SUPPLEMENTARY FILE

Urinary metabolite profiling to non-invasively monitor the omega-3 index: An exploratory secondary analysis of a randomized clinical trial in young adults.

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Table S1: Full annotation of the 17 urinary metabolites significantly associated with n3-LCPUFA supplementation or the O3I reported in Table 3.

Metabolite/Identifier[1]	*m/z*:RMT:mode[2]	Molecular Formula[2]	Mass Error[2] (ppm)
Choline	104.108:0.333:p	$C_5H_{14}NO$	2.9
Tiglylglycine (HMBD0000959)	156.066:0.843:n	$C_7H_{11}NO_3$	4.5
S-Carboxypropylcysteamine (HMDB0002169)	164.074:0.613:p	$C_6H_{13}NO_2S$	1.8
N1-Acetylspermidine (HMDB0001276)	188.175:0.259:p	$C_9H_{21}N_3O$	6.4
Quinic acid (HMDB0003072)	191.056:0.798:n	$C_7H_{12}O_6$	4.2
Glucuronic acid (HMDB0000127)	193.035:0.772:n	$C_6H_{10}O_7$	2.6
Tryptophan	205.098:0.899:p	$C_{11}H_{12}N_2O_2$	2.9
Unknown dianion $[M-H_2]^{2-}$	221.075:0.927:n	$C_{22}H_{28}N_4O_2S_2$	--
Carboxybutylhomocysteine	222.080:0.762:p	$C_8H_{15}NO_4S$	2.7
O-Butyrylcarnitine (HMDB0002013)	232.155:0.718:p	$C_8H_{10}NO_4$	4.7
Pyroglutamylisoleucine (HMDB0341381)	241.120:0.653:n	$C_{11}H_{18}N_2O_4$	6.7
Pyroglutamylleucine (HMDB0341382)	241.120:0.662:n	$C_{11}H_{18}N_2O_4$	3.0
Unknown cation isobar#2	258.110:0.788:p	$C_{11}H_{19}N_3S_2$	--
Unknown cation	300.215:0.841:p	$C_{17}H_{25}N_5$	--
Tetrahydroaldosterone glucuronide (HMDB0010357)	539.249:0.472:n	$C_{27}H_{40}O_{11}$	0.6
Unknown dianion $[M-H_2]^{2-}$	88.004:1.622:n	$C_4H_6N_2O_6$	5.7
Symmetric dimethylarginine	203.151:0.478:p	$C_8H_{18}N_4O_2$	3.4

[1]Putative urinary metabolite identified together with their corresponding HMDB ID# if reported.
[2]All urinary metabolites were annotated based on their accurate mass, relative migration time and ionization mode (m/z:RMT:mode), most likely molecular formulae, and mass error (if structure known).

Table S2: glmmLasso selected urinary metabolites on the Omega-3 Index (O3I) and EPA+DHA (ng/mg Hb) integrated datasets.

		O3I Outcome		EPA+DHA (ng/mg Hb) Outcome	
Metabolite[1]	***m/z*:RMT:mode**	**Estimate**	**P value**	**Estimate**	**P value**
Unknown dianion $[M-H_2]^{2-}$	221.068:0.927:n	1.079	< 0.001	0.832	< 0.001
S-Carboxypropylcysteamine	164.074:0.613:p	0.501	< 0.001	0.535	< 0.001
Tetrahydroaldosterone glucuronide	539.249:0.472:n	0.365	< 0.001	0.264	< 0.001
N1-Acetylspermidine	188.175:0.259:p	−0.538	< 0.001	−0.858	< 0.001
Symmetric dimethylarginine	203.151:0.478:p	-	-	0.551	< 0.001
Tryptophan	205.098:0.899:p	-	-	0.166	< 0.001
Carboxybutylhomocysteine	222.080:0.762:p	-	-	−0.234	< 0.001

[1]Putative urinary metabolite as annotated by its accurate mass, relative migration time and ionization mode (m/z:RMT:mode). Most likely molecular formulae are provided for unknown chemical structures.

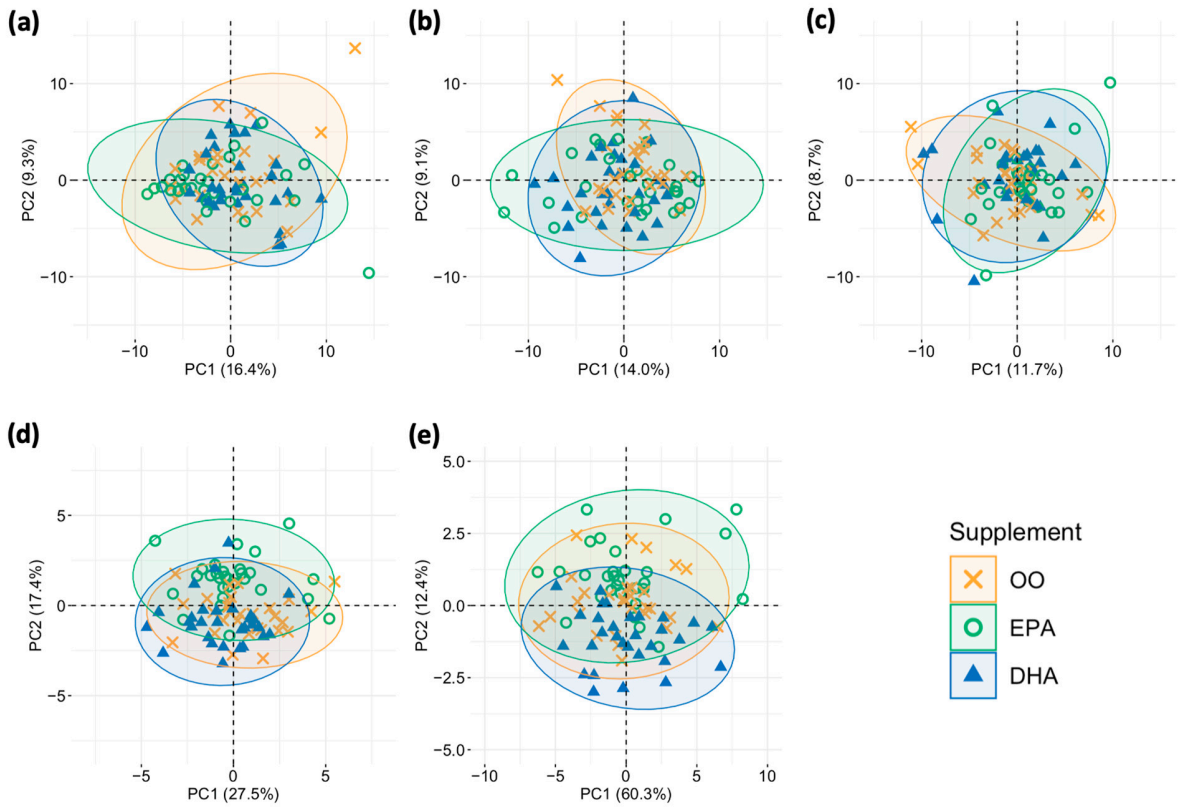

Figure S1. PCA plots of (**a**) baseline and (**b**) final (12 weeks) of 124 creatinine-normalized urinary metabolites for control (OO), EPA, and DHA groups (OO = orange, EPA = green, DHA = blue). Total variation explained in PC1 and PC2 plots was 25.7% and 23.1% respectively for each time point (**a, b**). (**c**) PCA plots for 88 participant delta values of 124 urinary metabolites using RPA; (**d**) 15 qualitative FA measurements (relative % FA), and (**e**) 15 quantitative FA measurements (ng FA/mg Hb). Total variation explained in PC1 and PC2 plots was 20.4%, 44.9%, 72.7% respectively for each dataset (**c-e**).

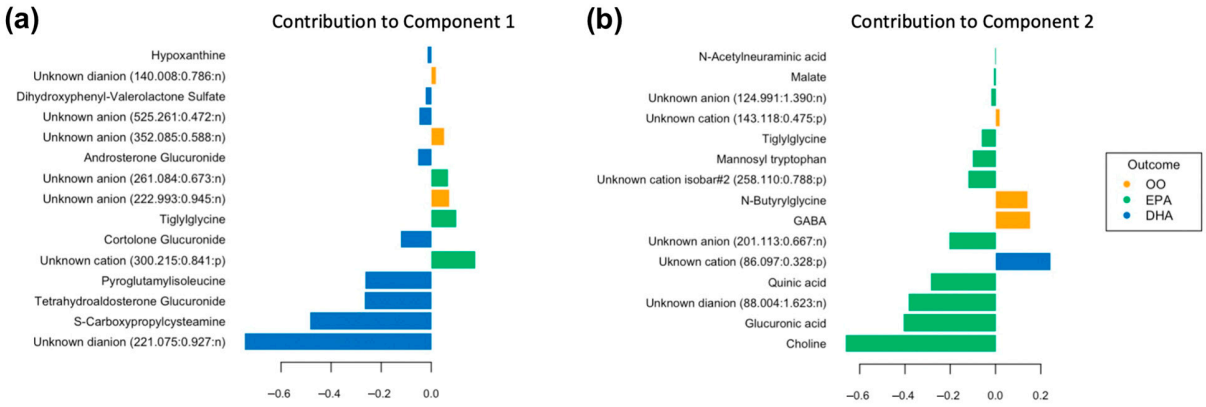

Figure S2. sPLS-DA loading weights reflecting the contribution for 15 urinary metabolites on (**a**) PC1 and (**b**) PC2 coloured by group (OO = orange, EPA = green, DHA = blue). All unknown metabolites are annotated based on their characteristic *m/z*:RMT:mode.

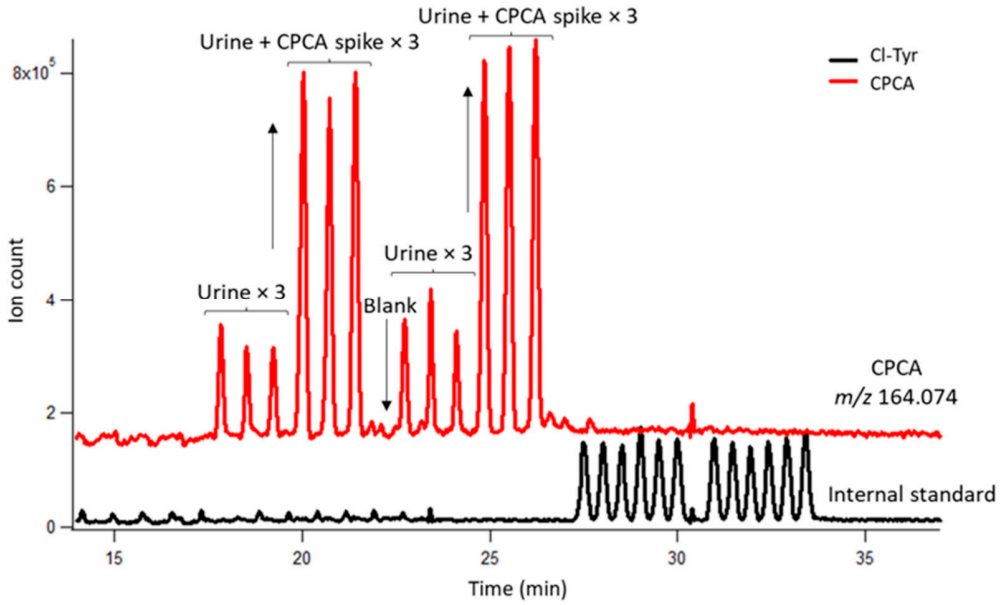

Figure S3. Tentative identification of CPCA based on spiking into pooled urine when using MSI-CE-MS with a 13 serial sample injection format, which confirmed its co-migration with the unknown sulfur-containing metabolite (*m/z* 164.074) under positive ion mode conditions.

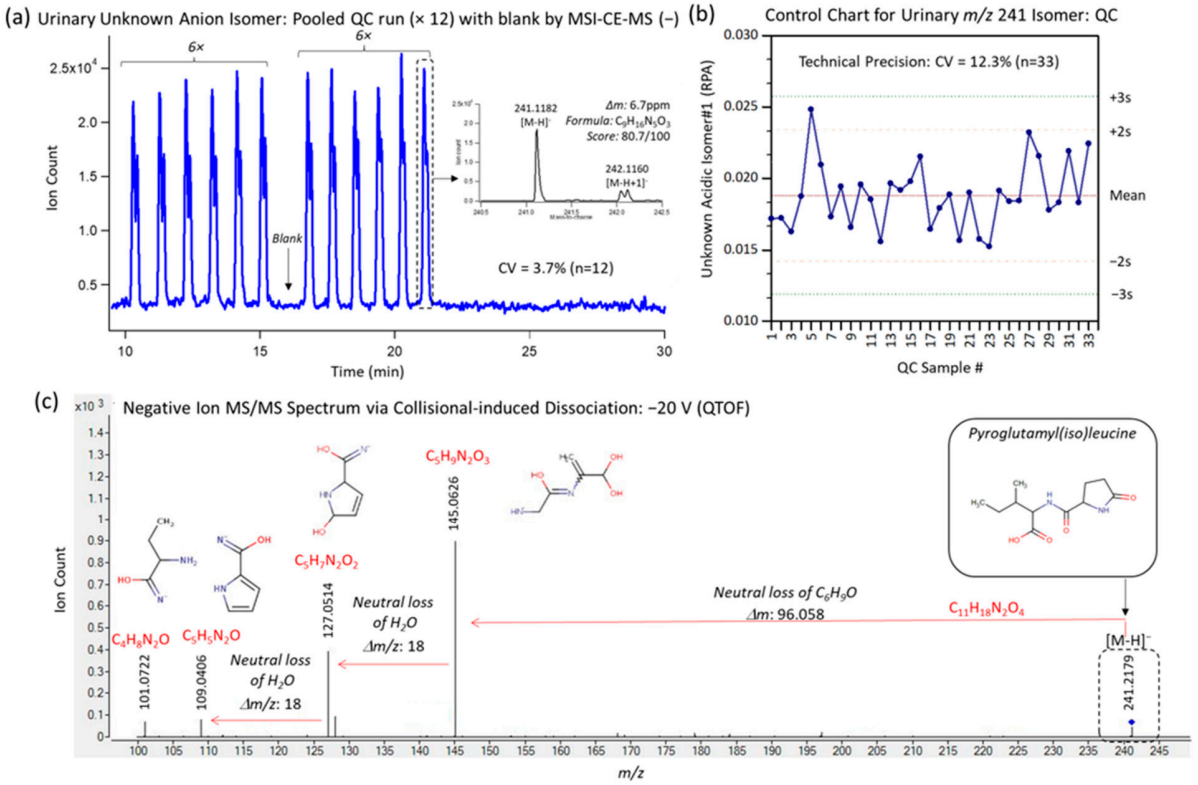

Figure S4. Tentative identification of two unknown anionic urinary metabolites that migrate as partially resolved isomers by MSI-CE-MS under negative ion mode conditions. (**a**) Representative extracted ion electropherogram (EIE) when using MSI-CE-MS under negative ion mode for the unknown anion isomer highlighting a serial injection of 12 urine samples and a blank in a single run. The first migrating isomer (241.120:0.653:n) was the determined as the third most significant urinary metabolite associated with the O3I notably following DHA ingestion. (**b**) Technical precision of first migrating unknown anion isomer in QC samples (CV = 12.3%, n = 33). (**c**) Annotation of the first migrating unknown anion isomer MS/MS at an optimal collision energy, tentatively identified as pyroglutamylisoleucine (pGlu-Ile), whereas its slower migrating isomer is likely pyroglutamylleucine (pGlu-Leu).

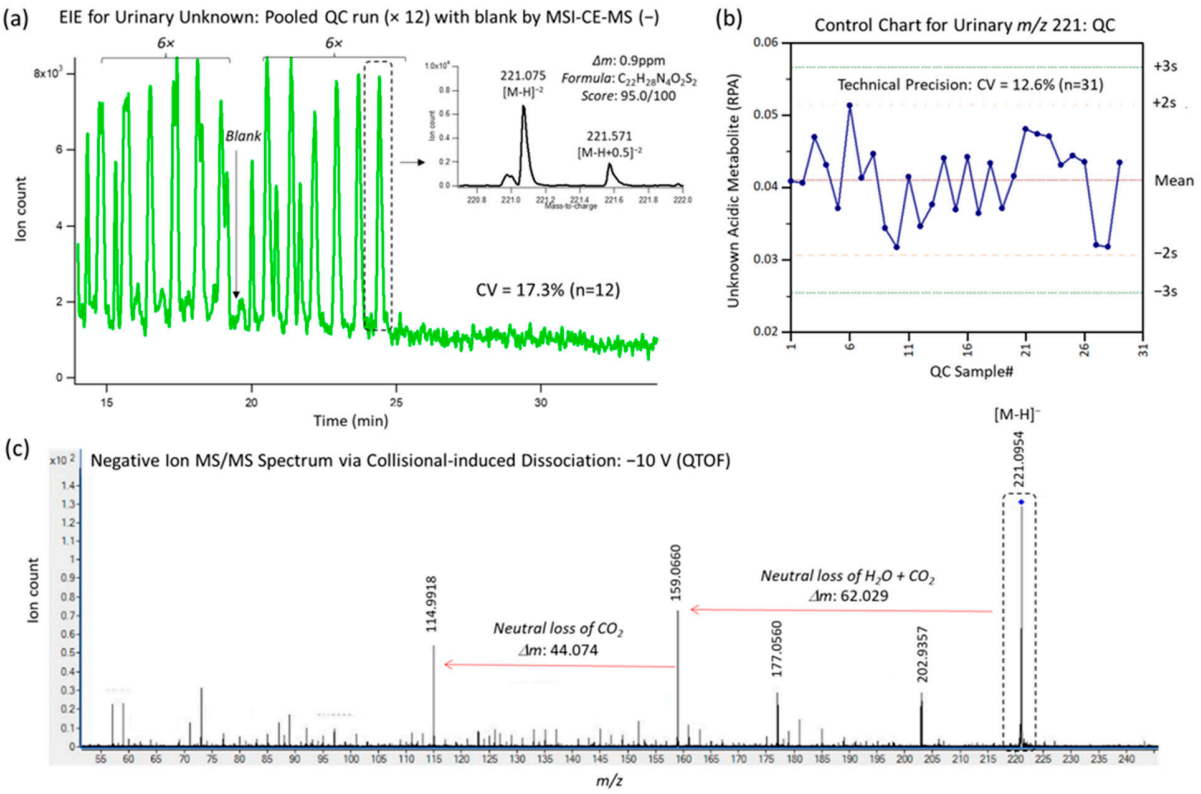

Figure S5. Annotation of an unknown urinary dianion (221.075:0.927:n; $[M-2H]^{2-}$) based on its (**a**) extracted ion electropherogram (EIE) when using MSI-CE-MS under negative ion mode demonstrating a charge state (i.e., isotopic pattern) that was associated with DHA supplementation. Although this acidic metabolite was measured with (**b**) adequate technical precision in QC samples (CV < 15%, n = 31), its low abundance prevented acquisition of a good quality MS/MS spectra under negative ion mode conditions. (**c**) Collisional-induced dissociation experiments confirmed the likely presence of two acidic functional moieties (-COOH) based on corresponding neutral mass losses.

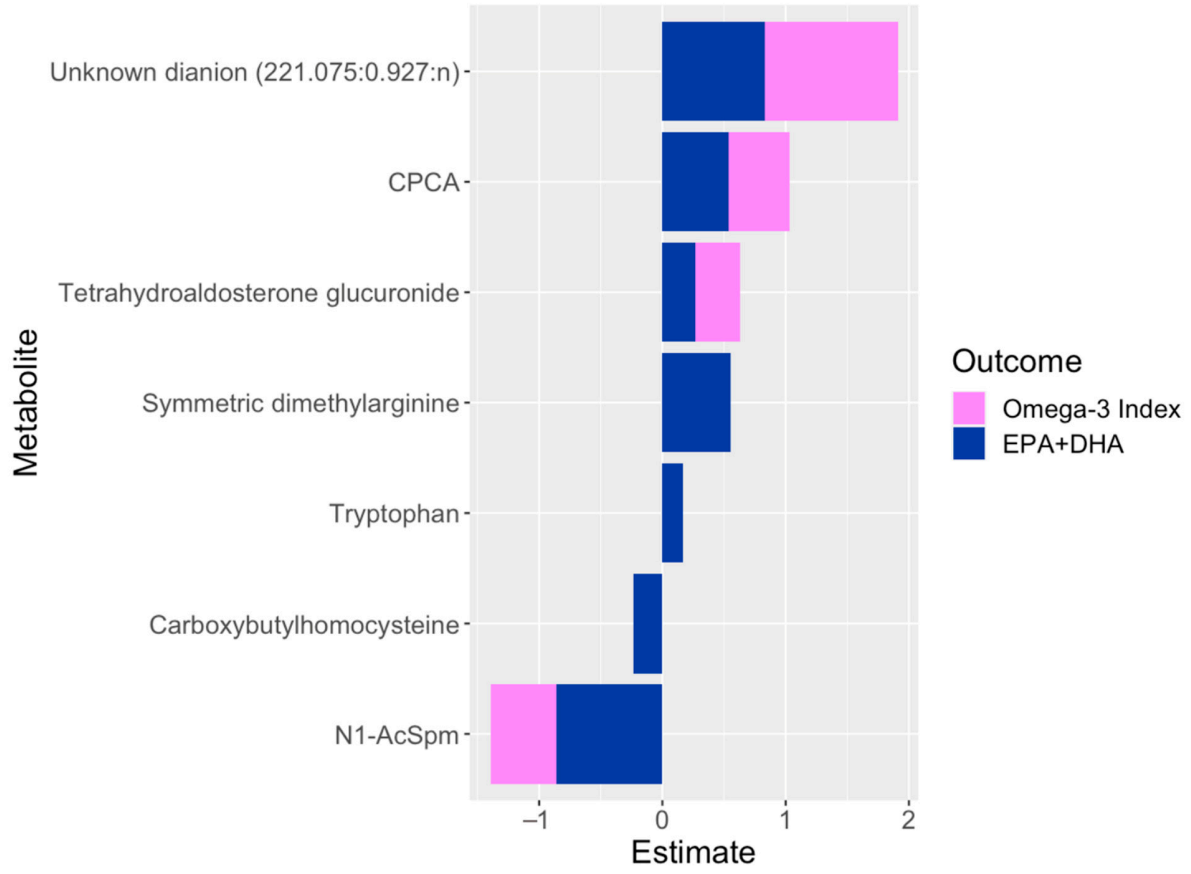

Figure S6. Estimate values for glmmLasso urinary metabolites from two integrated datasets with scaled from 0-10 or 80-700 urine metabolites as covariates and the O3I or EPA+DHA (ng/mg Hb), respectively, as outcome variables. Urinary metabolites with a positive estimate result indicate a positive association with the outcome variable and metabolites with a negative estimate result indicate a negative association with the outcome variable. Estimates from values from the O3I (pink) and EPA+DHA (ng/mg Hb) (navy) are depicted in stacked bars. Unknown metabolites were annotated based on their characteristic m/z:RMT:mode. CPCA = 2-Carboxypropylcysteamine, *N1*-AcSpm = *N1*-Acetylspermidine.

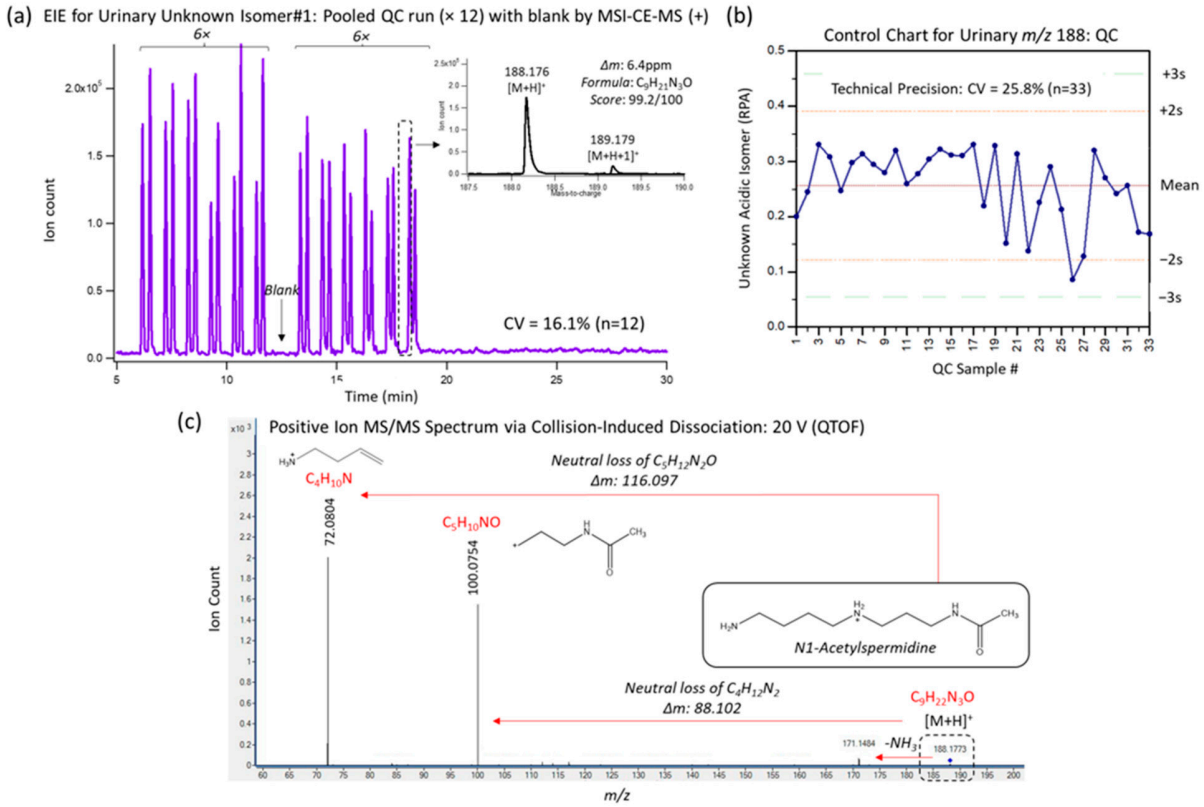

Figure S7. Tentative identification of two unknown cationic urinary metabolites that (**a**) migrate as partially resolved isomers by MSI-CE-MS under positive ion mode conditions. However, only the first migrating isomer (188.175:0.259:p) was associated with the O3I. (**b**) This unknown isomer was measurable with acceptable technical precision in QC samples. (**c**) Annotation of this first migrating isomer MS/MS at an optimal collision energy, tentatively identified as *N1*-acetylspermidine (*N1*-AcSpm), whereas its slower migrating isomer is likely *N8*-acetylspermidine (*N8*-AcSpm).

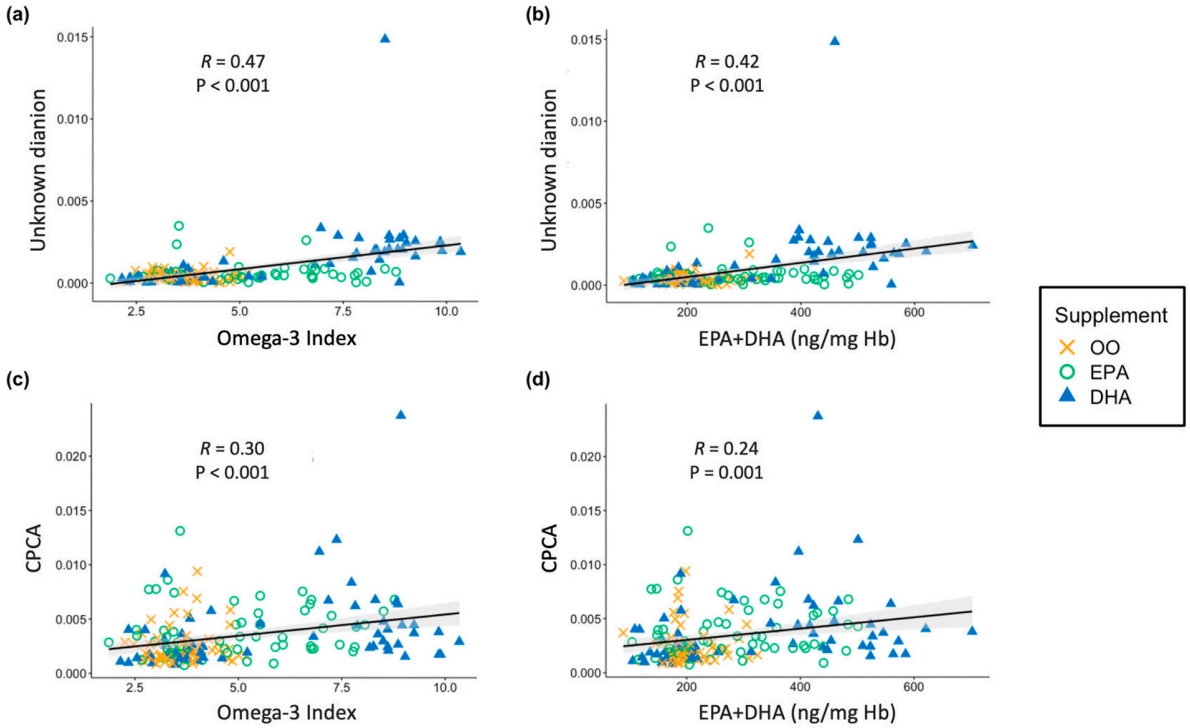

Figure S8. Correlation plots for the (**a**) unknown dianion (221.075:0.927:n) and the O3I (R = 0.47, P < 0.001); (**b**) unknown dianion (221.075:0.927:n) and the sum of EPA+DHA (ng/mg Hb) (R = 0.042, P < 0.001); (**c**) *S*-carboxypropylcysteamine (CPCA; 164.074:0.613:p) and the O3I (R = 0.30, P < 0.001); and (**d**) CPCA and the sum of EPA+DHA (ng/mg Hb) (R = 0.24, P = 0.001). OO = yellow, EPA = green, DHA = blue.