Supporting information for Pontoizeau et al.

Metabolomics analysis uncovers that dietary restriction buffers metabolic changes associated to aging in Caenorhabditis elegans

Table S1. Metabolite variations with age in WT, *slcf-1(tm2258)* and *eat-2(ad465)*.

Metabolite	Chemical Shift (ppm) and multiplicity	WT: A7 vs YA[a]	P[b]	*slcf-1*: A7 vs YA[a]	P[b]	*eat -2*: A7 vs YA[a]	P[b]
Alanine	1.48 (d)	↓	5.82E-07	ns	1.00E+00	ns	8.42E-01
Betaine	3.27 (s)	ns	2.29E-01	ns	1.00E+00	ns	6.57E-01
Choline	3.204 (s)	ns	1.00E+00	ns	1.00E+00	↑	2.92E-02
Cystathionine	3.145 (m)	↓	2.00E-40	↓	1.96E-10	↓	3.07E-06
Formate	8.46 (s)	↓	1.82E-11	↓	1.96E-10	ns	8.42E-01
GPC	3.235 (s)	↑	2.28E-28	↑	5.50E-14	↑	2.01E-03
Glutamate	2.355 (m)	↓	3.87E-11	↓	3.38E-04	ns	9.01E-02
Glutamine	2.465 (m)	↑	1.44E-07	↑	4.89E-03	ns	1.00E+00
Isoleucine	1.015 (d)	↓	1.12E-24	↓	5.08E-11	ns	7.38E-01
Leucine	0.96 (d)	↓	6.99E-18	↓	5.08E-11	↓	2.92E-02
Lysine	3.025 (t)	↓	1.22E-30	↓	6.33E-09	↓	6.25E-04
Phenylalanine	7.43 (t),	↓	5.83E-09	↓	5.41E-03	ns	1.00E+00
Phosphocholine	3.225 (s)	↑	1.85E-28	↑	1.77E-08	↑	8.65E-04
Succinate	2.41 (s)	ns	1.00E+00	ns	3.03E-01	ns	1.00E+00
Tyrosine	7.20 (d)	↓	2.11E-08	ns	2.00E-01	ns	1.00E+00
Trehalose	5.20 (d)	ns	4.13E-01	↑	8.20E-05	↑	2.92E-02
Valine	1.045 (d)	↓	8.47E-23	↓	1.01E-09	ns	8.42E-01
Arginine	3.246 (t)	↓	2.46E-20	↓	4.56E-02	ns	1.80E-01
Acetate	1.92 (s)	↓	2.39E-09	↓	2.50E-04	ns	5.33E-01
Lactate	1.33 (d)	↓	4.86E-13	ns	6.69E-01	ns	2.14E-01
Glycerol	3.56 (m)	↓	1.47E-13	↓	2.25E-06	ns	1.00E+00
Glycine	3.56 (s)	↑	6.39E-18	↑	2.96E-07	ns	1.80E-01

[a] ↑: increase in metabolite concentration with age; ↓: decrease in metabolite concentration with age; ns: non significant metabolite variation. [b] P-values obtained an unpaired two-tailed t-test, corrected with the Benjamini-Yekutieli method for multiple testing. P-values < 0.05 are significant. YA: young adult; A7: adult.

Table S2. Goodness-of-fit parameter values for the OPLS models discriminating young adults and adults for WT, *slcf-1(tm2258)* and *eat-2(ad465)* and for each age, discriminating WT from *slcf-1(tm2258)* or *eat-2(ad465)* .

OPLS model	Number of orthogonal components	R^2X	R^2Y	Q^2
WT: YA vs A7	3	0.846	0.978	0.956
slcf-1: YA vs A7	3	0.794	0.97	0.934
eat-2: YA vs A7	2	0.728	0.978	0.934
YA: WT vs *slcf-1*	5	0.827	0.987	0.96
A7: WT vs *slcf-1*	4	0.903	0.985	0.947
YA: WT vs *eat-2*	2	0.759	0.963	0.933
A7: WT vs *eat-2*	1	0.868	0.955	0.94

YA: 1-day old young adult; A7: 7-day old adult.

Table S3. Metabolite variations between WT and long-lived mutants *slcf-1(tm2258)* or *eat-2(ad465)* in young adults and 7 day-old adults.

Metabolite	Chemical Shift (ppm) and multiplicity	YA: *slcf-1* vs WT [a]	P[b]	A7: *slcf-1* vs WT [a]	P[b]	YA: *eat-2* vs WT [a]	P[b]	A7: *eat-2* vs WT [a]	P[b]
Alanine	1.48 (d)	ns	1.68E-01	↑	2.27E-06	ns	4.34E-01	ns	1.00E+00
Betaine	3.27 (s)	↓	5.05E-04	ns	1.00E+00	ns	1.00E+00	ns	4.67E-01
Choline	3.204 (s)	↑	3.65E-03	↑	7.79E-03	ns	2.05E-01	ns	1.29E-01
Cystathionine	3.145 (m)	↑	5.85E-03	↑	8.79E-07	↑	2.67E-11	↑	2.13E-20
Formate	8.46 (s)	ns	1.00E+00	ns	6.98E-02	↓	4.01E-10	↓	4.59E-06
GPC	3.235 (s)	ns	1.00E+00	ns	3.91E-01	↓	1.09E-04	↓	1.22E-12
Glutamate	2.355 (m)	ns	1.00E+00	ns	8.72E-02	↑	5.55E-12	↑	3.78E-15
Glutamine	2.465 (m)	ns	8.03E-01	ns	1.00E+00	↑	3.75E-11	↑	2.38E-08
Isoleucine	1.015 (d)	↓	3.26E-03	ns	1.00E+00	↓	1.76E-10	ns	1.00E+00
Leucine	0.96 (d)	ns	2.81E-01	↓	2.92E-04	↓	4.38E-10	↓	8.07E-08
Lysine	3.025 (t)	↑	6.47E-14	↑	3.08E-19	↑	2.04E-06	↑	2.13E-20
Phenylalanine	7.43 (t),	ns	1.68E-01	↑	7.79E-03	↓	3.07E-05	ns	9.41E-01
Phosphocholine	3.225 (s)	↓	3.65E-03	↓	1.68E-07	↓	1.94E-05	↓	1.78E-14
Succinate	2.41 (s)	↑	2.29E-03	ns	1.00E+00	↑	3.18E-10	↑	9.08E-08
Tyrosine	7.20 (d)	ns	1.00E+00	ns	9.81E-02	↓	2.01E-05	ns	1.00E+00
Trehalose	5.20 (d)	↓	4.74E-17	↓	2.17E-03	↓	3.55E-11	↓	5.29E-10
Valine	1.045 (d)	↓	1.42E-02	ns	1.00E+00	↓	5.55E-12	ns	4.42E-01
Arginine	3.246 (t)	ns	1.60E-01	↑	2.27E-06	↓	1.21E-03	↑	2.50E-10
Acetate	1.92 (s)	ns	1.00E+00	ns	1.00E+00	↓	2.78E-10	ns	9.41E-01
Lactate	1.33 (d)	↓	7.50E-05	ns	1.00E+00	↓	2.61E-10	ns	6.06E-01
Glycerol	3.56 (m)	ns	6.74E-02	ns	1.00E+00	↓	2.32E-05	ns	1.00E+00
Glycine	3.56 (s)	ns	8.80E-01	ns	1.00E+00	↑	8.21E-05	ns	1.00E+00

[a] ↑: increase in metabolite concentration in long-lived mutant (*slcf-1* or *eat-2*) by comparison to WT; ↓: decrease in metabolite concentration in long-lived mutant (*slcf-1* or *eat-2*) by comparison to WT; ns: non significant metabolite variation. [b] P-values obtained an unpaired two-tailed t-test, corrected with the Benjamini-Yekutieli method for multiple testing. P-values < 0.05 are significant. YA: young adult; A7: adult.

Table S4. Metabolite variations with age in *daf-18(e1375)* mutants and *daf-18(e1375)*; *slcf-1(tm2258)* double mutants.

Metabolite	Chemical Shift (ppm) and multiplicity	*daf-18*: A7 vs YA[a]	P[b]	*daf-18; slcf-1* : A7 vs YA [a]	P[b]
Alanine	1.48 (d)	↓	1.92E-08	↓	1.03E-06
Betaine	3.27 (s)	↓	9.63E-03	↑	1.44E-04
Choline	3.204 (s)	↑	6.74E-05	↑	1.82E-06
Cystathionine	3.145 (m)	↓	9.63E-03	↑	4.37E-05
Formate	8.46 (s)	↓	3.92E-10	↓	4.29E-08
GPC	3.235 (s)	↑	2.66E-08	↑	1.12E-11
Glutamate	2.355 (m)	ns		ns	
Glutamine	2.465 (m)	↑	1.48E-07	↑	2.33E-08
Isoleucine	1.015 (d)	↓	1.33E-13	↓	3.54E-11
Leucine	0.96 (d)	↓	8.53E-11	↓	3.10E-12
Lysine	3.025 (t)	↑	3.34E-02	↓	1.82E-06
Phenylalanine	7.43 (t),	↓	1.37E-07	↓	1.88E-07
Phosphocholine	3.225 (s)	↑	4.24E-06	↑	4.50E-24
Succinate	2.41 (s)	↑	1.69E-06	↑	1.19E-13
Tyrosine	7.20 (d)	ns		ns	
Trehalose	5.20 (d)	↓	1.09E-04	↑	1.38E-06
Valine	1.045 (d)	↓	1.01E-12	↓	1.09E-10
Arginine	3.246 (t)	ns		↓	
Acetate	1.92 (s)	↓	2.06E-07	↓	8.64E-07
Lactate	1.33 (d)	↓	3.22E-05	↓	4.88E-16
Glycerol	3.56 (m)	↓	2.06E-07	↓	1.63E-08
Glycine	3.56 (s)	ns		ns	

[a] ↑: increase in metabolite concentration with age; ↓: decrease in metabolite concentration with age; ns: non significant metabolite variation. [b] P-values obtained with an unpaired two-tailed t-test, corrected with the Benjamini-Yekutieli method for multiple testing. P-values < 0.05 are significant. YA: young adult; A7: adult. Acetate, lactate, glycerol and glycine p-values are not reliable due to signal overlaps.

Table S5. Metabolite variations between WT and *daf-18(e1375)* or *daf-18(e1375)*; *slcf-1(tm2258)* in young adults and adults.

Metabolite	YA: *daf-18*[a] vs WT[a]	P[b]	A7: *daf-18* vs WT[a]	P[b]	YA: *slcf-1; daf-18* vs *slcf-1*[a]	P[b]	A7: *slcf-1; daf-18* vs *slcf-1*[a]	P[b]
Alanine	↑	2.21E-08	↓	4.27E-03	ns	1.00E+00	↓	1.61E-11
Betaine	ns	4.09E-01	ns	1.00E+00	ns	1.00E+00	↑	1.35E-02
Choline	ns	1.00E+00	↑	4.57E-08	ns	7.20E-01	↑	1.54E-06
Cystathionine	ns	1.00E+00	↑	7.29E-14	ns	1.00E+00	↑	2.93E-14
Formate	ns	1.00E+00	↓	3.30E-07	ns	2.03E-01	↓	9.55E-08
GPC	ns	1.00E+00	ns	4.95E-01	ns	5.19E-01	↓	3.03E-07
Glutamate	ns	1.00E+00	↑	2.00E-05	↓	3.48E-04	ns	5.72E-01
Glutamine	↓	2.20E-02	↑	1.25E-03	ns	5.19E-01	↑	4.06E-03
Isoleucine	↑	3.26E-02	↑	4.74E-02	ns	5.19E-01	ns	1.38E-01
Leucine	↑	1.72E-03	↑	2.56E-03	↑	3.66E-02	↑	6.65E-05
Lysine	↑	2.54E-03	↑	9.91E-11	ns	1.00E+00	ns	1.00E+00
Phenylalanine	↑	4.20E-03	↑	2.00E-05	ns	8.54E-01	ns	6.42E-01
Phosphocholine	ns	5.39E-01	↑	3.30E-07	ns	1.00E+00	↑	3.93E-26
Succinate	ns	5.11E-02	↑	1.22E-13	ns	1.21E-01	↑	3.11E-14
Tyrosine	ns	4.75E-01	↑	2.46E-08	ns	2.03E-01	↑	2.24E-06
Trehalose	↓	2.96E-04	↓	1.54E-09	ns	8.83E-01	↓	4.58E-05
Valine	↑	4.94E-03	ns	1.00E+00	ns	1.00E+00	ns	1.00E+00
Arginine	↑	1.53E-04	↑	4.01E-06	↑	3.66E-02	↓	1.21E-06
Acetate	↑	1.54E-04	↑	1.37E-07	ns	1.00E+00	ns	1.00E+00
Lactate	ns	1.00E+00	↓	3.07E-19	↑	2.19E-02	↓	3.51E-17
Glycerol	↓	1.96E-03	↓	4.52E-05	ns	5.19E-01	↓	2.34E-04
Glycine	↑	3.79E-02	↑	1.85E-02	ns	1.00E+00	ns	1.00E+00

[a]↑: increase in metabolite concentration in *daf-18* or *daf-18; slcf-1* by comparison to WT or *slcf-1*; ↓: decrease in metabolite concentration in *daf-18* or *daf-18; slcf-1* by comparison to WT or *slcf-1*; ns: non significant metabolite variation. [b]P-values obtained with an unpaired two-tailed t-test, corrected with the Benjamini-Yekutieli method for multiple testing. P-values < 0.05 are significant. YA: young adult; A7: adult. Acetate, lactate, glycerol and glycine p-values are not reliable due to signal overlaps.

Table S6. Wild-type and mutants average lifespans and corresponding phosphocholine levels

Genotype	Average lifespan	Number of worms	Relative phosphocholine level (age)
wild-type	17,4	928	1,93E-03 (YA)
			3,77E-03(A7)
slcf-1(tm2258)	22,8	988	1,59E-03(YA)
			2,81E-03(A7)
daf-18(e1375); slcf-1(tm2258)	13,7	190	1,61E-03(YA)
			9,50E-03(A7)
daf-18(e1375)	12,8	68	1,76E-03(YA)
			5,94E-03(A7)
eat-2(ad465)	25,6	223	1,45E-03(YA)
			1,95E-03(A7)

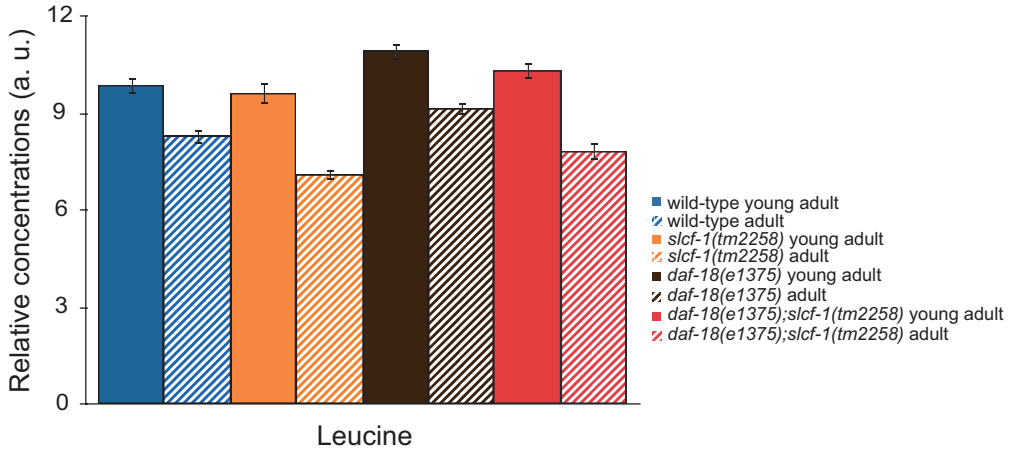

Figure S1. Relative concentrations in arbitrary units of leucine in young and 7 day-old adults WT, *slcf-1(tm2258)*, *daf-18(e1375)* and *daf-18(e1375)*;*slcf-1(tm2258*) double mutants. Results are reported with means and 95% confidence intervals.