

Metabolomics reveals the impact of saturation of dietary lipids on aging and longevity of *C. elegans*

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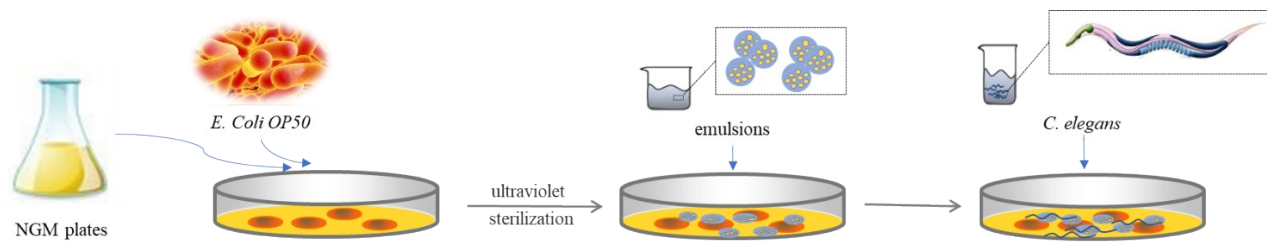


Figure S1. The delivery method of emulsions for *Caenorhabditis elegans* (*C. elegans*). One day prior to the experiment, test plates were coated with *E. coli OP50* solutions. The next day, the plates were treated with UV light to kill *E. coli OP50*, then 5mg different dietary oils were added onto each plate and 2-day-old synchronized worms (adult) were transferred to the test plates.

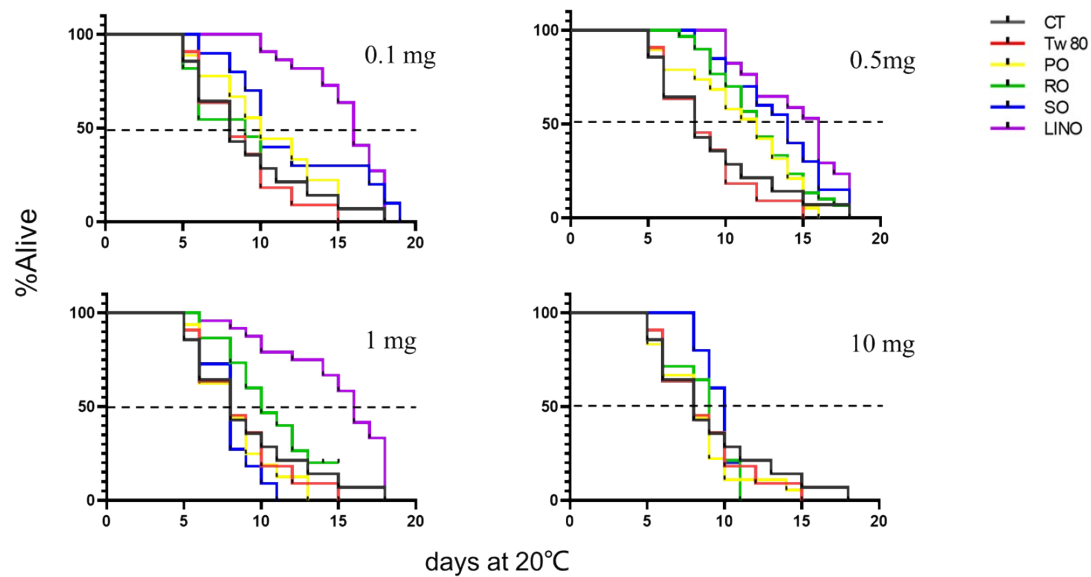


Figure S2. Effects of different dietary oils (concentration at 0.1, 0.5, 1, 10 mg/plate) on lifespan of *C. elegans* at 20 °C. *C. elegans* were synchronized before divided into different groups, all groups were treated with *E. coli* OP50, with or without 5mg different dietary oils (delivered by oil in water (O/W) emulsions using Tween 80 (Tw 80) as the emulsifier) in NGM plates containing 10 μ M 5-fluoro-2'-deoxyuracil (FUDR) and were stated at 20 °C. Each group had 3-5 biological replicates and each plate had at least 40 worms.

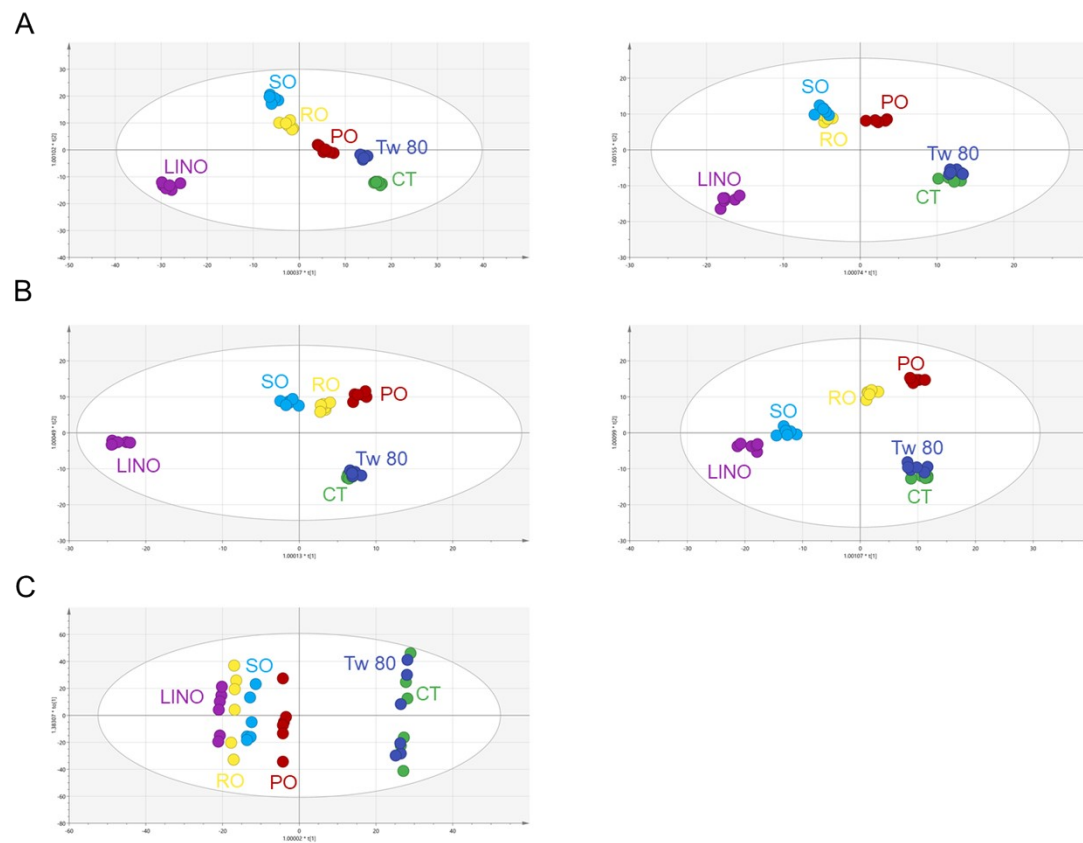


Figure S3. Statistical analysis of metabolomic profiles in *C. elegans*. (A) OPLS-DA scores of *C. elegans* samples detected by LC-MS using C18 positive and negative mode. (B) OPLS-DA

scores of *C. elegans* samples detected by LC-MS using HILIC positive and negative mode. (C) OPLS-DA scores of *C. elegans* samples detected by GC-MS.

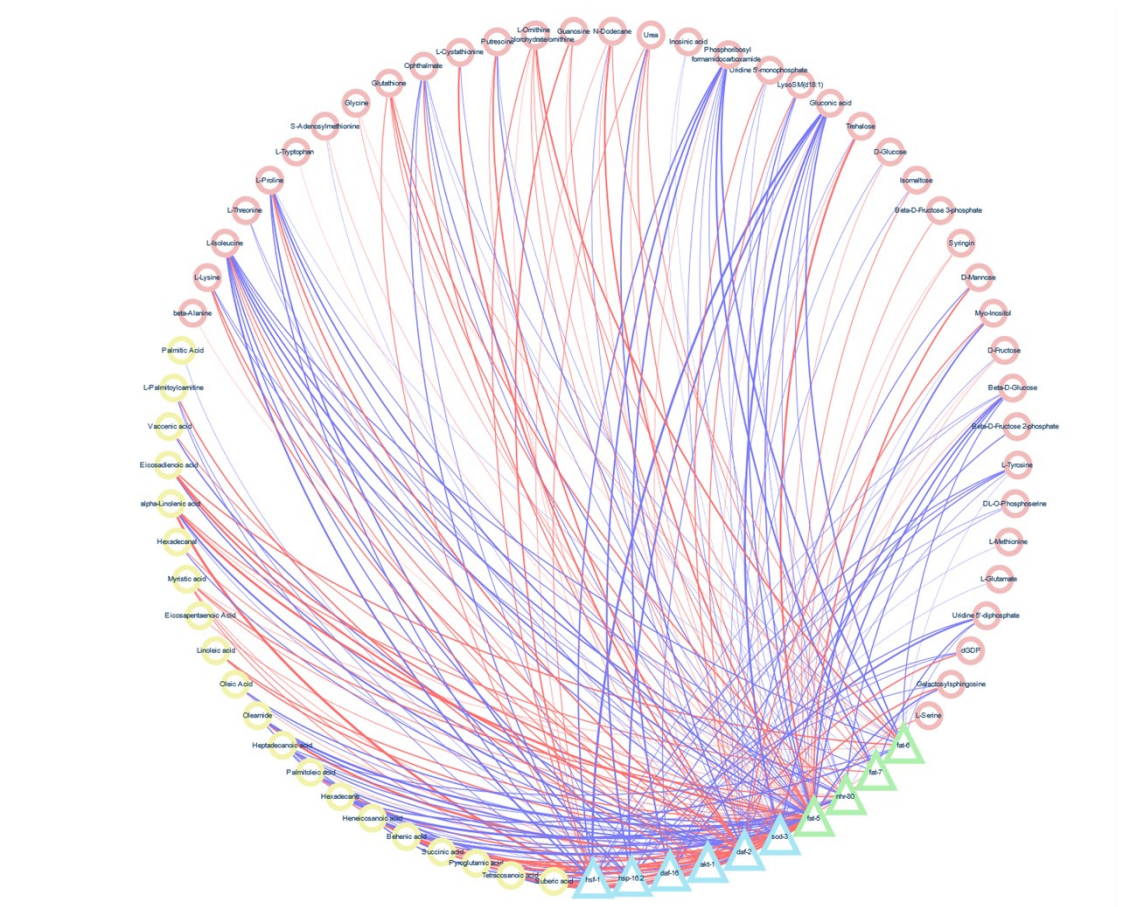


Figure S4. Correlation analysis between differential metabolites and gene expressions. Spearman bivariate correlate analysis was displayed between differential metabolites and gene expression levels altered by different dietary lipids ingestion. The bend lines portend a relation between metabolites and genes, the width means the strength of relevance. The color of lines states a positive (red) and a negative (blue) association. The circle shape delegate metabolites in IIS pathway (pink) and biosynthesis of USFAs metabolism pathway (yellow); the triangle shape represent genes in IIS pathway (blue) and biosynthesis of USFAs metabolism pathway (green).

Table S1. The fatty acids composition of dietary oils

Dietary oil constitution (%)	constitution (%)									
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C22:1	C20:4
PO	0.808	43.564	/	4.128	42.867	8.313	0.32	/	/	/
RO	/	4.359	0.113	1.61	66.932	18.412	7.573	0.36	0.186	0.456
SO	/	6.742	/	2.421	25.213	65.296	/	/	0.328	/
LINO	/	6.56	/	4.815	20.757	16.598	51.27	/	/	/

Table S2. Raw data of average lifespan of *C. elegans* treated with different dietary oils.

Group Name	Replicate 1 (days)	Replicate 2 (days)	Replicate 3 (days)
CT	16.00±3.63	16.31±3.59	15.56±3.87
Tw 80	15.71±3.12	17.38±4.51	17.68±4.50
PO	17.54±4.33	16.48±4.46	16.12±4.84
RO	17.61±3.68	18.67±4.65	18.63±4.68
SO	19.37±3.78	19.89±4.65	20.23±3.97
LINO	20.96±4.79	19.43±5.39	20.41±4.94

Table S3. Primer sequences

Gene Name	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
<i>act-1</i>	ACGAGGTTGCCGCTCTTGTTG	ACGAGTCCTTCTGTCCCATAACCG
<i>daf-2</i>	CCACGACGACGAGCACATCAC	GGCGGGTTTTCTCATAGCAGTC
<i>akt-1</i>	CGTTCTTGCCGGATACCTTCAC	TCGGCTCGTCATTTCAGATTCTTCATC
<i>daf-16</i>	CGCCGCTACCATCTGACATCAC	TCCGCCAAAAGAAGCCGAACG
<i>hsf-1</i>	AGGAGGACCATCTACATCATCTTCGG	TTCTATTCACCTGAGCCATTTGCCTA G
<i>hsp-16.2</i>	GATGTAGATGTTGGTGCAGTTGCTTC	CTTCCTGAACCGCTTCTTTCTTTGG
<i>sod-3</i>	TCACTATTGCGGTTCAAGGCTCTG	TTGCACAGGTGGCGATCTTCAAG
<i>nhr-80</i>	TTTGAAGTTTGCTAGGCGAGAGAGTG	TAGACAACGAGAGAGTGTGGTGGAG
<i>fat-5</i>	GCCCTCTCCGTTACTGCTTCAC	CTTCTCCGACTGCCGCAATAGATG
<i>fat-6</i>	TCGGAGAGGGAGGTCACAACCTTC	CGGTCGTAGACAAGTCCAAGAGC
<i>fat-7</i>	ATCGTTGCCATCACAAGTGGACTG	TTACGCACAAGAAGCCATCCCATG

Table S4. Raw data of differential metabolites. The original data of LC-MS and GC-MS were converted into mzData format before loading to MZmine 2.53 for baseline corrections, peak detection and data normalization. The vacant values were filled with half of the minimum value in each dataset.

Table S5. Differential metabolites affect specific parameters of main metabolic pathways. The verified metabolites were used for pathway enrichment using MetaboAnalyst 5.0.

Pathway Name	Total	Hits	Expect	P value	Holm P	FDR
Aminoacyl-tRNA biosynthesis	48	10	2.59	1.45E-4	0.0122	0.0122
Biosynthesis of unsaturated fatty acids	36	8	1.94	4.44E-4	0.0368	0.0186
Starch and sucrose metabolism	18	4	0.97	0.0134	1.0	0.311
Glutathione metabolism	28	5	1.51	0.0148	1.0	0.311
Glycine, serine and threonine metabolism	33	5	1.78	0.029	1.0	0.405
Cysteine and methionine metabolism	33	5	1.78	0.029	1.0	0.405
D-Glutamine and D-glutamate metabolism	6	2	0.323	0.0373	1.0	0.448
Galactose metabolism	27	4	1.45	0.0533	1.0	0.523
Purine metabolism	65	7	3.5	0.056	1.0	0.523
Valine, leucine and isoleucine biosynthesis	8	2	0.431	0.065	1.0	0.546
Neomycin, kanamycin and gentamicin biosynthesis	2	1	0.108	0.105	1.0	0.801
Arginine and proline metabolism	38	4	2.05	0.144	1.0	0.984
Alpha-Linolenic acid metabolism	13	2	0.7	0.152	1.0	0.984

Table S6. Raw data of correlations between differential metabolites and gene expressions. The spearman

bivariate correlate analysis was performed through SPSS version 20, *p*-values less than 0.05 were identified as significant.

Metabolites	Gene	<i>r</i>-value	<i>p</i>-value	Realation
Suberic acid	sod-3	0.654	0.001	positive
Tetracosanoic acid	sod-3	0.546	0.007	positive
Behenic acid	sod-3	0.553	0.006	positive
Heneicosanoic acid	sod-3	0.514	0.012	negative
Hexadecane	sod-3	0.501	0.015	negative
Heptadecanoic acid	sod-3	0.594	0.003	negative
Oleamide	sod-3	0.657	0.001	negative
Oleic Acid	sod-3	0.51	0.013	negative
Linoleic acid	sod-3	0.605	0.003	positive
Eicosadienoic acid	sod-3	0.699	0	positive
L-Isoleucine	sod-3	0.591	0.003	negative
L-Threonine	sod-3	0.47	0.024	negative
L-Proline	sod-3	0.524	0.01	negative
L-Tyrosine	sod-3	0.48	0.02	negative
L-Serine	sod-3	0.423	0.044	positive
Glutathione	sod-3	0.509	0.013	positive
Ophthalmate	sod-3	0.521	0.011	negative
L-Ornithine monochlorohydrate/ornithine	sod-3	0.498	0.016	positive
Guanosine	sod-3	0.441	0.035	positive
N-Dodecane	sod-3	0.456	0.029	positive
Urea	sod-3	0.461	0.027	positive
Phosphoribosyl formamidocarboxamide	sod-3	0.491	0.017	negative
LysoSM(d18:1)	sod-3	0.465	0.025	negative
Gluconic acid	sod-3	0.733	0	negative
Trehalose	sod-3	0.458	0.028	positive
Syringin	sod-3	0.432	0.039	positive
Myo-Inositol	sod-3	0.431	0.04	negative
D-Fructose	sod-3	0.421	0.045	positive
Beta-D-Glucose	sod-3	0.65	0.001	negative
Tetracosanoic acid	nhr-80	0.426	0.048	positive
Heneicosanoic acid	nhr-80	0.581	0.005	negative
Hexadecane	nhr-80	0.811	0	negative
Palmitoleic acid	nhr-80	0.442	0.039	negative
Heptadecanoic acid	nhr-80	0.507	0.016	negative
Myristic acid	nhr-80	0.598	0.003	positive

alpha-Linolenic acid	nhr-80	0.453	0.034	positive
L-Lysine	nhr-80	0.505	0.017	negative
L-Isoleucine	nhr-80	0.642	0.642	negative
L-Proline	nhr-80	0.528	0.012	negative
Glutathione	nhr-80	0.598	0.003	positive
Putrescine	nhr-80	0.467	0.028	negative
Inosinic acid	nhr-80	0.424	0.049	negative
Phosphoribosyl formamidocarboxamide	nhr-80	0.496	0.019	negative
LysoSM(d18:1)	nhr-80	0.474	0.026	negative
Gluconic acid	nhr-80	0.506	0.016	negative
Beta-D-Glucose	nhr-80	0.586	0.042	negative
Galactosylsphingosine	nhr-80	0.438	0.042	negative
L-Palmitoylcarnitine	hsp-16.2	0.495	0.027	negative
Palmitic Acid	hsp-16.2	0.453	0.045	negative
Suberic acid	hsp-16.2	0.503	0.024	positive
Heptadecanoic acid	hsp-16.2	0.518	0.019	negative
Myristic acid	hsp-16.2	0.519	0.019	positive
L-Isoleucine	hsp-16.2	0.592	0.006	negative
L-Proline	hsp-16.2	0.658	0.002	negative
L-Tyrosine	hsp-16.2	0.564	0.01	negative
Ophthalmate	hsp-16.2	0.515	0.02	negative
dGDP	hsp-16.2	0.514	0.02	negative
Phosphoribosyl formamidocarboxamide	hsp-16.2	0.679	0.001	negative
Uridine 5'-diphosphate	hsp-16.2	0.571	0.008	negative
Gluconic acid	hsp-16.2	0.704	0.001	negative
Beta-D-Glucose	hsp-16.2	0.477	0.033	negative
L-Palmitoylcarnitine	hsf-1	0.517	0.02	negative
Suberic acid	hsf-1	0.751	0	positive
Heneicosanoic acid	hsf-1	0.627	0.003	negative
Heptadecanoic acid	hsf-1	0.517	0.02	negative
Oleamide	hsf-1	0.58	0.007	negative
Myristic acid	hsf-1	0.519	0.019	positive
alpha-Linolenic acid	hsf-1	0.802	0	positive
L-Isoleucine	hsf-1	0.691	0.001	negative
L-Proline	hsf-1	0.591	0.001	negative
L-Tyrosine	hsf-1	0.57	0.009	negative
Glutathione	hsf-1	0.531	0.016	positive
Ophthalmate	hsf-1	0.594	0.006	negative
DL-O-Phosphoserine	hsf-1	0.486	0.03	negative
L-Ornithine monochlorohydrate/ornithine	hsf-1	0.555	0.011	positive
Guanosine	hsf-1	0.629	0.003	positive
N-Dodecane	hsf-1	0.463	0.04	positive
Urea	hsf-1	0.513	0.021	positive
dGDP	hsf-1	0.534	0.015	negative

Phosphoribosyl formamidocarboxamide	hsf-1	0.636	0.003	negative
Gluconic acid	hsf-1	0.774	0	negative
Beta-D-Glucose	hsf-1	0.485	0.03	negative
Galactosylsphingosine	hsf-1	0.523	0.018	negative
Suberic acid	fat-7	0.545	0.011	positive
alpha-Linolenic acid	fat-7	0.561	0.008	positive
L-Isoleucine	fat-7	0.54	0.012	negative
Ophthalmate	fat-7	0.439	0.046	negative
L-Ornithine monochlorohydrate/ornithine	fat-7	0.507	0.019	positive
Urea	fat-7	0.512	0.018	positive
Phosphoribosyl formamidocarboxamide	fat-7	0.686	0.001	negative
Uridine 5'-diphosphate	fat-7	0.591	0.005	negative
Uridine 5'-monophosphate	fat-7	0.462	0.035	negative
Gluconic acid	fat-7	0.512	0.018	negative
Suberic acid	fat-6	0.465	0.025	positive
Hexadecane	fat-6	0.643	0.001	negative
Heptadecanoic acid	fat-6	0.557	0.006	negative
Myristic acid	fat-6	0.445	0.033	positive
Eicosadienoic acid	fat-6	0.613	0.002	positive
L-Isoleucine	fat-6	0.579	0.004	negative
L-Proline	fat-6	0.437	0.037	negative
L-Tyrosine	fat-6	0.434	0.039	negative
Glycine	fat-6	0.423	0.044	positive
Glutathione	fat-6	0.481	0.02	positive
Ophthalmate	fat-6	0.485	0.019	negative
L-Ornithine monochlorohydrate/ornithine	fat-6	0.454	0.03	positive
N-Dodecane	fat-6	0.514	0.012	positive
Phosphoribosyl formamidocarboxamide	fat-6	0.679	0	negative
Uridine 5'-diphosphate	fat-6	0.477	0.021	negative
Gluconic acid	fat-6	0.648	0.001	negative
Beta-D-Glucose	fat-6	0.557	0.006	negative
Suberic acid	fat-5	0.632	0.001	positive
Tetracosanoic acid	fat-5	0.729	0	positive
Pyroglutamic acid	fat-5	0.392	0.048	positive
Succinic acid	fat-5	0.398	0.044	positive
Behenic acid	fat-5	0.682	0	positive
Heneicosanoic acid	fat-5	0.848	0	negative
Hexadecane	fat-5	0.601	0.001	negative
Palmitoleic acid	fat-5	0.635	0	negative
Heptadecanoic acid	fat-5	0.59	0.001	negative
Oleamide	fat-5	0.761	0	negative
Oleic Acid	fat-5	0.625	0.001	negative
Linoleic acid	fat-5	0.589	0.002	positive
Eicosapentaenoic Acid	fat-5	0.487	0.012	positive

Myristic acid	fat-5	0.5	0.009	positive
Hexadecanal	fat-5	0.517	0.007	positive
alpha-Linolenic acid	fat-5	0.759	0	positive
Eicosadienoic acid	fat-5	0.657	0	positive
Vaccenic acid	fat-5	0.528	0.006	positive
beta-Alanine	fat-5	0.421	0.032	positive
L-Lysine	fat-5	0.527	0.006	negative
L-Isoleucine	fat-5	0.67	0	negative
L-Threonine	fat-5	0.484	0.012	negative
L-Proline	fat-5	0.658	0	negative
L-Tryptophan	fat-5	0.422	0.032	positive
L-Serine	fat-5	0.522	0.006	positive
S-Adenosylmethionine	fat-5	0.443	0.024	positive
Glycine	fat-5	0.461	0.018	positive
Glutathione	fat-5	0.575	0.002	positive
Ophthalmate	fat-5	0.581	0.002	negative
L-Cystathionine	fat-5	0.491	0.011	negative
Putrescine	fat-5	0.575	0.002	negative
L-Ornithine monochlorohydrate/ornithine	fat-5	0.479	0.013	positive
Guanosine	fat-5	0.541	0.004	positive
N-Dodecane	fat-5	0.666	0	positive
Urea	fat-5	0.555	0.003	positive
Inosinic acid	fat-5	0.428	0.029	negative
Phosphoribosyl formamidocarboxamide	fat-5	0.479	0.013	negative
Uridine 5'-monophosphate	fat-5	0.476	0.014	negative
LysoSM(d18:1)	fat-5	0.607	0.001	negative
Gluconic acid	fat-5	0.607	0.001	negative
Trehalose	fat-5	0.703	0	positive
D-Glucose	fat-5	0.459	0.018	positive
Isomaltose	fat-5	0.513	0.007	positive
Beta-D-Fructose 3-phosphate	fat-5	0.499	0.009	positive
Syringin	fat-5	0.479	0.013	positive
D-Mannose	fat-5	0.637	0	positive
Myo-Inositol	fat-5	0.666	0	negative
D-Fructose	fat-5	0.448	0.022	positive
Beta-D-Glucose	fat-5	0.646	0	negative
Galactosylsphingosine	fat-5	0.634	0.001	negative
Suberic acid	daf-2	0.468	0.032	negative
Tetracosanoic acid	daf-2	0.648	0.001	negative
Pyroglutamic acid	daf-2	0.501	0.021	negative
Behenic acid	daf-2	0.63	0.002	negative
Heneicosanoic acid	daf-2	0.794	0	positive
Palmitoleic acid	daf-2	0.623	0.003	positive
Heptadecanoic acid	daf-2	0.441	0.046	positive

Oleamide	daf-2	0.771	0	positive
Oleic Acid	daf-2	0.454	0.039	positive
Hexadecanal	daf-2	0.573	0.007	negative
alpha-Linolenic acid	daf-2	0.691	0.001	negative
Eicosadienoic acid	daf-2	0.612	0.003	negative
Vaccenic acid	daf-2	0.621	0.003	negative
L-Lysine	daf-2	0.592	0.005	positive
L-Isoleucine	daf-2	0.563	0.008	positive
L-Proline	daf-2	0.634	0.002	positive
S-Adenosylmethionine	daf-2	0.442	0.045	negative
Ophthalmate	daf-2	0.578	0.006	positive
L-Cystathionine	daf-2	0.669	0.001	positive
Putrescine	daf-2	0.642	0.002	positive
N-Dodecane	daf-2	0.491	0.024	negative
Urea	daf-2	0.629	0.002	negative
Uridine 5'-monophosphate	daf-2	0.558	0.009	positive
LysoSM(d18:1)	daf-2	0.548	0.01	positive
Gluconic acid	daf-2	0.437	0.048	positive
Trehalose	daf-2	0.48	0.028	negative
D-Glucose	daf-2	0.483	0.026	negative
Isomaltose	daf-2	0.486	0.025	negative
Beta-D-Fructose 2-phosphate	daf-2	0.581	0.006	negative
D-Mannose	daf-2	0.562	0.008	negative
Myo-Inositol	daf-2	0.579	0.006	positive
D-Fructose	daf-2	0.467	0.033	negative
Beta-D-Glucose	daf-2	0.449	0.041	positive
Galactosylsphingosine	daf-2	0.613	0.003	positive
Heptadecanoic acid	daf-16	0.456	0.033	negative
Myristic acid	daf-16	0.463	0.03	positive
L-Methionine	daf-16	0.44	0.04	negative
L-Isoleucine	daf-16	0.624	0.002	negative
L-Glutamate	daf-16	0.44	0.04	negative
L-Tyrosine	daf-16	0.59	0.004	negative
DL-O-Phosphoserine	daf-16	0.479	0.024	negative
L-Ornithine monochlorohydrate/ornithine	daf-16	0.432	0.045	positive
Phosphoribosyl formamidocarboxamide	daf-16	0.666	0.001	negative
Uridine 5'-diphosphate	daf-16	0.691	0	negative
Gluconic acid	daf-16	0.542	0.009	negative
L-Palmitoylcarnitine	akt-1	0.606	0.004	positive
Heneicosanoic acid	akt-1	0.535	0.012	positive
Hexadecane	akt-1	0.595	0.004	positive
alpha-Linolenic acid	akt-1	0.473	0.03	negative
L-Lysine	akt-1	0.57	0.007	positive
L-Isoleucine	akt-1	0.521	0.015	positive

L-Proline	akt-1	0.507	0.019	positive
Putrescine	akt-1	0.468	0.032	positive
dGDP	akt-1	0.606	0.004	positive
Phosphoribosyl formamidocarboxamide	akt-1	0.451	0.04	positive
