Supporting Information

Development of a Method for the Determination of Acyl-CoA Compounds by Liquid Chromatography Mass Spectrometry to Probe the Metabolism of Fatty Acids

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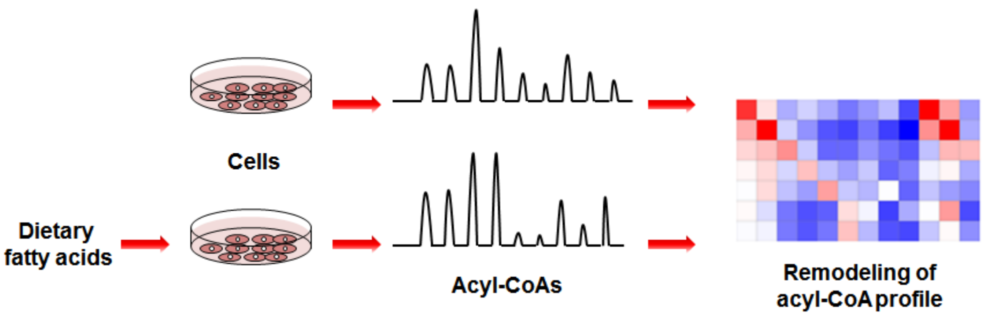

ABSTRACT: Acyl-Coenzyme As (acyl-CoAs) are a group of activated fatty acid molecules participating in multiple cellular processes including lipid synthesis, oxidative metabolism of fatty acids to produce ATP, transcriptional regulation and protein post-translational modification. Quantification of cellular acyl-CoAs is challenging due to their instability in aqueous solutions and lack of blank matrices. Here we demonstrate an LC-MS/MS analytical method which allows for absolute quantitation with broad coverage of cellular acyl-CoAs. This assay was applied to profile endogenous acyl-CoAs under the challenge of a variety of dietary fatty acids in prostate and hepatic cells. Additionally, this approach allowed for detection of multiple fatty acid metabolic processes including the biogenesis of acyl-CoAs, and their elongation, degradation, and desaturation. Hierarchical clustering in the remodeling of acyl-CoA profiles revealed a fatty acid-specific pattern across all tested cell lines, which provides a valuable reference for making predictions in other cell models. Individual acyl-CoAs were identified which were altered differentially by exogenous fatty acids in divergent tumorigenicity states of cells. These findings demonstrate the power of acyl-CoA profiling toward understanding the mechanisms for the progression of tumors or other diseases in response to fatty acids.

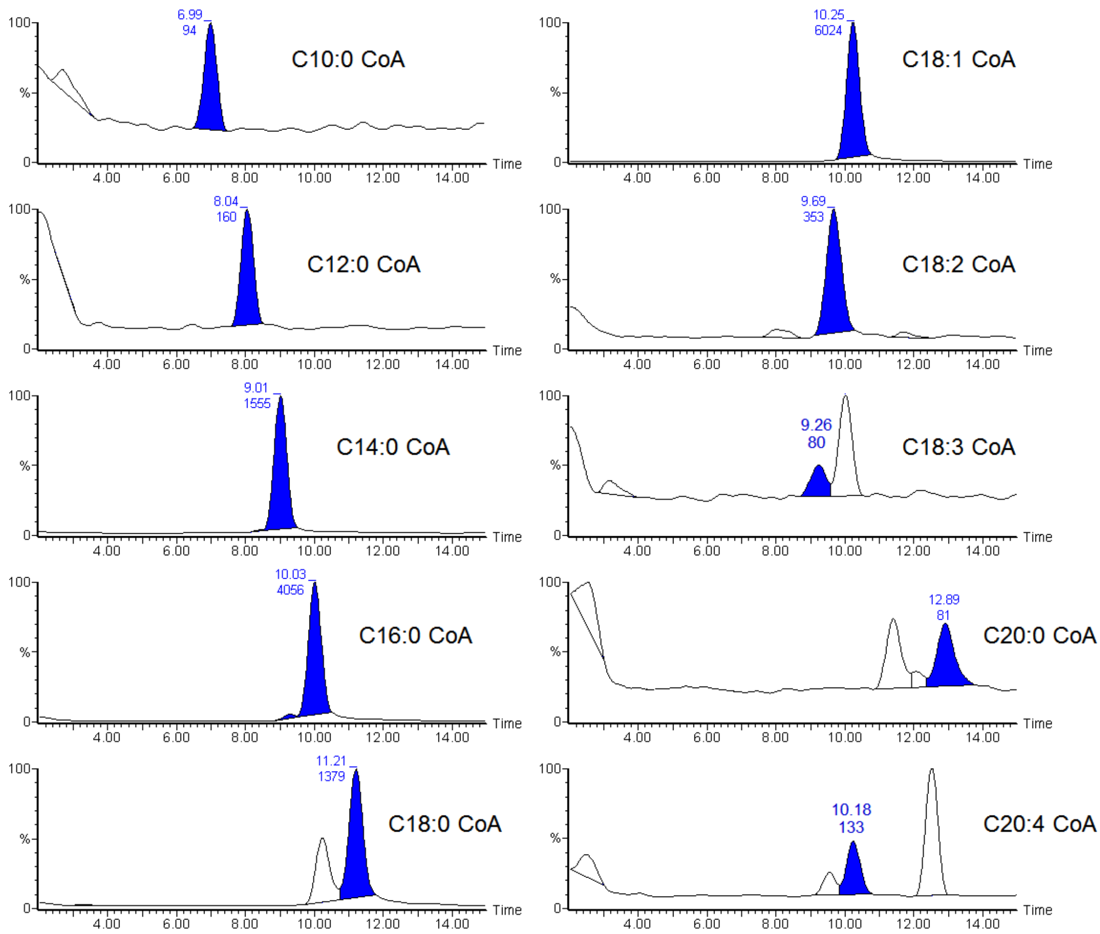

Figure S1. Representative chromatograms of acyl-CoA analytes in HepG2 cells. Retention times (min) and peak areas are labeled.

Name	Forward Sequence	Reverse Sequence
ACSL1	TCTTCCCCGTGGTTCCAA	TGGTGTTTGCTTGTCCGAAA
ACSL3	ACTCCACTGTGCGACAGCTTT	CACCACACAACAGGAGACGAA
ACSL4	ACTGGCCGACCTAAGGGAG	GCCAAAGGCAAGTAGCCAATA
ACSL5	TGGCTATCTTACAAACAGGTGTC	TCCACTCTGGCCTATTCTGAG
ACSL6	CGCTACATCATCAATACAGCGG	GCATGGACTTAATGACCACCC
GAPDH	CCACATCGCTCAGACACCAT	ACCAGGCGCCCAATACG

Table S1. Primer sets for real-time RT-PCR analysis of expression of ACSL genes.

PNT2	Control	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:1 EA	C20:0
10:0 CoA	2.40 ± 0.99	129.88 ± 35.13	26.61 ± 1.44	15.44 ± 0.80	10.27 ± 1.00	9.00 ± 2.99	9.74 ± 1.35	8.66 ± 2.42	2.81 ± 1.81
12:0 CoA	3.55 ± 1.22	19.66 ± 4.95	365.17 ± 44.89	31.28 ± 6.62	21.75 ± 7.12	21.61 ± 1.80	8.76 ± 1.42	11.42 ± 1.16	5.34 ± 1.66
14:0 CoA	22.49 ± 0.41	29.67 ± 6.21	48.38 ± 1.37	363.56 ± 55.25	50.23 ± 3.23	37.97 ± 2.32	14.47 ± 1.68	15.35 ± 3.20	16.02 ± 3.81
16:0 CoA	36.81 ± 1.56	77.10 ± 17.15	34.70 ± 4.57	72.05 ±13.08	316.58 ±17.96	39.31 ± 8.12	14.75 ± 0.58	15.83 ± 1.50	23.71 ± 2.12
18:0 CoA	23.33 ± 3.15	29.87 ± 7.66	9.65 ± 1.73	12.22 ± 1.89	39.95 ± 2.97	298.33 ±27.36	11.19 ± 1.83	15.14 ± 2.03	21.53 ± 1.63
18:1 CoA	92.25 ± 6.74	60.85 ± 16.74	30.55 ± 2.97	47.97 ± 11.38	103.78 ± 8.36	172.85 ± 9.33	545.17 ± 46.29	762.97 ± 24.58	63.04 ± 11.62
18:2 CoA	4.38 ± 4.48	4.37 ± 0.71	2.84 ± 0.67	4.23 ± 1.25	8.14 ± 1.38	6.47 ± 1.69	14.34 ± 1.61	6.73 ± 1.17	5.45 ± 2.55
20:0 CoA	2.28 ± 0.18	3.66 ± 1.05	0.72 ± 0.76	1.39 ± 1.14	1.17 ± 0.68	8.43 ± 2.15	0.74 ± 0.32	0.92 ± 0.43	38.94 ± 11.49
20:4 CoA	8.03 ± 2.09	2.92 ± 0.27	1.14 ± 0.66	3.41 ± 1.66	5.77 ± 2.28	4.16 ± 1.33	10.29 ± 2.81	2.84 ± 0.92	2.65 ± 1.57

DU145	Control	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:1 EA	C20:0
10:0 CoA	0.73 ± 0.38	184.82 ± 32.23	11.74 ± 0.81	1.23 ± 0.45	2.32 ± 0.58	1.31 ± 0.37	4.67 ± 1.15	1.34 ± 0.74	0.52 ± 0.10
12:0 CoA	0.58 ± 0.15	7.39 ± 0.72	233.04 ± 5.58	5.59 ± 1.76	2.47 ± 0.53	0.62 ± 0.59	0.78 ± 0.70	2.36 ± 1.68	0.56 ± 0.26
14:0 CoA	28.78 ±0.67	30.37 ± 2.56	36.67 ± 1.65	260.33 ± 30.37	36.66 ± 3.52	22.86 ± 2.84	15.34 ± 4.29	25.75 ± 7.58	10.16 ± 0.62
16:0 CoA	43.61 ±2.17	54.59 ± 6.13	37.48 ± 1.30	48.69 ± 4.42	555.89 ± 24.79	20.00 ± 2.45	6.28 ± 1.43	18.90 ± 2.81	10.57 ± 2.60
18:0 CoA	28.96 ±5.28	24.15 ± 3.67	7.62 ± 1.73	8.59 ± 0.98	42.07 ± 2.33	281.53 ± 13.10	5.97 ± 0.71	28.18 ± 8.73	6.27 ± 0.36
18:1 CoA	75.54 ±8.97	37.48 ± 6.31	25.37 ± 0.17	25.85 ± 2.44	94.76 ± 6.47	53.20 ± 1.25	532.53 ±31.64	552.21 ± 85.21	11.19 ± 1.48
18:2 CoA	7.49 ± 2.63	6.28 ± 0.41	3.41 ± 0.85	3.71 ± 0.99	8.89 ± 1.93	6.92 ± 1.21	7.37± 2.30	5.06 ± 1.40	5.49 ± 1.67
20:0 CoA	2.34 ±	3.80 ± 1.49	0.95 ± 0.23	1.08 ± 0.56	2.14 ± 0.85	11.17 ± 2.80	0.75 ± 0.47	2.29 ± 0.91	23.24 ± 1.47

	0.36								
20:4 CoA	3.70 ± 0.97	4.04 ± 0.38	1.94 ± 0.53	2.17 ± 0.45	3.23 ± 0.97	3.12 ± 0.74	8.39 ± 0.51	1.92 ± 0.22	3.78 ± 0.99

HepG2	Control	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:1 EA	C20:0
10:0 CoA	5.16 ± 1.07	132.57 ± 18.76	23.51 ± 1.69	7.92 ± 0.17	5.84 ± 0.49	5.61 ± 2.86	6.65 ± 1.34	5.97 ± 1.00	6.13 ± 1.67
12:0 CoA	6.90 ± 0.35	12.87 ± 2.36	155.32 ± 9.00	20.51 ± 3.52	9.32 ± 1.23	10.16 ± 3.19	8.70 ± 1.63	9.95 ± 0.28	10.04 ± 1.75
14:0 CoA	90.62 ± 11.26	54.91 ± 9.23	67.8 ± 2.29	232.91 ± 58.40	82.33 ± 5.63	104.6 ± 21.37	101.23 ± 7.87	110.71 ± 18.00	104.31 ± 10.77
16:0 CoA	143.89 ± 17.40	81.25 ± 8.43	92.46 ± 4.42	132.75 ± 5.73	237.55 ± 10.31	219.25 ± 42.27	147.12 ± 7.24	183.35 ± 19.71	179.17 ± 22.09
18:0 CoA	58.59 ± 9.10	36.84 ± 2.70	31.98 ± 2.64	36.81 ± 2.81	64.05 ± 7.14	208.47 ± 37.44	47.93 ± 4.61	67.40 ± 14.92	66.7 ± 12.69
18:1 CoA	262.57 ± 31.98	123.24 ± 7.97	151.9 ± 12.23	206.88 ± 17.71	275.4 ± 16.33	445.83 ± 86.15	526.67 ± 22.31	664.62 ± 99.36	332.83 ± 47.03
18:2 CoA	17.47 ± 2.84	6.82 ± 0.99	8.83 ± 0.92	15.24 ± 3.17	17.49 ± 1.74	21.99 ± 5.13	20.93 ± 3.40	27.84 ± 2.32	18.67 ± 3.34
18:3 CoA	1.35 ± 0.26	0.53 ± 0.30	0.83 ± 0.52	0.54 ± 0.47	0.69 ± 0.66	1.63 ± 1.01	0.86 ± 0.78	1.34 ± 0.74	1.46 ± 0.41
20:0 CoA	2.51 ± 0.20	2.29 ± 0.42	1.12 ± 0.07	1.85 ± 0.06	1.46 ± 0.39	4.56 ± 1.01	1.43 ± 1.26	1.56 ± 0.61	21.83 ± 2.24
20:4 CoA	5.32 ± 0.59	2.46 ± 0.45	2.45 ± 0.39	2.85 ± 1.78	3.43 ± 1.39	6.40 ± 2.87	9.75 ± 2.16	4.84 ± 4.10	6.78 ± 0.70

Hep3B	Control	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:1 EA	C20:0
10:0 CoA	3.61 ± 0.38	49.26 ± 6.96	13.12 ± 2.56	5.51 ± 0.66	5.14 ± 1.03	4.83 ± 0.30	4.73 ± 0.18	8.39 ± 2.36	4.74 ± 0.67
12:0 CoA	1.20 ± 0.57	7.51 ± 0.85	48.95 ± 1.88	8.19 ± 1.07	2.61 ± 0.60	2.44 ± 0.63	2.10 ± 0.22	3.14 ± 0.48	1.92 ± 0.82
14:0 CoA	31.51 ± 2.26	56.12 ± 2.81	78.95 ± 4.26	238.85 ± 12.68	41.08 ± 4.25	49.08 ± 2.10	51.32 ± 6.90	73.51 ± 6.54	50.61 ± 6.49
16:0 CoA	42.79 ± 9.33	61.11 ± 3.01	48.52 ± 1.03	84.26 ± 4.18	92.00 ± 9.78	49.87 ± 7.90	56.82 ± 10.20	57.57 ± 7.06	76.09 ± 5.33
18:0 CoA	7.73 ± 1.31	9.32 ± 3.34	4.51 ± 0.53	8.93 ± 0.45	11.43 ± 1.37	33.53 ± 2.86	12.25 ± 3.41	14.11 ± 0.72	12.25 ± 1.71
18:1 CoA	35.01 ± 11.58	40.64 ± 1.80	24.98 ± 1.93	43.22 ± 4.61	41.44 ± 4.39	50.35 ± 3.45	153.16 ± 22.16	187.26 ± 25.99	67.40 ± 8.07
18:2 CoA	28.29 ± 6.72	36.71 ± 0.83	27.51 ± 2.72	40.80 ± 6.42	34.27 ± 3.23	41.27 ± 3.96	46.86 ± 5.39	58.37 ± 10.08	45.90 ± 8.59

18:3 CoA	2.52 ± 1.03	3.58 ± 1.05	4.13 ± 0.20	6.19 ± 0.45	3.80 ± 0.58	3.06 ± 0.86	2.14 ± 0.61	2.12 ± 0.25	3.31 ± 0.50
20:0 CoA	0.53 ± 0.08	0.14 ± 0.02	0.50 ± 0.33	0.47 ± 0.22	0.39 ± 0.07	0.94 ± 0.45	1.20 ± 0.11	0.97 ± 0.23	1.75 ± 0.55
20:4 CoA	7.63 ± 2.53	10.26 ± 0.29	10.34 ± 1.12	17.44 ± 2.02	13.96 ± 1.67	15.75 ± 3.34	21.21 ± 2.54	16.44 ± 2.45	15.17 ± 3.75

Table S2. Amounts of acyl-CoAs (pmol/mg protein) in PNT2, DU145, HepG2 and Hep3B cells (n = 3) incubated with 400 μM fatty acids for 24 h.