Diminished nutrient supply stimulates endothelial metabolic rate turnover – impact of the cellular sex on glucose und glutamine metabolism

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Supplemental Table S1. Mass pairs (mass fragment m/z) analyzed after ^{13}C-glucose or ^{13}C-glutamine labeling of HUVEC. Citric acid in oxidative (oxy) and reductive (red) form.

Metabolite	Unlabeled	Labeling with ^{13}C-glucose	Labeling with ^{13}C-glutamine
alpha-Ketoglutaric acid	198	200	-
Citric acid	273	275	276 oxy
Citric acid	273	275	278 red
Fumaric acid	245	247	249
Glycerol	218	221	-
Lactic acid	219	222	-
Malic acid	245	247	249
Pyruvic acid	174	177	-
Succinic acid	247	249	251

Supplemental Table S2A. Annotated metabolites from ^{13}C-glucose labeling and number of HUVECs for the paired conditions serum starvation (0.5% FCS) and basal condition (20% FCS).

Metabolite	Time [h]	N	Mean [SE] basal	Mean [SE] starved	r [95% CI]	p-value / q-value
a-Ketoglutaric acid	0	24	7.94 [1.10]	13.17 [1.14]	0.73 [0.49-0.87]	3.75e-04 / 1.13e-03
a-Ketoglutaric acid	1.5	23	11.82 [0.92]	18.62 [0.82]	0.82 [0.7-0.88]	5.96e-06 / 1.39e-05
a-Ketoglutaric acid	3	24	12.35 [0.37]	21.14 [0.95]	0.87 [0.84-0.88]	2.38e-07 / 3.06e-07
Citric acid	0	20	1.38 [0.64]	2.25 [0.72]	0.24 [0.02-0.65]	2.94e-01 / 4.41e-01
Citric acid	1.5	21	11.95 [1.30]	25.10 [2.14]	0.73 [0.48-0.86]	2.93e-04 / 2.93e-04
Citric acid	3	21	16.19 [1.35]	34.61 [1.58]	0.87 [0.83-0.88]	1.91e-06 / 2.15e-06
Fumaric acid	0	27	0.41 [0.18]	0.42 [0.18]	0.19 [0.01-0.57]	6.15e-01 / 6.92e-01
Fumaric acid	1.5	26	3.22 [0.25]	5.48 [0.47]	0.70 [0.46-0.87]	1.09e-04 / 1.23e-04
Fumaric acid	3	27	5.77 [0.38]	10.46 [0.73]	0.86 [0.82-0.87]	4.47e-08 / 8.05e-08
Glycerol	0	27	2.27 [0.36]	2.96 [0.65]	0.50 [0.15-0.82]	7.59e-03 / 1.71e-02
Glycerol	1.5	26	6.75 [0.45]	9.67 [0.75]	0.74 [0.53-0.86]	3.76e-05 / 4.83e-05
Glycerol	3	27	8.41 [0.45]	11.71 [0.89]	0.76 [0.59-0.87]	1.35e-05 / 1.35e-05
Lactic acid	0	27	1.25 [0.66]	0.90 [0.34]	0.25 [0.01-0.62]	1.94e-01 / 3.49e-01
Lactic acid	1.5	26	13.50 [1.16]	33.67 [2.63]	0.81 [0.68-0.87]	2.09e-06 / 9.40e-06
Lactic acid	3	27	23.08 [1.29]	48.24 [2.27]	0.87 [0.87-0.88]	1.49e-08 / 6.70e-08
Malic acid	0	24	0.35 [0.20]	0.27 [0.13]	0.04 [0.01-0.49]	1.00e+00/ 1.00e+00
Malic acid	1.5	25	3.38 [0.19]	6.27 [0.54]	0.76 [0.54-0.88]	3.19e-05 / 4.79e-05
Malic acid	3	27	5.78 [0.43]	11.28 [0.68]	0.86 [0.81-0.87]	7.45e-08 / 1.12e-07
Pyruvic acid	0	27	10.31 [1.72]	9.82 [1.71]	0.13 [0.01-0.52]	5.54e-01 / 6.92e-01
Pyruvic acid	1.5	26	28.70 [2.38]	49.71 [2.63]	0.78 [0.62-0.87]	9.15e-06 / 1.65e-05
Pyruvic acid	3	27	34.52 [1.87]	61.42 [1.78]	0.87 [0.87-0.87]	1.49e-08 / 6.70e-08
Succinic acid	0	27	0.70 [0.11]	4.14 [0.35]	0.83 [0.73-0.87]	1.77e-05 / 1.59e-04
Succinic acid	1.5	26	1.72 [0.29]	6.83 [0.54]	0.79 [0.65-0.87]	6.17e-06 / 1.39e-05
Succinic acid	3	27	2.04 [0.32]	9.38 [0.60]	0.87 [0.85-0.87]	2.98e-08 / 6.70e-08

Significance was calculated using the paired Wilcoxon rank sum test. SE: standard error. r: effect size.
CI: confidence interval.

Supplemental Table S2B. Annotated metabolites from ^{13}C-glutamine labeling and number of HUVECs for the paired conditions serum starvation (0.5% FCS) and basal condition (20% FCS).

Metabolite	Time [h]	N	Mean [SE] basal	Mean [SE] starved	r [95% CI]	p-value / q-value
Citric acid oxidative	0	26	0.34 [0.07]	1.86 [0.56]	0.78 [0.62-0.86]	2.00e-04 / 2.50e-04
Citric acid oxidative	3	31	11.76 [1.25]	20.32 [0.88]	0.85 [0.78-0.87]	1.30e-08 / 6.50e-08
Citric acid oxidative	6	30	19.69 [1.25]	24.81 [0.75]	0.57 [0.25-0.80]	1.23e-03 / 2.05e-03
Citric acid reductive	0	26	0.32 [0.14]	1.35 [0.32]	0.68 [0.43-0.83]	2.09e-03 / 2.09e-03
Citric acid reductive	3	31	4.50 [0.58]	8.59 [0.65]	0.73 [0.50-0.87]	8.02e-06 / 1.03e-05
Citric acid reductive	6	30	7.02 [0.57]	10.29 [0.44]	0.64 [0.35-0.84]	2.09e-04 / 5.23e-04
Fumaric acid	0	26	2.03 [0.65]	4.68 [0.98]	0.77 [0.57-0.87]	1.33e-05 / 6.65e-05
Fumaric acid	3	30	15.32 [1.68]	24.03 [0.84]	0.82 [0.70-0.87]	1.64e-07 / 4.10e-07
Fumaric acid	6	29	24.42 [1.73]	26.60 [0.76]	0.22 [0.01-0.54]	2.30e-01 / 2.30e-01
Malic acid	0	27	1.49 [0.31]	4.80 [0.79]	0.76 [0.52-0.87]	1.13e-04 / 2.22e-04
Malic acid	3	31	16.63 [1.57]	25.57 [0.84]	0.80 [0.67-0.87]	8.26e-06 / 1.03e-05
Malic acid	6	30	26.12 [1.34]	29.59 [0.65]	0.42 [0.10-0.70]	1.97e-02 / 2.46e-02
Succinic acid	0	27	0.47 [0.12]	2.64 [0.28]	0.77 [0.55-0.87]	1.33e-04 / 2.22e-04
Succinic acid	3	31	4.03 [0.72]	11.14 [1.1]	0.78 [0.64-0.86]	1.42e-05 / 1.42e-05
Succinic acid	6	30	7.64 [1.23]	14.75 [1.03]	0.65 [0.40-0.83]	1.70e-04 / 5.23e-04

Significance was calculated using the paired Wilcoxon rank sum test. SE: standard error. r: effect size.
CI: confidence interval.

Supplemental Table S3A. Annotated metabolites from ^{13}C-glucose labeling and number of biological replicates for female and male HUVECs (twin pairs) under basal and starved conditions.

Metabolite	Time [h]	FCS	N (pairs)	Mean [SE] female	Mean [SE] male	r [95% CI]	p-value / q-value
a-Ketoglutaric acid	0	0.5	12	16.08 [1.75]	10.27 [0.90]	0.86 [0.75-0.89]	9.77e-04 / 8.79e-03
a-Ketoglutaric acid	1.5	0.5	11	18.70 [1.17]	18.92 [1.24]	0.13 [0.00-0.75]	7.00e-01 / 9.10e-01
a-Ketoglutaric acid	3	0.5	12	19.72 [1.31]	22.57 [1.30]	0.54 [0.07-0.89]	6.40e-02 / 2.88e-01
a-Ketoglutaric acid	0	20	12	8.26 [1.88]	7.62 [1.24]	0.09 [0.00-0.66]	7.91e-01 / 8.93e-01
a-Ketoglutaric acid	1.5	20	12	10.23 [0.84]	13.21 [1.48]	0.59 [0.09-0.84]	4.25e-02 / 3.83e-01
a-Ketoglutaric acid	3	20	12	12.35 [0.54]	12.35 [0.54]	0.14 [0.02-0.73]	6.66e-01 / 9.46e-01
Citric acid	0	0.5	9	2.21 [1.12]	2.10 [1.13]	0.06 [0.02-0.70]	9.10e-01 / 9.10e-01
Citric acid	1.5	0.5	9	24.21 [3.77]	27.81 [2.95]	0.45 [0.02-0.89]	2.03e-01 / 9.03e-01
Citric acid	3	0.5	8	36.67 [3.06]	35.85 [2.04]	0.1 [0.00-0.75]	8.44e-01 / 8.44e-01
Citric acid	0	20	13	0.57 [0.14]	2.00 [0.96]	0.05 [0.01-0.61]	8.93e-01 / 8.93e-01
Citric acid	1.5	20	12	9.56 [0.70]	12.56 [1.94]	0.32 [0.02-0.77]	3.01e-01 / 6.84e-01
Citric acid	3	20	12	13.09 [0.98]	17.14 [1.75]	0.48 [0.05-0.82]	1.10e-01 / 9.46e-01
Fumaric acid	0	0.5	13	0.56 [0.33]	0.31 [0.16]	0.31 [0.01-0.75]	2.86e-01 / 8.58e-01
Fumaric acid	1.5	0.5	12	5.46 [0.72]	5.62 [0.75]	0.07 [0.00-0.64]	8.50e-01 / 9.10e-01
Fumaric acid	3	0.5	13	10.07 [0.97]	11.04 [1.17]	0.18 [0.01-0.71]	5.42e-01 / 8.44e-01
Fumaric acid	0	20	13	0.10 [0.07]	0.61 [0.34]	0.43 [0.04-0.82]	1.42e-01 / 3.19e-01
Fumaric acid	1.5	20	13	3.30 [0.38]	3.05 [0.30]	0.25 [0.01-0.87]	4.56e-01 / 6.84e-01
Fumaric acid	3	20	13	5.72 [0.54]	5.64 [0.55]	0.15 [0.01-0.67]	6.35e-01 / 9.46e-01
Glycerol	0	0.5	13	3.14 [0.97]	2.96 [0.95]	0.11 [0.01-0.63]	7.35e-01 / 9.10e-01
Glycerol	1.5	0.5	12	9.60 [1.29]	9.74 [0.93]	0.07 [0.00-0.66]	8.50e-01 / 9.10e-01
Glycerol	3	0.5	13	11.95 [1.34]	11.85 [1.26]	0.07 [0.01-0.69]	8.39e-01 / 8.44e-01
Glycerol	0	20	13	1.80 [0.45]	2.60 [0.57]	0.49 [0.07-0.85]	8.03e-02 / 3.19e-01
Glycerol	1.5	20	13	7.26 [0.68]	6.73 [0.58]	0.11 [0.01-0.63]	7.35e-01 / 8.27e-01
Glycerol	3	20	13	8.57 [0.54]	8.43 [0.76]	0.18 [0.01-0.71]	5.42e-01 / 9.46e-01
Lactic acid	0	0.5	13	1.00 [0.52]	0.86 [0.50]	0.04 [0.01-0.60]	9.06e-01 / 9.10e-01

Lactic acid	1.5	0.5	12	32.29 [4.35]	36.12 [3.52]	0.36 [0.02-0.75]	2.33e-01 / 9.03e-01
Lactic acid	3	0.5	13	46.44 [3.01]	50.16 [3.68]	0.28 [0.01-0.75]	3.40e-01 / 7.65e-01
Lactic acid	0	20	13	0.14 [0.04]	1.73 [1.17]	0.45 [0.03-0.86]	1.16e-01 / 3.19e-01
Lactic acid	1.5	20	13	12.61 [1.30]	13.10 [1.38]	0.07 [0.01-0.63]	8.39e-01 / 8.39e-01
Lactic acid	3	20	13	22.20 [1.55]	22.82 [1.90]	0.05 [0.01-0.63]	8.93e-01 / 9.46e-01
Malic acid	0	0.5	12	0.35 [0.23]	0.18 [0.12]	0.41 [*]	1.78e-01 / 8.01e-01
Malic acid	1.5	0.5	12	5.94 [0.82]	6.54 [0.81]	0.32 [0.02-0.82]	3.01e-01 / 9.03e-01
Malic acid	3	0.5	13	11.12 [0.89]	11.61 [1.10]	0.15 [0.01-0.71]	6.35e-01 / 8.44e-01
Malic acid	0	20	12	0.06 [0.04]	0.64 [0.38]	0.56 [0.15-0.83]	7.59e-02 / 3.19e-01
Malic acid	1.5	20	12	3.45 [0.17]	3.24 [0.31]	0.27 [0.00-0.77]	3.80e-01 / 6.84e-01
Malic acid	3	20	13	5.61 [0.53]	5.67 [0.66]	0.05 [0.01-0.63]	8.93e-01 / 9.46e-01
Pyruvic acid	0	0.5	13	8.91 [2.17]	9.87 [2.76]	0.16 [0.01-0.71]	6.66e-01 / 9.10e-01
Pyruvic acid	1.5	0.5	12	48.99 [4.60]	51.30 [3.09]	0.25 [0.02-0.75]	4.24e-01 / 9.10e-01
Pyruvic acid	3	0.5	13	59.74 [2.76]	62.70 [2.48]	0.53 [0.07-0.83]	5.74e-02 / 2.88e-01
Pyruvic acid	0	20	13	10.29 [2.14]	9.05 [2.61]	0.19 [0.01-0.70]	5.05e-01 / 8.93e-01
Pyruvic acid	1.5	20	13	26.01 [3.13]	29.55 [2.79]	0.26 [0.01-0.71]	3.76e-01 / 6.84e-01
Pyruvic acid	3	20	13	33.30 [2.24]	33.33 [2.07]	0.03 [0.01-0.59]	9.46e-01 / 9.46e-01
Succinic acid	0	0.5	13	4.65 [0.56]	3.91 [0.38]	0.15 [0.01-0.65]	6.35e-01 / 9.10e-01
Succinic acid	1.5	0.5	12	7.07 [0.69]	6.89 [0.85]	0.05 [0.02-0.66]	9.10e-01 / 9.10e-01
Succinic acid	3	0.5	13	8.93 [0.94]	9.98 [0.80]	0.13 [0.01-0.65]	6.85e-01 / 8.44e-01
Succinic acid	0	20	13	0.74 [0.20]	0.67 [0.10]	0.07 [0.01-0.65]	8.34e-01 / 8.93e-01
Succinic acid	1.5	20	13	1.39 [0.19]	1.62 [0.37]	0.15 [0.01-0.65]	6.35e-01 / 8.16e-01
Succinic acid	3	20	13	1.62 [0.17]	2.00 [0.44]	0.15 [0.01-0.61]	6.35e-01 / 9.46e-01

Significance was calculated using the paired Wilcoxon rank sum test. SE: standard error. r: effect size.
CI: confidence interval. *Confidence intervals for the Wilcoxon effect size could not be estimated due to zero variance in pairwise differences.

Supplemental Table S3B. Annotated metabolites from ^{13}C-glutamine labeling and number of biological replicates for female and male HUVECs (twin pairs) under basal and starved conditions.

Metabolite	Time [h]	FCS	N (pairs)	Mean [SE] female	Mean [SE] male	r [95% CI]	p-value / q-value
Citric acid oxidative	0	0.5	12	0.85 [0.22]	0.97 [0.45]	0.21 [0.01-0.72]	6.36e-01 / 6.73e-01
Citric acid oxidative	3	0.5	16	20.34 [1.50]	20.46 [0.87]	0.16 [0.01-0.66]	5.62e-01 / 8.60e-01
Citric acid oxidative	6	0.5	15	24.93 [1.23]	24.41 [0.89]	0.19 [0.01-0.68]	4.78e-01 / 5.97e-01
Citric acid oxidative	0	20	14	0.34 [0.08]	0.32 [0.08]	0.07 [0.01-0.62]	7.84e-01 / 8.55e-01
Citric acid oxidative	3	20	15	11.88 [1.76]	11.48 [1.95]	0.06 [0.01-0.59]	8.47e-01 / 8.47e-01
Citric acid oxidative	6	20	15	19.91 [1.76]	19.31 [1.85]	0.22 [0.01-0.66]	4.21e-01 / 5.26e-01
Citric acid reductive	0	0.5	12	0.82 [0.24]	0.93 [0.50]	0.13 [0.01-0.70]	6.73e-01 / 6.73e-01
Citric acid reductive	3	0.5	16	9.18 [0.99]	8.03 [0.78]	0.36 [0.03-0.78]	1.59e-01 / 7.95e-01
Citric acid reductive	6	0.5	15	10.38 [0.63]	10.03 [0.64]	0.19 [0.01-0.71]	4.78e-01 / 5.97e-01
Citric acid reductive	0	20	14	0.27 [0.20]	0.23 [0.17]	0.02 [*]	8.55e-01 / 8.55e-01
Citric acid reductive	3	20	15	4.72 [0.92]	4.34 [0.79]	0.14 [0.01-0.60]	5.72e-01 / 7.15e-01
Citric acid reductive	6	20	15	7.15 [0.80]	6.92 [0.85]	0.37 [0.01-0.78]	1.69e-01 / 2.82e-01
Fumaric acid	0	0.5	12	2.53 [0.37]	4.80 [1.71]	0.32 [0.02-0.75]	3.01e-01 / 6.73e-01
Fumaric acid	3	0.5	15	24.05 [1.43]	24.19 [0.91]	0.07 [0.00-0.65]	8.04e-01 / 8.60e-01
Fumaric acid	6	0.5	14	27.31 [1.32]	25.94 [0.84]	0.32 [0.03-0.73]	2.08e-01 / 5.97e-01
Fumaric acid	0	20	14	1.25 [0.22]	2.61 [1.18]	0.29 [0.03-0.72]	2.96e-01 / 7.40e-01
Fumaric acid	3	20	15	15.04 [2.12]	15.25 [2.65]	0.18 [0.01-0.72]	5.24e-01 / 7.15e-01
Fumaric acid	6	20	15	25.99 [2.05]	22.15 [2.66]	0.63 [0.21-0.88]	1.25e-02 / 6.25e-02
Malic acid	0	0.5	13	3.21 [0.53]	4.00 [1.01]	0.21 [0.01-0.66]	4.56e-01 / 6.73e-01
Malic acid	3	0.5	16	25.06 [1.30]	26.00 [1.02]	0.05 [0.01-0.60]	8.60e-01 / 8.60e-01
Malic acid	6	0.5	15	29.84 [1.01]	28.93 [0.85]	0.28 [0.01-0.71]	3.03e-01 / 5.97e-01
Malic acid	0	20	14	1.18 [0.37]	1.62 [0.44]	0.32 [0.02-0.75]	2.45e-01 / 7.40e-01
Malic acid	3	20	15	16.74 [2.00]	16.29 [2.62]	0.26 [0.01-0.73]	3.30e-01 / 7.15e-01
Malic acid	6	20	15	26.68 [1.83]	25.29 [2.06]	0.44 [0.04-0.81]	9.46e-02 / 2.37e-01
Succinic acid	0	0.5	13	2.31 [0.42]	2.24 [0.44]	0.14 [0.01-0.64]	6.66e-01 / 6.73e-01

Succinic acid	3	0.5	16	11.60 [1.67]	10.86 [1.38]	0.09 [0.01-0.57]	7.44e-01 / 8.60e-01
Succinic acid	6	0.5	15	14.76 [1.38]	14.47 [1.52]	0.15 [0.01-0.60]	5.99e-01 / 5.99e-01
Succinic acid	0	20	14	0.34 [0.06]	0.36 [0.06]	0.20 [0.01-0.70]	5.55e-01 / 8.55e-01
Succinic acid	3	20	15	4.70 [1.27]	3.62 [0.76]	0.46 [0.03-0.84]	9.35e-02 / 4.68e-01
Succinic acid	6	20	15	7.77 [1.68]	7.66 [1.83]	0.11 [0.01-0.64]	7.80e-01 / 7.80e-01

Significance was calculated using the paired Wilcoxon rank sum test. SE: standard error. r: effect size.
CI: confidence interval. *Confidence intervals for the Wilcoxon effect size could not be estimated due to zero variance in pairwise differences.