

Supplemental information

Fluge et al, “*Metabolic profiling indicates impaired pyruvate dehydrogenase function in Myalgic Encephalopathy/Chronic Fatigue Syndrome*”.

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Serum levels of category I and II amino acids in non-fasting ME/CFS patients versus healthy controls, by gender and physical activity level

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Supplemental Table S11

Serum concentrations of amino acids converted to pyruvate (category I), or to acetyl-CoA (category II), in non-fasting ME/CFS patients and healthy controls, by gender and by health-related quality of life.

Supplemental Methods

Storage of serum samples. Control analyses for metabolite stability were performed for all metabolites analyzed, comparing healthy control samples harvested in 2012 versus in 2015, and ME/CFS patient samples obtained in 2010 versus 2014/15, by gender. There was a significantly higher mean serum level of arginine (Arg) measured in samples from healthy controls 2012 relative to 2015 (data not shown), which is a known effect of storage, and in agreement with in-house data (<http://www.bevital.no>). Hence, for Arg data the 62 healthy control samples from 2012, and 19 ME/CFS samples from 2010 were excluded from the statistical analyses. Similarly, glutamine (Gln) converts to Glu, and asparagine (Asn) to aspartate (Asp), during storage. These data were therefore analyzed as the sum of Glu and Gln (Glx) and sum of Asp and Asn (Asx), respectively. For all other amino acids, there were no systematic significant differences between serum levels among healthy controls sampled in 2012 versus 2015, or patient samples sampled in 2010 versus 2014/15. These data were therefore analyzed without further precautions regarding metabolite stability.

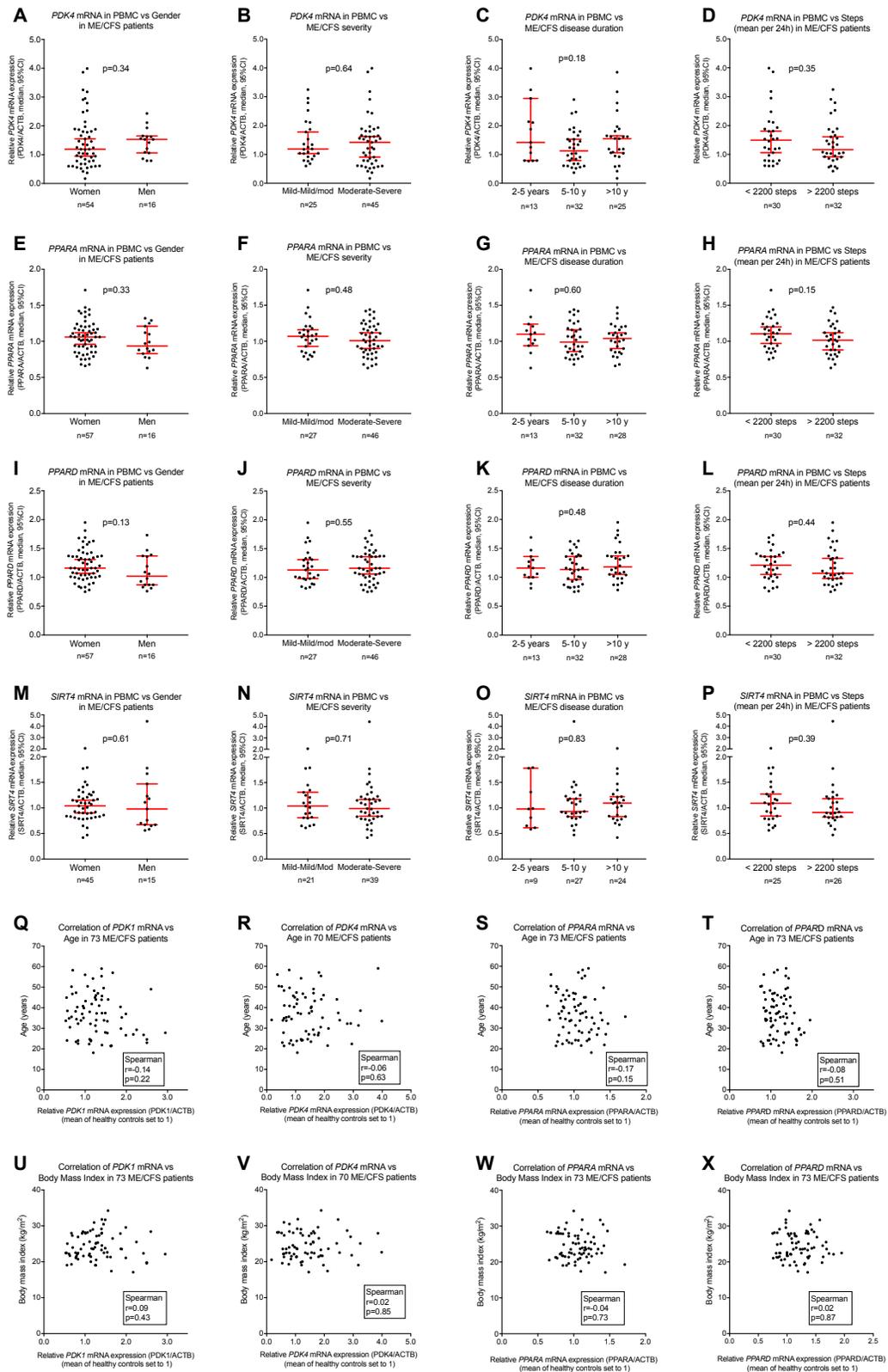
Clinical variables. As demonstrated, we observed significant effects of gender on serum amino acid profile. The data were therefore analyzed in the complete non-fasting ME/CFS group and healthy controls, but also with separate analyses in men and women (Table 1 and Table 2). Correlation matrix (Pearson's coefficients) was made for ME/CFS patients, in women and men, between different amino acids and also to the clinical variables age, Body Mass Index (BMI), physical activity (mean number of steps per 24 hours), and "SF-36mean5" (Supplemental Table S3 for amino acids that may convert to acetyl-CoA (category II) or to pyruvate (category I), and Supporting Table S4 for anaplerotic (category III) amino acids).

To address potential associations between serum levels of amino acids and patient or disease characteristics, ANOVA (or Kruskal-Wallis) tests were performed, for each gender, after categorization of variables such as BMI (Supplemental Tables S5 and S6), disease severity (Supplemental Table S7), ME/CFS disease duration (Supplemental Table S8), physical activity (Supplemental Tables S9 and S10), and self-reported quality of life (SF36 short form (Norwegian translation) (Supplemental Table S11). The levels of physical activity were assessed in the home setting using continuous registration with Sensewear electronic armbands for 5-7 consecutive days.

Total RNA extraction from PBMC, cDNA synthesis and quantitative RT-PCR

Baseline blood samples from ME/CFS patients and healthy individuals were collected by venous puncture in Tempus™ Blood RNA Tubes (Applied Biosystems, Foster City, CA, USA) and stored at -80 °C until RNA isolation. Total RNA used for qRT-PCR was extracted from the tubes using Tempus™ Spin RNA isolation Kit (Applied Biosystems, Foster City, CA, USA), including DNase treatment using RNase-Free DNase Set (cat.no 79254, Qiagen). Quantity and purity of the extracted RNA were checked using NanoDrop spectrophotometer (NanoDrop technologies, Inc., Wilmington, DE, USA). A quantity of 500 nanograms of total RNA was converted to cDNA using qScript cDNA SuperMix, (Quanta Bioscience). All qRT-PCR amplifications were performed on ABI Prism Sequence Detector 7900 HT (Applied Biosystems, Foster City, USA). For the duplex qRT-PCR amplifications (target gene and ACTB internal control) the Quantify Probe PCR Kit (Qiagen) was used.

The following TaqMan assays were used to assess the respective mRNA expression levels of the genes in peripheral blood mononuclear cells (PBMC): *PDK1* (assay ID: Hs00176853_m1); *PDK2* (assay ID: Hs00176865_m1.); *PDK3* (assay ID: Hs001768440_m1), *PDK4* (assay ID: Hs01037712_m1); peroxisome proliferator-activated receptor α (*PPARA*) (assay ID: Hs00947536_m1); *PPARG* (assay ID: Hs00234592_m1); *PPARD* (assay ID: Hs04187066_m1); pyruvate dehydrogenase E1, subunit α (*PDHA*, assay ID: Hs01049345_m1); mitochondrial pyruvate carrier-1 (*MPC1*, assay Hs00211484_m1); *MPC2* (assay Hs00209889_m1.); peroxisomal acyl-CoA oxidase 1 (*ACOX1*, assay ID: Hs01074241_m1); sirtuin 4 (*SIRT4*, assay ID: Hs_00202033_m1); hypoxia-inducible factor-1 α (*HIF1A*, assay ID: Hs_00153153_m1). β -Actin (*ACTB*, assay Hs01060665_m1) was used as internal control for target genes. The comparative $2^{-\Delta\Delta C_t}$ method was used to quantify the relative mRNA expression, and shown relative to mean level 1.0 among the healthy controls.



Supplemental Figure S1. mRNA expression levels in peripheral blood mononuclear cells (PBMC) from non-fasting ME/CFS patients, and associations to clinical variables.

The PBMC mRNA expression data were normalized to coamplified internal β -Actin (*ACTB*) in duplex qRT-PCR, and calculated relative to mean of healthy controls. All subjects were non-fasting at blood sampling. P-values from Mann-Whitney U-test for independent samples, from Kruskal-Wallis test (panels C, G, K, and O), or from Spearman correlation analyses (panels Q-X). Error bars indicate median with 95% confidence intervals (CI). (A) Pyruvate dehydrogenase kinase (*PDK4*) mRNA versus gender (B) *PDK4* mRNA versus disease severity (C) *PDK4* mRNA versus disease duration (D) *PDK4* mRNA versus steps per 24 hours (E) Peroxisomal proliferator-activated receptor α (*PPARA*) mRNA versus gender (F) *PPARA* mRNA versus severity (G) *PPARA* mRNA versus disease duration (H) *PPARA* mRNA versus steps per 24 hours (I) *PPARD* mRNA versus gender (J) *PPARD* mRNA versus disease severity (K) *PPARD* mRNA versus disease duration (L) *PPARD* mRNA versus steps per 24 hours (M) Sirtuin 4 (*SIRT4*) mRNA versus gender (N) *SIRT4* mRNA versus disease severity (O) *SIRT4* mRNA versus disease duration (P) *SIRT4* mRNA versus steps per 24 hours (Q) Correlation between *PDK1* mRNA and age (R) Correlation between *PDK4* mRNA and age (S) Correlation between *PPARA* mRNA and age (T) Correlation between *PPARD* mRNA and age (U) Correlation between *PDK1* mRNA and Body Mass Index (BMI) (V) Correlation between *PDK4* mRNA and BMI (W) Correlation between *PPARA* mRNA and BMI (X) Correlation between *PPARD* mRNA and BMI.

Supplemental Table S1. Serum levels of amino acids converted to pyruvate (category I), or to acetyl-CoA (category II), in ME/CFS patients. Effects of fasting and gender.

	All ME-patients			Female ME patients			Male ME patients		
	Fasting ^A	Non-fasting ^B	P-val. ^C	Fasting ^A	Non-fasting ^B	P-val. ^C	Fasting ^A	Non-fasting ^B	P-val. ^C
μM, mean (SD)	n=47	n=153		n=38	n=124		n=9	n=29	
Category I (to Pyr)									
Gly	286.1 (86.6)	290.7 (79.9)	0.75	293.3 (92.2)	295.4 (85.6)	0.90	255.9 (50.2)	270.5 (43.3)	0.45
Ser	144.4 (27.3)	143.2 (28.5)	0.81	146.2 (28.8)	143.7 (29.0)	0.64	136.7 (19.0)	141.4 (26.6)	0.56
Cys	266.2 (25.2)	259.0 (26.0)	0.094	260.9 (23.2)	257.8 (25.5)	0.49	288.4 (21.8)	264.0 (28.1)	0.014
Thr	142.5 (32.3)	135.9 (32.7)	0.22	144.8 (33.0)	134.5 (33.8)	0.10	132.9 (28.9)	141.7 (26.8)	0.43
Category II (to ac-CoA)									
Lys	180.9 (32.6)	179.8 (37.7)	0.85	177.3 (31.6)	175.5 (36.1)	0.78	195.9 (34.2)	197.9 (39.5)	0.88
Leu	119.4 (25.5)	123.5 (31.6)	0.36	111.8 (18.4)	118.0 (28.2)	0.12	151.2 (27.6)	147.3 (34.6)	0.74
Phe	63.9 (8.3)	68.2 (13.1)	0.008	63.0 (8.3)	67.5 (12.5)	0.012	67.8 (7.6)	71.4 (15.1)	0.35
Tyr	57.9 (11.9)	63.1 (15.9)	0.019	57.0 (11.6)	62.1 (15.8)	0.033	61.8 (12.9)	67.3 (16.4)	0.32
Ile	64.5 (14.4)	70.3 (20.0)	0.029	61.2 (11.7)	67.0 (17.3)	0.019	78.3 (17.1)	84.4 (24.8)	0.42
Trp	67.6 (15.6)	73.4 (14.6)	0.027	66.8 (14.3)	72.0 (14.4)	0.054	71.1 (20.8)	79.3 (14.2)	0.29
Ala ^D	382.8 (86.1)	423.5 (93.5)	0.007	383.9 (89.6)	420.3 (91.0)	0.033	378.4 (74.0)	437.1 (104.1)	0.077

^AOvernight fasting ME/CFS patients; ^Bnon-fasting ME/CFS patients; ^Cp-values from independent t-tests (equal variances not assumed); ^DAla was excluded from category I, since it is involved in the glucose-Ala cycle transporting amino groups from peripheral tissues to liver via blood. This cycle exerts major influence on the serum concentration of Ala, masking potential effects due to Ala oxidation.

Supplemental Table S2. Serum levels of anaplerotic amino acids (category III), metabolites that may affect endothelial function, and markers of protein catabolism, in ME/CFS patients. Effects of fasting and gender.

	All ME-patients			Female ME patients			Male ME patients		
	Fasting ^A	Non-fasting ^B	P-val. ^C	Fasting ^A	Non-fasting ^B	P-val. ^C	Fasting ^A	Non-fasting ^B	P-val. ^C
μ M, mean (SD)	n=47	n=153		n=38	n=124		n=9	n=29	
Category III (anaplerotic)									
Val	236.5 (36.6)	241.7 (51.5)	0.45	228.3 (33.9)	233.8 (77.8)	0.44	271.3 (27.1)	275.4 (51.2)	0.76
Met	26.7 (4.4)	28.3 (7.1)	0.06	26.3 (3.8)	27.5 (6.1)	0.14	28.3 (6.2)	31.6 (9.8)	0.24
Asx	76.5 (11.2)	76.5 (16.0)	0.99	76.9 (11.8)	76.5 (15.6)	0.84	74.5 (8.8)	76.5 (17.5)	0.65
His	82.6 (11.0)	83.0 (12.7)	0.84	82.1 (9.3)	82.3 (13.4)	0.91	85.0 (16.8)	86.9 (8.8)	0.87
Pro	201.5 (71.9)	225.1 (74.5)	0.054	194.6 (60.6)	213.9 (67.8)	0.10	230.4 (107.6)	272.8 (83.8)	0.30
Glx	570.0 (84.0)	572.4 (77.3)	0.87	562.6 (87.5)	559.3 (76.8)	0.84	601.8 (61.3)	628.4 (50.8)	0.26
Other metabolites									
ADMA	0.52 (0.10)	0.51 (0.11)	0.45	0.51 (0.07)	0.51 (0.11)	0.98	0.57 (0.17)	0.51 (0.10)	0.28
SDMA	0.59 (0.11)	0.58 (0.11)	0.43	0.57 (0.10)	0.57 (0.11)	0.82	0.70 (0.10)	0.61 (0.10)	0.026
hArg	2.05 (0.96)	2.10 (0.84)	0.73	1.95 (0.89)	2.04 (0.87)	0.56	2.47 (1.17)	2.34 (0.65)	0.77
1-MHis	6.04 (5.93)	5.77 (6.94)	0.74	5.67 (5.92)	5.65 (7.07)	0.85	7.62 (6.07)	6.27 (6.47)	0.99
3-MHis	3.44 (0.89)	3.47 (0.99)	0.85	3.31 (0.85)	3.29 (0.95)	0.93	3.99 (0.86)	4.22 (0.78)	0.50
Stored < 15 months	n=47	n=134		n=38	n=108		n=9	n=26	
Arg ^D	80.8 (19.5)	86.5 (18.0)	0.086	81.0 (18.2)	85.9 (17.8)	0.15	80.3 (25.5)	88.9 (19.0)	0.37

^AOvernight fasting ME/CFS patients; ^Bnon-fasting ME/CFS patients; ^Cp-values from independent t-tests (equal variances not assumed), except for 1-MHis analyzed by nonparametric Mann-Whitney test for independent samples; ^Dinitial analyses demonstrated significantly higher mean serum levels of Arg in healthy controls sampled in 2012 vs those sampled in 2015, and similarly in ME/CFS patient samples from the KTS2 study (sampled in 2010) versus serum samples harvested from ME/CFS patients in 2015. Thus, analyses for Arg were performed excluding samples from 2010 and 2012, with remaining samples harvested during late 2014 and 2015, from 134 non-fasting ME/CFS patients and 47 overnight fasting ME/CFS patients.

Supplemental Table S3. Correlation matrix for serum concentrations of amino acids (category I and II), and age, body mass index, physical activity level, and quality of life, in non-fasting ME/CFS patients, by gender.

Female ME-pts n=124^A	Lys	Leu	Ile	Phe	Tyr	Trp	Gly	Ser	Cys	Thr	Age^B	BMI^C	Steps^D	SF36 mean5^E
Lys		0.63**	0.60**	0.46**	0.56**	0.44**	0.18	0.43**	0.26**	0.47**	0.19*	0.07	-0.03	0.05
Leu			0.93**	0.68**	0.69**	0.53**	-0.01	0.20*	0.25**	0.14	0.18*	0.28**	-0.01	-0.07
Ile				0.58**	0.69**	0.55**	0.04	0.20*	0.16	0.21*	0.18*	0.27**	-0.04	-0.08
Phe					0.56**	0.45**	-0.12	0.24**	0.18	0.17	0.13	0.23*	-0.01	0.03
Tyr						0.47**	0.14	0.24*	0.12	0.28**	0.33**	0.24**	0.08	0.01
Trp							-0.02	0.08	0.08	0.15	0.03	-0.02	-0.02	0.01
Gly								0.53**	0.18*	0.18*	0.37**	-0.07	0.02	0.12
Ser									0.02	0.52**	0.07	-0.21*	0.06	0.21*
Cys										-0.03	0.31**	0.34**	-0.27**	-0.10
Thr											-0.01	-0.24**	0.01	0.19*
Male ME-pts n=29^A	Lys	Leu	Ile	Phe	Tyr	Trp	Gly	Ser	Cys	Thr	Age^B	BMI^C	Steps^D	SF36 mean5^E
Lys		0.71**	0.55**	0.59**	0.61**	0.36	0.28	0.64**	0.05	0.60**	0.03	0.03	0.06	-0.16
Leu			0.88**	0.68**	0.71**	0.40*	-0.01	0.35	-0.28	0.36	-0.06	0.14	0.15	-0.24
Ile				0.54**	0.73**	0.49**	-0.12	0.30	-0.38*	0.29	-0.17	-0.01	-0.03	-0.22
Phe					0.63**	0.61**	0.22	0.40*	0.01	0.41*	0.09	0.26	0.18	-0.30
Tyr						0.61**	-0.01	0.18	-0.19	0.40*	0.07	0.34	0.19	-0.22
Trp							0.20	0.14	-0.25	0.33	-0.28	-0.15	0.05	-0.02
Gly								0.48**	0.38*	0.62**	0.23	-0.16	0.10	-0.17
Ser									-0.03	0.56**	-0.04	-0.20	-0.13	-0.09
Cys										0.19	0.54**	0.25	0.26	-0.02
Thr											0.15	-0.15	-0.03	-0.30

^AData for amino acids concentrations were available from 124 non-fasting women with ME/CFS, for BMI from 122, for SF36 from 119, and for steps per 24h from 105 women. Data for amino acid concentrations and BMI were available from 29 non-fasting men with ME/CFS, for SF36 from 28, for steps per 24h from 26 men; ^Bage at baseline (years); ^CBody Mass Index (BMI) expressed as kg/m²; ^Dmean steps per 24h, assessed for 5-7 consecutive days at baseline, for patients included in RituxME and CycloME clinical trials; ^ESF36mean5 score was the mean of raw scores (scale 0-100) for the five subdimensions Physical Function, Bodily pain, Vitality, General Health and Social Function, from the SF36 questionnaire (SF36 ver. 1.2, Norwegian translation), assessed at baseline for ME/CFS patients included in the clinical trials RituxME (SF36 data missing for one patient) and CycloME. Out of 19 patients from KTS-2-2010, baseline SF36 data were available from 14 patients. Thus, there were baseline SF36 data available from 194 ME/CFS patients (157 women and 37 men) out of 200 patients included for analyses of metabolites and amino acids, and from in 147 non-fasting ME/CFS patients (119 women and 28 men). Correlations were expressed as Pearson's r, with indication of two-sided significance levels. * p<0.05; ** p<0.01.

Supplemental Table S4. Correlation matrix for serum concentrations of category III amino acids, metabolites that may affect endothelial function, and markers of protein catabolism, with category I and II amino acids, age, body mass index, activity level, and quality of life, in non-fasting ME/CFS patients, by gender.

Female ME-pts n=124 ^A	Lys	Leu	Phe	Tyr	Val	Pro	Gly	Ser	Cys	Thr	Age ^B	BMI ^C	Steps ^D	SF36 mean5 ^E
Val	0.61**	0.89**	0.59**	0.66**		0.26**	-0.03	0.16	0.19*	0.12	0.24**	0.37**	0.03	-0.04
Met	0.61**	0.58**	0.38**	0.67**	0.53**	0.36**	0.25**	0.48**	0.01	0.48**	0.17	-0.14	0.08	0.07
His	0.44**	0.29**	0.35**	0.21*	0.27**	0.29**	-0.02	0.29**	0.04	0.46**	-0.11	-0.12	0.07	0.10
Pro	0.27**	0.30**	0.34**	0.53**	0.26**		0.15	0.12	-0.02	0.33**	0.14	0.15	0.03	-0.03
Arg ^F	0.40*	0.19	0.26*	0.21*	0.15	0.15	0.12	0.20*	0.04	0.27*	0.10	0.09	-0.04	0.17
ADMA	0.33**	0.13	0.14	0.17	0.10	0.24**	0.20*	0.19*	0.17	0.28**	0.10	0.13	0.03	0.03
SDMA	0.06	-0.02	0.10	-0.08	-0.02	-0.10	0.13	0.14	0.28**	0.14	0.04	0.01	0.09	0.13
1-MHis	0.19*	0.14	0.04	0.04	0.26**	-0.14	0.15	0.08	0.25**	0.04	0.18*	0.02	0.16	0.01
3-MHis	0.19*	0.16	0.04	0.01	0.26**	-0.16	0.10	-0.04	0.40**	-0.01	0.33**	0.25**	-0.02	-0.03
Male ME-pts n=29 ^A	Lys	Leu	Phe	Tyr	Val	Pro	Gly	Ser	Cys	Thr	Age ^B	BMI ^C	Steps ^D	SF36 mean5 ^E
Val	0.53**	0.91**	0.52**	0.60**		0.38*	-0.05	0.26	-0.39*	0.38*	-0.10	0.10	0.18	-0.18
Met	0.61**	0.72**	0.59**	0.78**	0.65**	0.32	0.17	0.45*	-0.14	0.61**	-0.08	0.04	-0.06	-0.27
His	0.55**	0.35	0.31	0.30	0.36	0.06	0.55**	0.49**	0.02	0.74**	-0.07	-0.24	0.10	-0.20
Pro	0.06	0.34	0.16	0.36	0.38*		0.15	0.03	-0.30	0.28	-0.12	-0.06	-0.16	-0.27
Arg ^F	0.60**	0.52**	0.54**	0.56**	0.41*	0.19	0.43*	0.53*	0.21	0.59**	0.25	0.10	0.20	-0.52**
ADMA	0.29	0.14	0.20	0.40*	0.04	0.21	0.11	-0.01	-0.09	0.14	0.05	0.23	0.02	0.07
SDMA	0.18	0.11	0.27	0.11	-0.06	0.01	0.28	-0.05	0.33	0.16	0.20	0.15	0.05	-0.22
1-MHis	0.40*	0.45*	0.32	0.32	0.35	0.06	0.05	0.02	-0.01	0.13	-0.09	0.26	0.30	-0.20
3-MHis	0.14	0.06	0.01	0.01	0.05	-0.13	0.07	-0.21	0.22	0.06	0.20	0.20	0.05	-0.25

^AData for serum amino acids concentrations were available from 124 non-fasting women with ME/CFS, for BMI from 122, for SF36 from 119, and for steps per 24 hours from 105 women. Data for serum amino acid concentrations and BMI were available from 29 non-fasting men with ME/CFS, for SF36 from 28, and for steps per 24 hours from 26 men; ^Bage at baseline (years); ^CBody Mass Index (BMI) expressed as kg/m²; ^Dmean steps per 24 hours, assessed for 5-7 consecutive days at baseline, for patients included in RituxME and CycloME clinical trials; ^ESF36mean5 score was the mean of raw scores (scale 0-100) for the five subdimensions Physical Function, Bodily pain, Vitality, General Health and Social Function, from the SF36 questionnaire (SF36 ver. 1.2, Norwegian translation), assessed at baseline for ME/CFS patients included in the clinical trials RituxME and CycloME. There were available baseline SF36 data from 147 non-fasting ME/CFS patients (119 women and 28 men) out of 153 non-fasting patients included for analyses of metabolites and amino acids; ^Finitial analyses demonstrated significantly higher mean serum levels of Arg in healthy controls sampled in 2012 vs those sampled in 2015, and similarly in ME/CFS patient samples from the KTS2 study (sampled in 2010) versus serum samples harvested from ME/CFS patients in 2015. Analyses for Arg were performed excluding samples from ME/CFS patients in the KTS-2010 trial, with remaining 108 samples from non-fasting female, and 26 male ME/CFS patients, harvested during late 2014 and 2015. Correlations were expressed as Pearson's r (except for 1-MHis analyzed by Spearman's analyses), with indication of two-sided significance levels. * p<0.05; ** p<0.01.

Supplemental Table S5. Serum levels of amino acids converted to pyruvate (category I), or to acetyl-CoA (category II), in non-fasting ME/CFS patients and healthy controls, by gender and by body mass index (BMI).

	Women						Men					
	Female ME/CFS patients					Healthy women	Male ME/CFS patients					Healthy men
	<20	20-25	25-30	>30	P-val. ^B		<20	20-25	25-30	>30	P-val. ^B	
BMI^A												
μ M, mean (SD)	N=20	N=53	N=29	N=20		N=67	N=2	N=13	N=12	N=2		N=35
Category I (to Pyr)												
Gly	274.3 (78.8)	310.6 (88.3)	304.8 (89.6)	272.3 (72.8)	0.20 ^C 0.73 ^D	285.5 (83.5)	273.7 (47.9)	269.5 (41.1)	271.9 (49.5)	265.3 (53.1)	0.99 ^C 0.95 ^D	249.9 (54.1)
Ser	150.8 (34.5)	148.6 (27.7)	135.8 (26.6)	136.9 (28.5)	0.12 0.031	155.4 (30.3)	145.1 (13.5)	144.1 (24.1)	141.0 (32.9)	123.2 (3.5)	0.79 0.43	136.9 (16.3)
Cys	241.2 (25.4)	256.8 (23.2)	262.0 (22.1)	272.0 (28.7)	0.001 <0.001	257.8 (29.2)	233.6 (1.9)	255.8 (22.1)	277.0 (31.2)	270.0 (25.6)	0.099 0.027	261.0 (22.1)
Thr	150.0 (37.7)	140.1 (35.1)	120.7 (27.9)	126.5 (26.6)	0.008 0.003	149.2 (38.8)	146.7 (6.7)	140.9 (29.3)	144.4 (28.7)	125.4 (6.4)	0.83 0.70	131.8 (19.8)
Category II (to ac-CoA)												
Lys	169.3 (38.0)	180.0 (41.1)	170.4 (30.8)	179.6 (24.2)	0.53 0.75	196.6 (42.5)	199.2 (6.0)	196.2 (41.3)	200.0 (45.5)	195.6 (18.3)	0.99 0.94	197.4 (28.1)
Leu	105.7 (30.9)	116.0 (26.3)	124.4 (29.6)	128.2 (24.3)	0.042 0.005	139.6 (40.9)	134.2 (2.2)	149.4 (37.4)	146.0 (38.4)	155.5 (2.4)	0.94 0.77	151.6 (22.6)
Phe	65.1 (14.3)	65.5 (13.0)	70.1 (11.4)	71.8 (9.8)	0.13 0.026	77.0 (12.7)	60.8 (8.6)	68.5 (12.9)	75.3 (18.3)	77.7 (9.5)	0.48 0.13	75.8 (10.0)
Tyr	58.0 (21.7)	61.0 (14.3)	63.7 (15.1)	68.4 (11.7)	0.16 0.026	72.3 (21.6)	60.2 (5.0)	64.3 (19.7)	69.3 (13.8)	81.7 (1.1)	0.49 0.15	74.1 (14.8)
Ile	60.2 (18.2)	66.4 (16.2)	68.8 (18.5)	73.8 (15.9)	0.090 0.013	77.9 (26.3)	81.8 (7.2)	90.0 (29.0)	78.7 (23.8)	84.4 (1.5)	0.75 0.54	86.0 (15.4)
Trp	72.2 (13.6)	72.9 (13.8)	73.0 (15.9)	70.1 (11.4)	0.89 0.65	76.7 (12.7)	83.1 (8.5)	82.2 (15.1)	74.2 (13.9)	87.5 (12.5)	0.43 0.52	79.9 (10.3)

^ABody mass index (BMI) expressed as kg/m², BMI data was available from 122 out of 124 non-fasting female ME/CFS patients, and from 29 non-fasting male ME/CFS patients; ^Bp-values from ANOVA between BMI-groups in ME/CFS patients, analyses between groups^C and for linearity^D. In women, there was significant linearity between higher BMI categories and higher serum levels of amino acids (except for Gly, Lys, and Trp). Still, when comparing the 20 obese female ME/CFS patients (BMI >30) with the 67 healthy women (by independent t-tests, equal variances not assumed), there were higher serum levels in healthy women versus the obese ME/CFS patients for amino acids catabolized to acetyl-CoA (category II): Lys (196.6 vs 179.6, p=0.03), Leu (139.6 vs 128.1, p=0.013), Trp (76.7 vs 70.1, p=0.035), with trend for Phe (77.0 vs 71.8, p=0.06), not significant for Tyr (72.3 vs 68.4, p=0.29), and Ile (77.9 vs 73.8, p=0.39). Other abbreviations: Pyr, pyruvate; ac-CoA, acetyl-CoA.

Supplemental Table S6. Serum concentrations of anaplerotic (category III) amino acids, metabolites that may affect endothelial function, and markers of protein catabolism, in non-fasting ME/CFS patients and healthy controls, by gender and body mass index (BMI).

	Women					Men						
	ME/CFS women				Healthy women	ME/CFS men				Healthy men		
BMI ^A	<20	20-25	25-30	>30		P-val. ^B	<20	20-25	25-30		>30	P-val. ^B
μM, mean (SD)	N=20	N=53	N=29	N=20		N=67	N=2	N=13	N=12	N=2		N=35
Category III												
To succ-CoA												
Val	210.3 (44.0)	228.3 (47.5)	246.0 (50.5)	258.1 (39.3)	0.005 ^C <0.001 ^D	259.8 (55.2)	269.7 (8.7)	282.0 (57.3)	266.6 (53.3)	290.6 (19.7)	0.87 ^C 0.88 ^D	279.1 (29.3)
Met	29.0 (8.1)	28.3 (5.9)	26.5 (5.9)	26.2 (4.2)	0.30 0.068	31.3 (8.9)	29.1 (1.1)	32.6 (12.6)	31.1 (8.0)	30.3 (6.0)	0.96 0.88	29.5 (5.9)
To OAA												
Asx	78.5 (14.8)	78.3 (18.5)	74.7 (13.9)	72.9 (10.2)	0.50 0.15	87.2 (13.7)	63.9 (0.5)	80.7 (21.4)	75.1 (14.9)	70.0 (4.9)	0.57 0.80	81.4 (9.7)
To α-KG												
His	82.3 (14.8)	85.0 (14.2)	79.2 (12.5)	78.8 (6.6)	0.15 0.10	90.0 (13.3)	88.8 (2.0)	86.6 (10.3)	85.1 (8.8)	84.2 (2.0)	0.93 0.53	88.7 (10.2)
Pro	201.3 (79.0)	215.0 (70.2)	208.5 (59.8)	234.9 (63.4)	0.43 0.21	256.2 (79.0)	330.0 (139.8)	261.3 (96.7)	282.3 (68.3)	233.3 (30.5)	0.64 0.64	260.1 (48.1)
Glx	556.0 (103.8)	571.4 (72.5)	542.0 (70.5)	554.2 (68.3)	0.42 0.44	579.0 (67.6)	607.4 (43.9)	635.8 (43.6)	633.5 (56.2)	570.8 (64.5)	0.36 0.52	620.2 (72.2)
Other metabolites												
ADMA	0.48 (0.10)	0.51 (0.13)	0.49 (0.05)	0.54 (0.11)	0.27 0.25	0.54 (0.13)	0.58 (0.28)	0.46 (0.07)	0.52 (0.07)	0.62 (0.10)	0.082 0.19	0.53 (0.13)
SDMA	0.58 (0.11)	0.57 (0.11)	0.56 (0.11)	0.59 (0.09)	0.73 0.84	0.62 (0.12)	0.63 (0.10)	0.57 (0.08)	0.63 (0.11)	0.69 (0.08)	0.26 0.15	0.65 (0.11)
hArg	2.07 (0.88)	1.98 (0.83)	1.91 (0.62)	2.30 (1.18)	0.42 0.47	2.01 (0.71)	2.30 (1.22)	2.48 (0.83)	2.25 (0.37)	2.13 (0.23)	0.82 0.47	2.34 (0.77)
1-MHis	5.52 (6.66)	5.20 (6.45)	6.60 (8.49)	6.08 (7.36)	0.45 ^E	5.04 (5.24)	1.07 (0.36)	7.43 (8.80)	6.09 (3.87)	5.05 (1.79)	0.26 ^E	5.64 (4.94)
3-MHis	3.13 (0.88)	3.14 (0.73)	3.46 (1.00)	3.72 (1.30)	0.08 0.08	3.32 (0.65)	3.65 (0.11)	4.19 (0.33)	4.30 (0.67)	4.51 (0.98)	0.71 0.31	3.63 (0.70)

^ABody mass index expressed as kg/m², BMI data was available from 122 (out of 124) non-fasting female ME/CFS patients, and from 29 non-fasting male ME/CFS patients; ^Bp-values from ANOVA between BMI-groups in ME/CFS patients, analyses between groups^C and for linearity^D; ^Ep-value from Kruskal-Wallis nonparametric test. Other abbreviations: succ-CoA, succinyl-CoA; OAA, Oxaloacetate; α-KG, α-ketoglutarate.

Supplemental Table S7. Serum concentrations of amino acids converted to pyruvate (category I), or to acetyl-CoA (category II), in non-fasting ME/CFS patients and healthy controls, by gender and disease severity.

Severity ^A	Women					Men				
	ME/CFS women				Healthy women	ME/CFS men				Healthy men
	Mild/ Mild-mod.	Mod.	Mod.-sev./ Severe	P-val. ^B		Mild/ Mild-mod.	Mod.	Mod.-sev./ Severe	P-val. ^B	
μM, mean (SD)	N=51	N=38	N=34		N=67	N=9	N=13	N=7		N=35
Category I (to Pyr)										
Gly	306.7 (82.4)	297.0 (93.7)	279.2 (80.9)	0.35 ^C 0.16 ^D	285.5 (83.5)	251.2 (36.5)	285.7 (44.8)	267.0 (43.3)	0.18 ^C 0.38 ^D	249.9 (54.1)
Ser	147.9 (28.3)	136.8 (26.9)	145.6 (32.0)	0.19 0.58	155.4 (30.3)	129.9 (12.7)	143.6 (31.2)	152.4 (28.0)	0.23 0.093	136.9 (16.3)
Cys	256.0 (22.3)	258.3 (28.5)	260.0 (27.3)	0.77 0.48	257.8 (29.2)	253.2 (26.9)	274.9 (31.3)	257.7 (16.7)	0.16 0.63	261.0 (22.1)
Thr	135.8 (37.9)	130.9 (28.9)	137.7 (33.0)	0.68 0.89	149.2 (38.8)	126.5 (18.3)	152.0 (33.0)	142.0 (12.5)	0.086 0.18	131.8 (19.8)
Category II (to ac-CoA)										
Lys	175.0 (38.2)	176.5 (29.2)	177.2 (39.7)	0.96 0.79	196.6 (42.5)	189.8 (44.1)	197.6 (44.2)	209.1 (23.2)	0.64 0.36	197.4 (28.1)
Leu	117.6 (28.7)	117.5 (27.1)	120.1 (29.2)	0.91 0.71	139.6 (40.9)	138.2 (28.6)	143.1 (38.5)	167.0 (30.6)	0.22 0.12	151.6 (22.6)
Phe	67.2 (13.8)	68.1 (11.7)	67.5 (11.7)	0.95 0.90	77.0 (12.7)	68.0 (14.9)	69.9 (12.7)	78.7 (19.2)	0.34 0.18	75.8 (10.0)
Tyr	62.1 (15.7)	61.7 (14.3)	63.1 (17.5)	0.93 0.81	72.3 (21.6)	66.3 (13.6)	69.1 (19.1)	65.3 (16.1)	0.87 0.95	74.1 (14.8)
Ile	66.3 (17.7)	68.0 (17.5)	67.6 (16.7)	0.89 0.71	77.9 (26.3)	82.9 (10.5)	82.7 (32.8)	89.4 (22.8)	0.84 0.64	86.0 (15.4)
Trp	72.9 (15.2)	71.6 (13.2)	72.5 (12.4)	0.91 0.85	76.7 (12.7)	83.6 (16.5)	79.4 (13.8)	73.7 (11.8)	0.40 0.18	79.9 (10.3)

^AME/CFS disease severity, categorized as Mild/Mild-Moderate, Moderate, Moderate/Severe-Severe, data was available from 123 (out of 124) non-fasting female and from 29 non-fasting male ME/CFS patients; ^Bp-values from ANOVA between severity-groups in ME/CFS-patients, analyses between groups^C and for linearity^D. Other abbreviations: Pyr, pyruvate; ac-CoA, acetyl-CoA.

Supplemental Table S8. Serum concentrations of amino acids converted to pyruvate (category I), or to acetyl-CoA (category II), in non-fasting ME/CFS patients and healthy controls, by gender and disease duration.

Disease Duration ^A	Women					Men				
	Female ME/CFS patients				Healthy women	Male ME/CFS patients				Healthy men
	2-5 y	5-10 y	>10 y	P-val. ^B		2-5 y	5-10 y	>10 y	P-val. ^B	
μM , mean (SD)	N=23	N=60	N=40		N=67	N=5	N=16	N=8		N=35
Category I (to Pyr)										
Gly	287.3 (100.0)	283.4 (77.0)	320.2 (86.3)	0.093 ^C 0.079 ^D	285.5 (83.5)	254.7 (31.8)	272.6 (47.5)	276.2 (43.1)	0.67 ^C 0.44 ^D	249.9 (54.1)
Ser	144.1 (26.5)	137.7 (26.1)	152.8 (32.9)	0.038 0.12	155.4 (30.3)	134.9 (23.5)	142.4 (24.5)	143.7 (34.5)	0.84 0.61	136.9 (16.3)
Cys	255.4 (19.8)	255.1 (29.3)	263.2 (22.1)	0.27 0.17	257.8 (29.2)	267.5 (32.4)	266.8 (30.1)	256.1 (22.7)	0.67 0.44	261.0 (22.1)
Thr	141.0 (27.7)	131.8 (30.6)	135.8 (41.1)	0.53 0.69	149.2 (38.8)	127.0 (12.2)	146.5 (32.5)	141.2 (17.9)	0.38 0.47	131.8 (19.8)
Category II (to ac-CoA)										
Lys	171.6 (31.4)	176.7 (37.5)	177.7 (36.4)	0.80 0.55	196.6 (42.5)	194.3 (24.9)	197.4 (46.5)	201.4 (35.1)	0.95 0.76	197.4 (28.1)
Leu	110.0 (18.8)	120.9 (30.9)	119.1 (27.9)	0.28 0.31	139.6 (40.9)	146.3 (46.6)	141.3 (36.5)	160.0 (21.0)	0.48 0.40	151.6 (22.6)
Phe	61.3 (6.5)	68.4 (12.0)	70.0 (14.7)	0.023 0.013	77.0 (12.7)	75.1 (25.8)	67.9 (12.2)	76.1 (12.6)	0.40 0.73	75.8 (10.0)
Tyr	57.4 (12.5)	63.8 (16.3)	62.7 (16.3)	0.25 0.30	72.3 (21.6)	63.6 (16.2)	66.2 (19.0)	71.9 (10.4)	0.63 0.36	74.1 (14.8)
Ile	62.6 (10.8)	68.5 (19.3)	67.9 (17.0)	0.37 0.33	77.9 (26.3)	85.3 (32.7)	82.7 (26.6)	87.1 (17.5)	0.92 0.85	86.0 (15.4)
Trp	71.1 (10.7)	73.6 (15.1)	71.3 (13.5)	0.64 0.89	76.7 (12.7)	82.9 (22.4)	77.5 (11.4)	80.8 (15.0)	0.73 0.90	79.9 (10.3)

^AME/CFS disease duration, categorized in 2-5 years, 5-10 years, and >10 years, with data available from 123 (out of 124) non-fasting female and from all 29 non-fasting male ME/CFS patients; ^Bp-values from ANOVA between duration-groups in ME/CFS-patients, analyses between groups^C and for linearity^D. Other abbreviations: Pyr, pyruvate; ac-CoA, acetyl-CoA.

Supplemental Table S9. Serum concentrations of amino acids converted to pyruvate (category I), or to acetyl-CoA (category II), in non-fasting ME/CFS patients and healthy controls, by gender and by physical activity level expressed as steps per 24 hours.

	Women					Men				
	ME/CFS women				Healthy women	ME/CFS men				Healthy men
	< 1600	1600-4000	> 4000	P-val. ^B		< 1600	1600-4000	> 4000	P-val. ^B	
μM , mean (SD)	N=24	N=48	N=33		N=67	N=5	N=14	N=7		N=35
Category I (to Pyr)										
Gly	260.9 (81.9)	305.2 (87.5)	295.4 (79.6)	0.11 ^C 0.17 ^D	285.5 (83.5)	253.1 (26.3)	272.2 (36.2)	276.7 (60.4)	0.62 ^C 0.38 ^D	249.9 (54.1)
Ser	136.4 (25.1)	144.8 (31.4)	146.0 (26.4)	0.40 0.24	155.4 (30.3)	141.8 (18.5)	138.5 (25.8)	140.6 (23.7)	0.96 0.96	136.9 (16.3)
Cys	264.4 (29.8)	260.3 (23.0)	250.7 (24.7)	0.10 0.038	257.8 (29.2)	252.6 (21.5)	262.8 (26.5)	272.9 (39.0)	0.51 0.25	261.0 (22.1)
Thr	133.8 (28.6)	133.6 (36.0)	135.9 (29.3)	0.89 0.64	149.2 (38.8)	132.0 (24.6)	146.3 (24.4)	139.4 (38.6)	0.62 0.75	131.8 (19.8)
Category II (to ac-CoA)										
Lys	174.7 (40.1)	173.4 (32.1)	178.2 (32.7)	0.82 0.66	196.6 (42.5)	183.5 (29.9)	193.2 (29.9)	195.1 (52.8)	0.85 0.62	197.4 (28.1)
Leu	123.7 (28.6)	114.6 (28.0)	120.8 (27.1)	0.37 0.81	139.6 (40.9)	132.7 (27.5)	148.9 (38.6)	148.5 (38.7)	0.68 0.51	151.6 (22.6)
Phe	68.6 (12.5)	66.2 (11.1)	66.0 (10.1)	0.64 0.43	77.0 (12.7)	61.3 (6.3)	73.9 (16.4)	69.8 (14.2)	0.27 0.41	75.8 (10.0)
Tyr	62.7 (16.4)	59.9 (15.6)	62.8 (14.2)	0.65 0.91	72.3 (21.6)	52.0 (10.1)	71.4 (17.2)	65.7 (14.5)	0.077 0.22	74.1 (14.8)
Ile	71.7 (18.0)	64.9 (17.2)	68.0 (16.3)	0.28 0.52	77.9 (26.3)	79.0 (8.6)	87.1 (30.3)	82.6 (26.7)	0.83 0.88	86.0 (15.4)
Trp	72.3 (12.7)	72.6 (13.4)	71.6 (13.8)	0.95 0.82	76.7 (12.7)	71.6 (10.0)	81.9 (15.6)	78.3 (16.1)	0.42 0.53	79.9 (10.3)

^AMean number of steps per 24 hours, assessed at baseline from continuous registration for 5-7 consecutive days using Sensewear electronic armbands, were available from 178 out of 181 ME/CFS patients in the RituxME and CycloME clinical trials. Activity registrations at baseline were not performed for the 19 patients in the KTS-2-2010 trial. Activity data was thus available from 105 out of 124 non-fasting female, and from 26 out of 29 non-fasting male ME/CFS patients; ^Bp-values from ANOVA between ME/CFS groups (< 1600, 1600-4000, > 4000 mean steps per 24 hours), analyses between groups^C and for linearity^D. Other abbreviations: Pyr, pyruvate; ac-CoA, acetyl-CoA.

Supplemental Table S10. Serum concentrations of anaplerotic (category III) amino acids, metabolites that may affect endothelial function, and markers of protein catabolism, in non-fasting ME/CFS patients and healthy controls, by gender and by physical activity level expressed as steps per 24 hours.

	Women				Men					
	Female ME/CFS patients			Healthy women	Male ME/CFS patients			Healthy men		
Steps per 24 hours ^A	< 1600	1600-4000	> 4000		P-val. ^B	< 1600	1600-4000		> 4000	P-val. ^B
μM, mean (SD)	N=24	N=48	N=33		N=67	N=5	N=14	N=7		N=35
Category III										
To succ-CoA										
Val	237.3 (49.7)	229.7 (45.9)	242.1 (51.5)	0.52 ^C 0.63 ^D	259.8 (55.2)	255.6 (32.4)	280.3 (57.2)	284.0 (62.7)	0.64 ^C 0.42 ^D	279.1 (29.3)
Met	27.2 (5.2)	27.3 (6.2)	28.8 (5.9)	0.45 0.28	31.3 (8.9)	28.6 (7.5)	33.0 (11.7)	30.3 (9.5)	0.69 0.87	29.5 (5.9)
To OAA										
Asx	75.3 (12.6)	76.1 (12.0)	74.1 (10.5)	0.77 0.66	87.2 (13.7)	70.0 (9.1)	78.9 (21.4)	72.7 (11.2)	0.56 0.89	81.4 (9.7)
To α-KG										
His	83.5 (9.9)	79.4 (10.6)	85.4 (10.2)	0.032 0.33	90.0 (13.3)	85.3 (11.9)	84.6 (7.4)	88.3 (10.1)	0.67 0.52	88.7 (10.2)
Pro	206.1 (66.4)	205.6 (68.0)	216.3 (66.0)	0.75 0.54	256.2 (79.0)	210.0 (33.9)	321.2 (86.7)	237.9 (66.2)	0.012 0.82	260.1 (48.1)
Glx	548.6 (66.3)	556.8 (80.9)	568.3 (68.5)	0.60 0.32	579.0 (67.6)	599.0 (19.7)	637.9 (54.4)	628.1 (62.0)	0.38 0.43	620.2 (72.2)
Other metabolites										
ADMA	0.45 (0.07)	0.51 (0.11)	0.51 (0.09)	0.024 0.036	0.54 (0.13)	0.46 (0.06)	0.54 (0.11)	0.46 (0.08)	0.11 0.82	0.53 (0.13)
SDMA	0.55 (0.08)	0.58 (0.09)	0.58 (0.09)	0.56 0.38	0.62 (0.12)	0.62 (0.09)	0.61 (0.11)	0.60 (0.10)	0.92 0.69	0.65 (0.11)
hArg	2.01 (0.91)	1.92 (0.74)	2.22 (1.01)	0.32 0.31	2.01 (0.71)	2.67 (0.83)	2.16 (0.54)	2.25 (0.74)	0.34 0.35	2.34 (0.77)
1-MHis	3.36 (5.30)	6.64 (7.99)	6.84 (7.87)	0.024 ^E	5.04 (5.24)	6.32 (6.63)	3.94 (3.68)	11.06 (9.87)	0.086 ^E	5.64 (4.94)
3-MHis	3.20 (0.82)	3.35 (1.09)	3.24 (0.82)	0.77 0.93	3.32 (0.65)	4.63 (1.01)	3.97 (0.73)	4.32 (0.80)	0.27 0.64	3.63 (0.70)

^AMean number of steps per 24 hours, assessed at baseline from continuous registration for 5-7 consecutive days using Sensewear electronic armbands, were available from 178 out of 181 ME/CFS patients in the RituxME and CycloME clinical trials. Activity registrations at baseline were not performed for the 19 patients in the KTS-2-2010 trial. Activity data was thus available from 105 out of 124 non-fasting female, and from 26 out of 29 non-fasting male ME/CFS patients; ^Bp-values from ANOVA between ME/CFS groups (< 1600, 1600-4000, > 4000 mean steps per 24 hours), analyses between groups^C and for linearity^D. ^Ep-values from nonparametric Kruskal-Wallis test (compare medians) for independent groups. Other abbreviations: succ-CoA, succinyl-CoA; OAA, Oxaloacetate; α-KG, α-ketoglutarate.

Supplemental Table S11. Serum concentrations of amino acids converted to pyruvate (category I), or to acetyl-CoA (category II), in non-fasting ME/CFS patients and healthy controls, by gender and by health-related quality of life.

	Women				Men					
	ME/CFS women			Healthy women	ME/CFS men				Healthy men	
	< 20	20-30	> 30		P-val. ^B	< 20	20-30	> 30		P-val. ^B
μM, mean (SD)	N=30	N=41	N=48		N=67	N=4	N=10	N=14		N=35
Category I (to Pyr)										
Gly	265.6 (60.7)	296.9 (95.2)	303.9 (80.9)	0.12 ^C 0.056 ^D	285.5 (83.5)	291.8 (59.5)	272.3 (27.4)	257.6 (43.7)	0.33 ^C 0.14 ^D	249.9 (54.1)
Ser	136.4 (30.5)	140.7 (25.7)	151.3 (30.6)	0.064 0.023	155.4 (30.3)	142.0 (28.3)	143.3 (27.8)	135.2 (20.5)	0.71 0.48	136.9 (16.3)
Cys	253.0 (28.9)	264.0 (23.1)	255.6 (25.7)	0.16 0.86	257.8 (29.2)	272.7 (42.2)	255.0 (21.0)	268.0 (29.6)	0.46 0.85	261.0 (22.1)
Thr	129.2 (28.6)	130.8 (33.2)	142.9 (37.4)	0.13 0.065	149.2 (38.8)	153.9 (51.8)	146.0 (18.6)	134.3 (23.8)	0.38 0.16	131.8 (19.8)
Category II (to ac-CoA)										
Lys	171.7 (36.8)	177.6 (33.7)	178.7 (37.2)	0.68 0.42	196.6 (42.5)	204.4 (64.3)	201.6 (34.8)	189.9 (35.8)	0.71 0.43	197.4 (28.1)
Leu	122.7 (31.4)	117.5 (26.3)	117.2 (28.5)	0.67 0.43	139.6 (40.9)	136.6 (39.9)	166.8 (41.7)	135.7 (23.1)	0.08 0.36	151.6 (22.6)
Phe	66.9 (10.6)	68.6 (13.0)	67.7 (13.7)	0.86 0.85	77.0 (12.7)	73.4 (18.4)	74.9 (16.2)	66.2 (11.3)	0.32 0.21	75.8 (10.0)
Tyr	64.1 (18.5)	60.3 (15.2)	62.6 (14.6)	0.59 0.78	72.3 (21.6)	66.7 (20.3)	71.4 (21.6)	64.6 (11.8)	0.63 0.58	74.1 (14.8)
Ile	70.7 (19.4)	66.1 (15.9)	66.2 (17.5)	0.48 0.31	77.9 (26.3)	81.0 (14.1)	95.9 (36.2)	77.7 (14.3)	0.21 0.38	86.0 (15.4)
Trp	73.2 (12.5)	71.3 (13.8)	73.3 (14.8)	0.78 0.91	76.7 (12.7)	79.9 (15.0)	79.8 (15.5)	78.2 (14.5)	0.96 0.80	79.9 (10.3)

^ASF36mean5 score was the mean of raw scores (scale 0-100) for the five subdimensions Physical Function, Bodily pain, Vitality, General Health and Social Function, from the SF36 questionnaire (SF36 ver. 1.2, Norwegian translation) assessed at baseline for ME/CFS patients included in the clinical trials RituxME (SF36 data missing for one patient) and CycloME. Out of 19 patients from KTS-2-2010, baseline SF36 data were available from 14 patients. Thus, there were baseline SF36 data from 194 ME/CFS patients out of 200 patients included for metabolite analyses, and from 147 (119 female and 28 male) out of 153 non-fasting ME/CFS patients; ^Bp-values from ANOVA between ME/CFS groups (SF36mean5 score < 20, 20-30, and >30), analyses between groups^C and for linearity^D. Other abbreviations: Pyr, pyruvate; ac-CoA, acetyl-CoA.