

## Supplementary materials

**Table S1.** Retention times, m/z transition and MS settings of the (stable isotope labeled) amino acids.

Amino acid	Internal standard	Ret.time (min)	MRM transition (m/z)	DP/CE <sup>1</sup>
glycine / <sup>13</sup> C <sub>2</sub> <sup>15</sup> N-glycine		5.7	76->76 / 79->79	25/5
alanine / <sup>13</sup> C <sub>3</sub> <sup>15</sup> N-alanine		5.2	90->44 / 94->47	25/18
serine / <sup>13</sup> C <sub>3</sub> <sup>15</sup> N <sub>3</sub> -serine		6.2	106->60 / 110->63	40/15
proline / <sup>13</sup> C <sub>5</sub> <sup>15</sup> N-proline		4.0	116->70 / 122->75	30/20
threonine / <sup>13</sup> C <sub>4</sub> <sup>15</sup> N-threonine		5.5	120->74 / 125->78	30/20
valine / <sup>13</sup> C <sub>5</sub> <sup>15</sup> N-valine		4.0	118->72 / 124->77	30/13
leucine / <sup>13</sup> C <sub>6</sub> <sup>15</sup> N-leucine		2.9	132->86 / 139->92	30/13
isoleucine / <sup>13</sup> C <sub>6</sub> <sup>15</sup> N-isoleucine		3.1	132->86 / 139->92	30/13
aspartate/ <sup>13</sup> C <sub>4</sub> <sup>15</sup> N-aspartate		7.2	134->74 / 139->77	30/20
glutamate / <sup>13</sup> C <sub>5</sub> <sup>15</sup> N-glutamate		6.4	148->84 / 154->89	30/16
histidine / <sup>13</sup> C <sub>6</sub> <sup>15</sup> N <sub>3</sub> -histidine		7.4	156->110 / 165/118	30/20
lysine / <sup>13</sup> C <sub>6</sub> <sup>15</sup> N <sub>2</sub> -lysine		7.9	147->84 / 155->90	25/18
phenylalanine / <sup>13</sup> C <sub>9</sub> <sup>15</sup> N-phenylalanine		2.8	166->120 / 176->129	30/18
tyrosine / <sup>13</sup> C <sub>5</sub> <sup>15</sup> N-tyrosine		4.2	182->136 / 192->145	30/20
arginine/ <sup>13</sup> C <sub>6</sub> <sup>15</sup> N <sub>4</sub> -arginine		7.6	175->116 / 185->122	30/20
citrulline / D4-citrulline		6.5	176->159 / 180->163	30/18
glutamine/ <sup>13</sup> C <sub>3</sub> <sup>15</sup> N-alanine		6.1	147->84 / 94->47	30/17
ornithine / <sup>13</sup> C <sub>6</sub> <sup>15</sup> N <sub>2</sub> -lysine		8.0	133->70 / 155->90	35/14
taurine / <sup>13</sup> C <sub>3</sub> <sup>15</sup> N-alanine		4.3	126->108 / 94->47	58/20
hydroxyproline / <sup>13</sup> C <sub>5</sub> <sup>15</sup> N-proline		4.2	132->86 / 122->75	20/21
tryptophan / <sup>13</sup> C <sub>9</sub> <sup>15</sup> N-phenylalanine		2.9	205->188 / 176->129	30/17
asparagine / <sup>13</sup> C <sub>3</sub> <sup>15</sup> N-alanine		11.5	133->74 / 94->47	25/18
cystine / <sup>13</sup> C <sub>6</sub> <sup>15</sup> N <sub>2</sub> -cystine		13.8	241->152 / 249->156	30/18
methionine / <sup>13</sup> C <sub>5</sub> <sup>15</sup> N-methionine		9.8	150->104 / 156-> 109	30/13

retention times are represented in minutes, MRM transitions (multiple reaction monitoring) indicate first transition/second transition and quantifying/qualifying used. DP= declustering potential; CE= collision energy.

**Table S2.** Limits of detection and quantification, intra-and inter-assay precision of 23 amino acids in the control samples.

			DBS						Low commercial QC						Vendor Range	high commercial QC						Vendor range
Amino acid	LOD	LOQ	intra-assay CV (%)			inter-assay CV (%)			intra-assay CV (%)			inter-assay CV (%)				intra-assay CV (%)			inter-assay CV (%)			
	μM	μM	Mean (μM)	SD	CV (%)	Mean (μM)	SD	CV (%)	Mean (μM)	SD	CV (%)	Mean (μM)	SD	CV (%)	μmol/L	Mean (μM)	SD	CV (%)	Mean (μM)	SD	CV (%)	μmol/L
alanine	2.4	8.1	405.2	26.9	6.6	402.2	4.7	1.2	779.0	43.3	5.6	822.9	65.6	8.0	460-955	1638.0	101.9	6.2	1711.4	122.0	7.1	1007-1869
arginine	3.7	12.3	27.1	2.2	8.3	17.9	3.0	17.0	13.6	1.9	13.8	9.3	4.1	43.7	4.90-23.1	206.7	15.6	7.5	37.9	12.1	32.0	44.5-178
asparagine	1.6	5.4	72.7	2.5	3.5	82.4	2.8	3.4	63.9	4.6	7.1	51.7	9.4	18.1	*	62.0	3.5	5.6	49.2	10.5	21.4	*
aspartate	4.3	14.2	31.6	9.3	29.5	30.6	3.5	11.3	32.2	6.7	20.9	20.2	6.3	31.0	21.4-64.1	211.0	32.0	15.2	184.0	41.3	22.5	128-299
citrulline	1.6	5.3	31.0	2.5	8.2	29.4	4.1	13.9	28.1	1.4	4.9	22.7	4.9	21.5	13.8-36.3	305.9	14.5	4.7	286.4	45.0	15.7	108-370
glutamine	3.3	11.0	566.4	62.4	11.0	448.9	5.3	1.1	69.7	6.0	8.6	5.7	2.9	50.0	*	68.4	5.4	7.9	17.7	6.7	38.2	*
glutamate	0.4	1.2	103.7	7.0	6.7	80.7	4.8	6.0	732.7	21.8	3.0	713.1	82.4	11.6	416-772	1196.0	70.5	5.9	1096.4	153.8	14.0	638-1186
glycine	3.6	12.0	259.2	24.9	9.6	227.5	13.1	4.7	595.2	45.8	7.7	607.4	61.9	10.2	380-705	1577.1	405.2	9.3	1699.7	142.5	8.4	967-1796
histidine	2.8	9.3	88.9	12.6	14.2	75.3	3.0	3.9	117.3	11.3	9.6	83.1	13.3	16.0	*	105.0	5.5	5.2	82.9	14.0	16.9	*
hydroxyproline	0.5	1.6	11.4	7.0	61.2	11.3	4.1	36.3	22.7	8.8	38.6	21.8	7.9	36.2	*	19.5	7.3	37.7	23.9	7.9	33.2	*
isoleucine	0.9	2.8	52.6	3.6	6.9	53.5	2.5	4.8	107.4	5.5	5.1	106.7	7.1	6.7	*	113.8	10.4	9.2	119.6	10.0	8.3	*
leucine	2.8	9.3	120.0	7.4	6.2	119.4	10.8	9.1	225.5	8.3	3.7	234.2	22.2	9.5	162-301	728.1	61.7	8.5	763.4	43.3	5.7	462-859
lysine	0.9	2.9	162.6	8.6	5.3	90.4	5.3	5.9	232.9	12.5	5.4	93.9	32.5	34.6	*	229.2	11.0	4.8	95.6	33.4	34.9	*
methionine	2.5	8.5	22.9	2.1	9.4	24.3	0.9	3.8	38.1	2.4	6.3	36.0	3.3	9.2	27.6-57.2	399.5	24.9	6.2	405.5	20.6	5.1	256-531
ornithine	3.5	11.5	72.7	2.5	3.5	84.2	25.2	30.0	63.9	4.6	7.1	109.3	42.1	38.5	125-292	62.0	3.5	5.6	321.7	112.8	35.1	267-623
phenylalanine	2.1	7.1	60.6	5.6	9.2	59.1	2.0	3.4	132.2	9.9	7.5	132.4	14.7	11.1	70.3-164	849.4	56.6	6.7	837.2	59.6	7.1	447-1043
proline	1	3.2	208.1	9.9	4.8	227.4	14.1	6.2	279.6	12.2	4.4	298.7	24.7	8.3	184-342	595.2	31.3	5.3	620.6	31.5	5.1	376-698

<b>serine</b>	4.4	14.7	103.8	8.5	8.2	110.5	21.4	19.3	267.9	19.5	7.0	262.6	25.0	9.5	*	248.2	19.3	7.8	247.6	34.0	13.7	*
<b>taurine</b>	7.2	24.0	215.2	16.4	7.6	170.9	6.7	3.9	163.6	17.1	10.5	151.3	22.6	14.9	*	155.1	14.6	9.4	134.5	18.9	14.0	*
<b>threonine</b>	1.8	6.0	130.1	14.1	10.9	126.9	0.8	0.7	193.9	11.4	5.9	188.6	16.1	8.5	*	183.0	18.3	10.0	179.1	11.1	6.2	*
<b>tryptophan</b>	2.9	9.7	47.4	4.3	9.0	41.1	5.9	14.5	63.1	3.7	5.9	52.8	8.9	16.9	*	59.5	4.9	8.2	49.0	8.8	18.0	*
<b>tyrosine</b>	3.4	11.4	116.6	9.0	3.8	72.7	2.1	2.8	116.6	9.0	7.7	108.7	6.4	5.9	50.7-134	715.7	40.3	5.6	698.6	59.4	8.5	335-781
<b>valine</b>	1.8	6.0	207.8	17.7	8.5	240.9	10.4	4.3	272.7	15.3	5.6	293.8	22.5	7.7	122-323	682.8	53.8	7.9	<u><b>774.7</b></u>	55.2	7.1	330-770

LOD= limit of detection. calculated as 3.3\*standard deviation (SD)/ slope of the calibration curve; LOQ= limit of quantification. calculated as 10\*SD/slope of calibration curve;  
CV= coefficient of variation. calculated as the ratio of the SD to the mean (sd/mean\*100%). The inter-assay precision per amino acid. calculated from quality control samples (n = 30 in ten experiments). Mean concentrations for Arginine and Glutamine in the low QC samples were below the limit of quantification (in. bold. italic. underlined). Underlined and bold AA in the table have intra- and/or interassay CV >25% in at least one of the QC samples. Bias compared to the established concentrations by the vendor is presented underlined and bold.

\*No established range was supplied for this amino acid by the vendor

**Table S3.** Mann-Whitney U for the annual percentile changes in controls compared to deceased children

Amino acid	P-value	Amino acid	P-value
Alanine	0.834	Lysine	0.548
Arginine	<0.01	Methionine	0.640
Asparagine	0.690	Ornithine	0.841
Aspartate	<0.05	Phenylalanine	0.095
Citrulline	<0.05	Proline	0.295
Glutamine	0.908	Serine	0.402
Glutamate	<0.05	Taurine	0.295
Glycine	0.310	Threonine	0.642
Histidine	<0.05	Tryptophan	0.920
Hydroxyproline	0.841	Tyrosine	0.660
Isoleucine	<0.05	Valine	0.295
Leucine	0.421		

*Mann-Whitney U test were done to compare annual percentile changes in DBS AA concentrations of controls and deceased children. Most annual percentile changes in AA between groups were comparable. Some AA showed different annual percentile change rates. Upon plotting (see Figure S1), these deviations were seen to be attributed to the control group, probably due to the loss of power from the smaller sample size.*

Figures: Plotting of annual percentile changes in AA concentrations between controls and deceased children

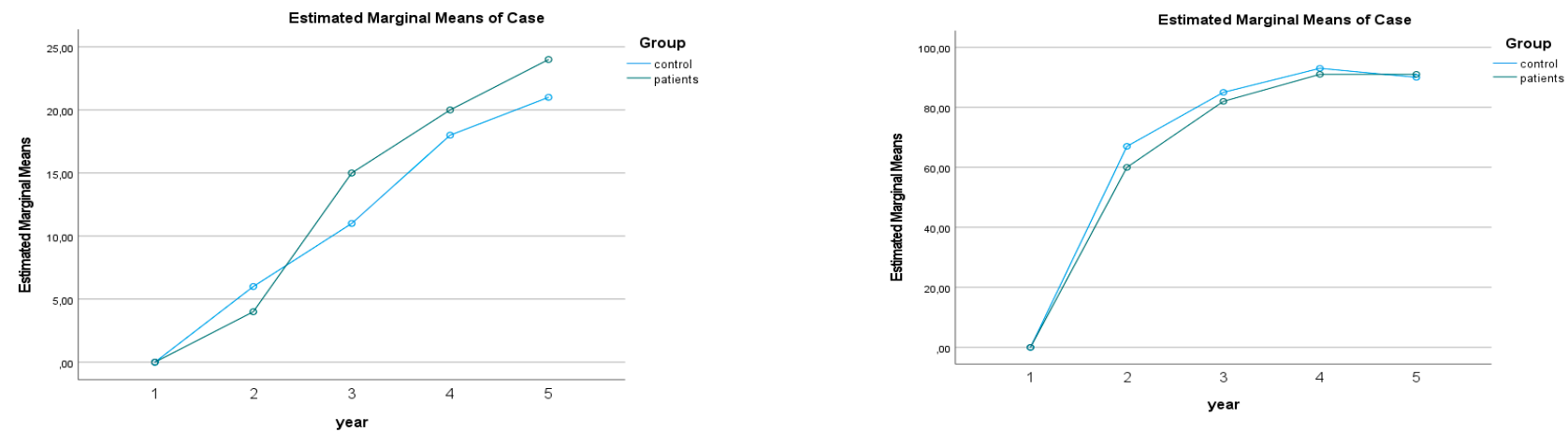


Figure S1. Examples of good agreement. Leucine and Glutamine

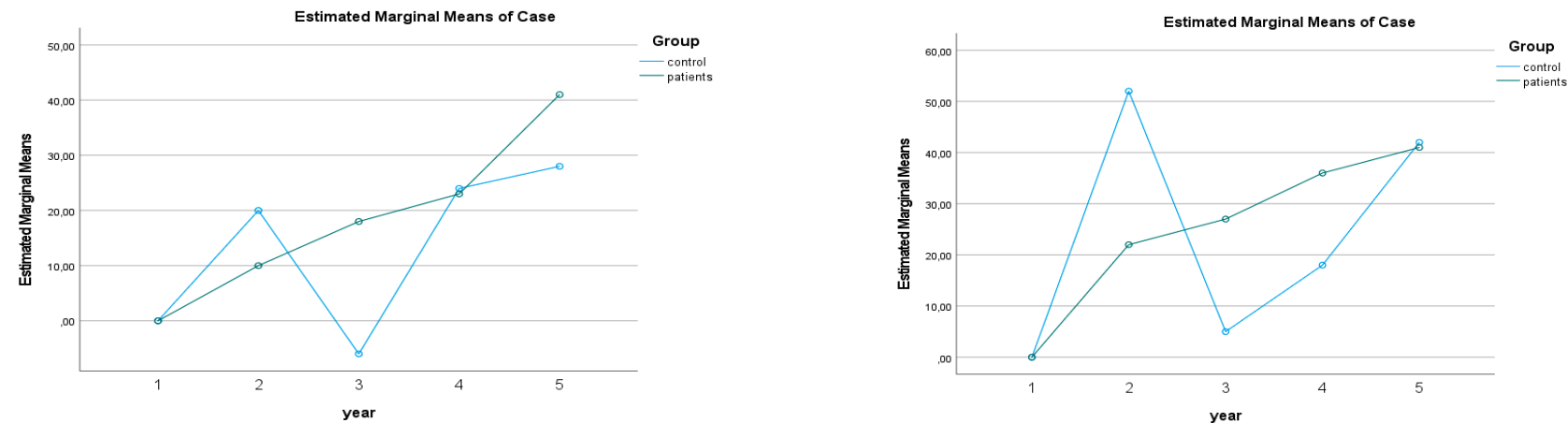


Figure S2. Examples of poor agreement. Citrulline and Arginine