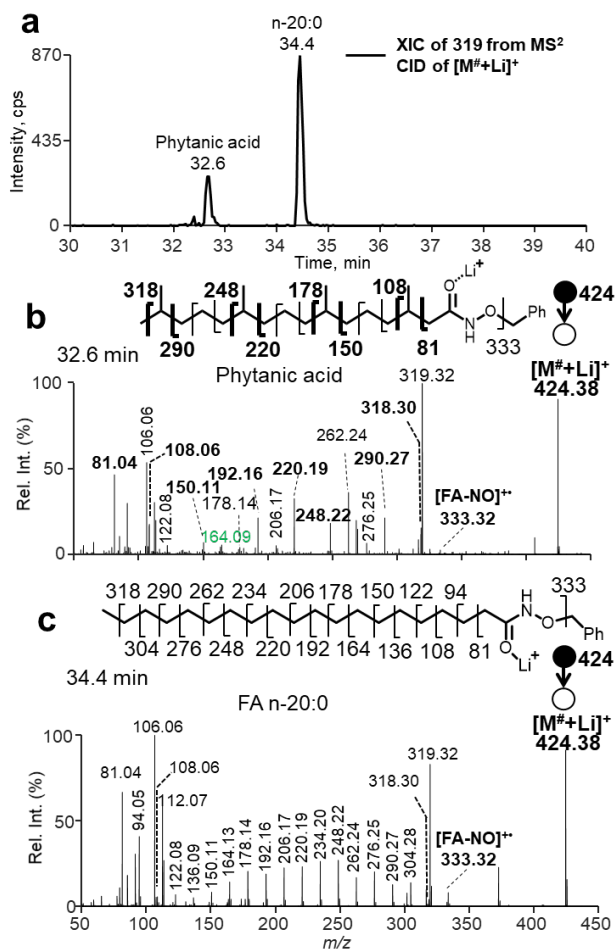


Figure S3. Identification of FA 20:0 isomers in yak milk powder. (a) XIC of m/z 319 from MS² CID of [M[#]+Li]⁺. MS² CID spectra of FA 20:0 ([M[#]+Li]⁺, m/z 424) from peaks eluted at (b) 32.6 min (phytanic acid) and (c) 34.4 min (FA n-20:0) collected on a QTOF mass spectrometer.

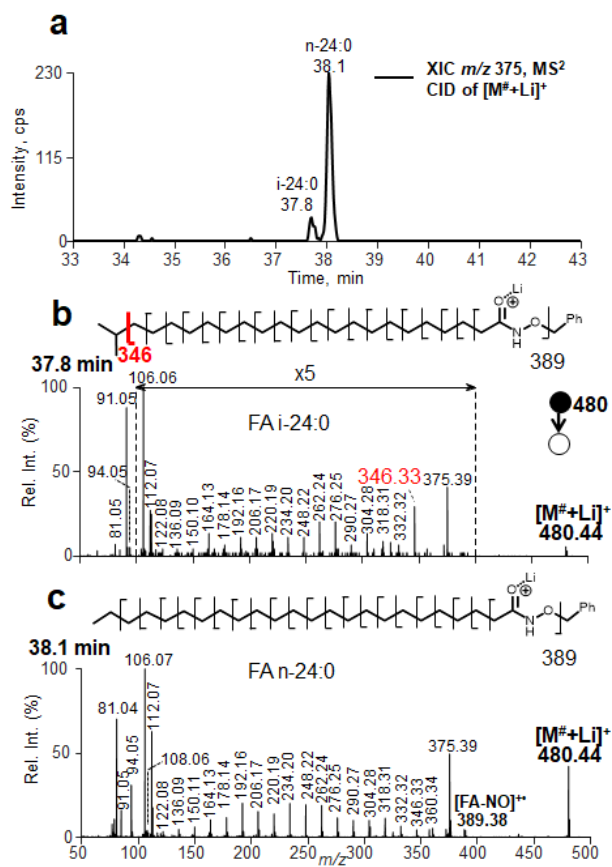


Figure S4. Identification of FA 24:0 isomers in human plasma. (a) XIC of the FA 24:0 isomers (m/z 375 from MS² CID of $[M^{\#}+Li]^+$). MS² CID spectra of $[M^{\#}+Li]^+$ (m/z 480) from peaks eluted at (b) 37.8 min (FA i-24:0) and (c) 38.1 min (FA n-24:0).

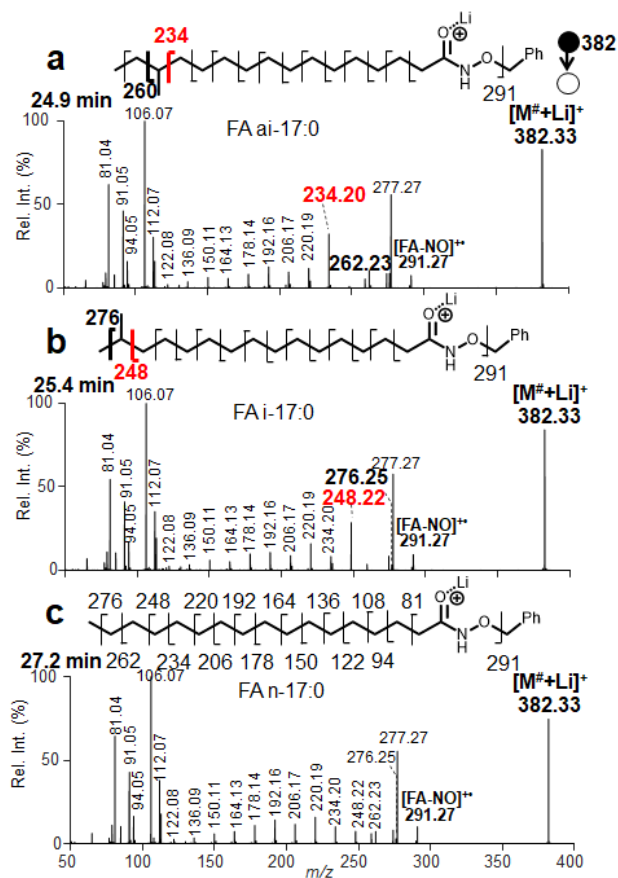


Figure S5. Identification of FA 17:0 isomers in human plasma. MS² CID spectra of [M[#]+Li]⁺ (*m/z* 382) from peaks eluted at (a) 24.9 min (FA ai-17:0), (b) 25.4 min (FA i-17:0) and (c) 27.2 min (n-17:0) collected on a QTOF mass spectrometer.

LC Method(All flow rate: 0.300 mL/min)

Method 1 (For standard mixture)

Table S1. LC Method 1 Gradient

Time (minute)	Mobile phase B (%)
0.0	30
2.0	30
8.0	45
24.5	45
31.5	95
34.0	95
34.5	30
37.0	30

Method 2 (For biological sample)

Table S2. LC Method 2 Gradient

Time (minute)	Mobile phase B (%)
0.0	30
2.0	30
8.0	45
24.5	45
31.5	95
41.5	95
42.0	100
46.5	100
47.0	30
50.0	30

Table S3. Accurate mass measurement of fragment ions generated from MS² CID of FA n-17:0 ([M[#]+Li]⁺) on a QTOF mass spectrometer.

Molecular formula	Theoretical <i>m/z</i>	Measured <i>m/z</i>	Error (ppm)
C ₂₄ H ₄₁ LiNO ₂ ⁺	382.3292	382.3292	0
C ₁₇ H ₃₄ LiNO ₂ ⁺	291.2744	291.2746	+0.7
C ₁₇ H ₃₄ LiO ₂ ⁺	277.2713	277.2713	0
C ₁₆ H ₃₁ LiNO ₂ ⁺	276.2509	276.2513	+1.3
C ₁₇ H ₃₃ LiNO ⁺	274.2717	274.2717	+1.0
C ₁₅ H ₂₉ LiNO ₂ ⁺	262.2353	262.2353	0
C ₁₇ H ₃₂ LiO ⁺	259.2608	259.2610	+0.8
C ₁₄ H ₂₇ LiNO ₂ ⁺	248.2196	248.2198	+0.7
C ₁₃ H ₂₅ LiNO ₂ ⁺	234.2040	234.2041	+1.3
C ₁₂ H ₂₃ LiNO ₂ ⁺	220.1883	220.1884	+0.4
C ₁₁ H ₂₁ LiNO ₂ ⁺	206.1727	206.1730	+1.5
C ₁₀ H ₁₉ LiNO ₂ ⁺	192.1570	192.1571	+0.5
C ₉ H ₁₇ LiNO ₂ ⁺	178.1414	178.1414	0
C ₈ H ₁₅ LiNO ₂ ⁺	164.1257	164.1255	-1.2
C ₇ H ₁₃ LiNO ₂ ⁺	150.1101	150.1100	0
C ₆ H ₁₁ LiNO ₂ ⁺	136.0944	136.0944	0
C ₅ H ₉ LiNO ₂ ⁺	122.0788	122.0787	0
C ₇ H ₈ LiN ⁺	113.0811	113.0811	-0.3
C ₇ H ₇ LiN ⁺	112.0733	112.0733	-0.8
C ₄ H ₇ LiNO ₂ ⁺	108.0631	108.0630	-1.2
C ₇ H ₈ N ⁺	106.0651	106.0652	+0.7
C ₃ H ₅ LiNO ₂ ⁺	94.0475	94.0473	-2.1
C ₇ H ₇ ⁺	91.0542	91.0543	+0.8
C ₆ H ₆ Li ⁺	85.0624	85.0623	-1.1
C ₂ H ₄ LiNO ₂ ⁺	81.0397	81.0395	-2.5

Table S4. A list of identified saturated FAs from yak milk powder.

Sum composition	$[M^{#+H}]^+$, <i>m/z</i>	Rel. Composition (%)	$[M^{#+Li}]^+$, <i>m/z</i>	Retention time, min
FA 6:0	222	n-6:0 (100%)	228.1571	2.6
FA 7:0	236	n-7:0 (100%)	242.1727	3.0
FA 8:0	250	n-8:0 (100%)	256.1884	3.4
FA 9:0	264	n-9:0 (100%)	270.204	4.1
FA 10:0	278	n-10:0 (100%)	284.2196	5.1
FA 11:0	292	n-11:0 (100%)	298.2353	6.6
FA 12:0	306	n-12:0 (100%)	312.2509	8.4
FA 13:0	320	ai-13:0 (6.6%)	326.2666	9.8
		i-13:0 (19.2%)		10.0
		n-13:0 (74.2%)		10.5
FA 14:0	334	i-14:0 (2.1%)	340.2822	12.4
		n-14:0 (97.9%)		13.4
FA 15:0	348	ai-15:0 (36.0%)	354.2979	15.2
		i-15:0 (15.9%)		15.6
		n-15:0 (48.1%)		16.5
FA 16:0	362	i-16:0 (1.7%)	368.3135	19.9
		n-16:0 (98.3%)		21.1
FA 17:0	376	ai-17:0 (31.9%)	382.3292	25.4
		i-17:0 (22.3%)		26
		n-17:0 (46.8%)		27.8
FA 18:0	390	i-18:0 (0.5 %)	396.3448	31.0
		n-18:0 (99.5%)		31.6
FA 19:0	404	n-19:0 (100%)	410.3605	33.3
FA 20:0	418	Phytanic acid (29.1%)	424.3762	32.5
		n-20:0 (70.9%)		34.4
FA 21:0	432	n-21:0 (100%)	438.3918	35.3
FA 22:0	446	n-22:0 (100%)	452.4075	36.1
FA 23:0	460	n-23:0 (100%)	466.4231	37.1
FA 24:0	474	n-24:0 (100%)	480.4388	38.2

Analysis of total fatty acids in pooled human plasma

Table S5. A list of identified saturated fatty acids from pooled human plasma.

Sum composition	[M [#] +H] ⁺ , m/z	Rel. Composition (%)	[M [#] +Li] ⁺ , m/z	Retention time, min
FA 6:0	222	n-6:0 (100%)	228.1571	2.6
FA 7:0	236	n-7:0 (100%)	242.1727	3.0
FA 8:0	250	n-8:0 (100%)	256.1884	3.4
FA 9:0	264	n-9:0 (100%)	270.204	4.1
FA 10:0	278	n-10:0 (100%)	284.2196	5.1
FA 12:0	306	n-12:0 (100%)	312.2509	8.4
FA 13:0	320	ai-13:0 (25.2%)	326.2666	9.8
		i-13:0 (9.7%)		10.0
		n-13:0 (65.1%)		10.5
FA 14:0	334	i-14:0 (1.9%)	340.2822	12.4
		n-14:0 (98.1%)		13.4
FA 15:0	348	ai-15:0 (28.4%)	354.2979	15.1
		i-15:0 (9.4%)		15.4
		n-15:0 (62.2%)		16.3
FA 16:0	362	i-16:0 (0.3%)	368.3135	19.4
		n-16:0 (99.7%)		20.6
FA 17:0	376	n-5 (4.7%)	382.3292	24.3
		ai-17:0 (16.5%)		24.9
		i-17:0 (14.0%)		25.4
		n-17:0 (64.7%)		27.2
FA 18:0	390	i-18:0 (0.1 %)	396.3448	30.7
		n-18:0 (99.9%)		31.4
FA 19:0	404	n-19:0 (100%)	410.3605	33.2
FA 20:0	418	n-20:0 (100%)	424.3762	34.3
FA 21:0	432	n-21:0 (100%)	438.3918	35.3
FA 22:0	446	n-22:0 (100%)	452.4075	36.1
FA 23:0	460	n-23:0 (100%)	466.4231	37.1
FA 24:0	474	i-24:0 (10.3%)	480.4388	37.8
		n-24:0 (89.7%)		38.1
FA 26:0	502	n-26:0 (100%)	508.4701	40.7