

SUPPLEMENTARY ONLINE MATERIALS

Table S1: Sensitivity and specificity of the employed ELISA test kits		
	Sensitivity (Detection limit [$\mu\text{mol/l}$])	Specificity (<i>Cross reactivity</i>)
Tryptophan	7	5-HTP (5-Hydroxy-Tryptophan) < 0,5% L-Phenylalanine < 0,1% L-Tyrosine < 0,1%
Phenylalanine	3,75	L-Tyrosine < 0,02%
Tyrosine	3,6	L-Phenylalanine < 2% L-Tryptophan < 0,5%
BCAA	5,8	No cross reactivity with L-Methionine was found.

Formula for TRP influx calculations across the blood-brain barrier:

$$\text{TRP influx} = \frac{V_{\max}C}{(K_m[1 + \sum(C_i/K_i)] + C)} + K_dC$$

C = TRP plasma concentration

V_{max} = Maximum rate of conversion

K_m = Michaelis constant

C_i = Plasma concentration of competing amino acids (TRP, PHE, TYR, BCAA)

K_i = Affinity constants of competing amino acids (TRP, PHE, TYR, BCAA)

K_d = Diffusion constant

Table S2: Mean TRP Influx values of the four sample groups	
Group	Mean TRP Influx (nmol/min/g)
ATD	7.005
BAL	15.583
ATD _{PHE/LEU}	4.616
BAL _{PHE/LEU}	9.830

Table S3: Bonferroni post-hoc analyses rmANOVA between the four sample groups		
df		3, 68
F		97.577
Group	Group	<i>p</i>-value
ATD	BAL	< 0.001
	ATD _{PHE/LEU}	0.004
	BAL _{PHE/LEU}	0.001
BAL	ATD	< 0.001
	ATD _{PHE/LEU}	< 0.001
	BAL _{PHE/LEU}	< 0.001
ATD _{PHE/LEU}	ATD	0.004
	BAL	< 0.001
	BAL _{PHE/LEU}	< 0.001
BAL _{PHE/LEU}	ATD	0.001
	BAL	< 0.001
	BAL _{PHE/LEU}	< 0.001

Table S4: Results of the item analyses of the somatic complaints score								
Item	Mixture	Baseline (T ₀)	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
Health	ATD	2.94 ± 0.04	2.94 ± 0.11	2.83 ± 0.11	3.00 ± 0.06	3.00 ± 0.09	3.00 ± 0.07	3.00 ± 0.09
	BAL	3.00 ± 0.04	2.83 ± 0.11	2.94 ± 0.11	3.00 ± 0.06	2.83 ± 0.09	3.00 ± 0.07	3.00 ± 0.09
	ATD_{PHE/LEU}	2.94 ± 0.04	2.94 ± 0.11	2.89 ± 0.11	2.94 ± 0.06	2.94 ± 0.09	2.89 ± 0.07	2.83 ± 0.09
	BAL_{PHE/LEU}	3.00 ± 0.04	2.72 ± 0.11	2.67 ± 0.11	2.72 ± 0.06	2.94 ± 0.09	2.72 ± 0.07	2.72 ± 0.09
Headache	ATD	0.11 ± 0.09	0.00 ± 0.05	0.22 ± 0.10	0.11 ± 0.07	0.00 ± 0.06	0.00 ± 0.06	0.11 ± 0.10
	BAL	0.06 ± 0.09	0.06 ± 0.05	0.00 ± 0.10	0.06 ± 0.07	0.06 ± 0.06	0.06 ± 0.06	0.06 ± 0.10
	ATD_{PHE/LEU}	0.06 ± 0.09	0.06 ± 0.05	0.17 ± 0.10	0.06 ± 0.07	0.11 ± 0.06	0.06 ± 0.06	0.17 ± 0.10
	BAL_{PHE/LEU}	0.11 ± 0.09	0.06 ± 0.05	0.17 ± 0.10	0.11 ± 0.07	0.11 ± 0.06	0.11 ± 0.06	0.28 ± 0.10
Dizziness	ATD	0.00 ± 0.08	0.06 ± 0.08	0.06 ± 0.09	0.00 ± 0.03	0.00 ± 0.04	0.00 ± 0.05	0.00 ± 0.04
	BAL	0.17 ± 0.08	0.06 ± 0.08	0.11 ± 0.09	0.00 ± 0.03	0.00 ± 0.04	0.00 ± 0.05	0.00 ± 0.04
	ATD_{PHE/LEU}	0.00 ± 0.08	0.00 ± 0.08	0.00 ± 0.09	0.00 ± 0.03	0.00 ± 0.04	0.06 ± 0.05	0.00 ± 0.04
	BAL_{PHE/LEU}	0.00 ± 0.08	0.22 ± 0.08	0.28 ± 0.09	0.06 ± 0.03	0.11 ± 0.04	0.11 ± 0.05	0.11 ± 0.04
Sweating	ATD	0.00 ± 0.03	0.00 ± 0.03	0.00 ± 0.03	0.00 ± 0.04	0.00 ± 0.03	0.00 ± 0.03	0.00 ± 0.00
	BAL	0.00 ± 0.03	0.00 ± 0.03	0.00 ± 0.03	0.00 ± 0.04	0.00 ± 0.03	0.00 ± 0.03	0.00 ± 0.00
	ATD_{PHE/LEU}	0.00 ± 0.03	0.00 ± 0.03	0.06 ± 0.03	0.00 ± 0.04	0.00 ± 0.03	0.06 ± 0.03	0.00 ± 0.00
	BAL_{PHE/LEU}	0.06 ± 0.03	0.06 ± 0.03	0.00 ± 0.03	0.11 ± 0.04	0.06 ± 0.03	0.00 ± 0.03	0.00 ± 0.00
Nausea	ATD	0.00 ± 0.03	0.44 ± 0.15	0.17 ± 0.12	0.00 ± 0.04	0.00 ± 0.00	0.00 ± 0.10	0.00 ± 0.08
	BAL	0.00 ± 0.03	0.11 ± 0.15	0.17 ± 0.12	0.06 ± 0.04	0.00 ± 0.00	0.00 ± 0.10	0.00 ± 0.08
	ATD_{PHE/LEU}	0.00 ± 0.03	0.28 ± 0.15	0.22 ± 0.12	0.00 ± 0.04	0.00 ± 0.00	0.28 ± 0.10	0.17 ± 0.08
	BAL_{PHE/LEU}	0.06 ± 0.03	0.56 ± 0.15	0.33 ± 0.12	0.06 ± 0.04	0.00 ± 0.00	0.17 ± 0.10	0.11 ± 0.08

Legend to Tab. S4: Results of the itemizing analyses of the somatic complaints score after the administration of four different amino acid mixtures: ATD (Acute Tryptophan Depletion; challenge condition), BAL (control condition for ATD), ATD_{PHE/LEU} (newly developed challenge condition) and BAL_{PHE/LEU} (control condition for ATD_{PHE/LEU}). Mean scores ± standard error are presented for the different groups at the seven time points of completion of the tolerance questionnaire (T₀-T₆). Self-evaluation of headache, dizziness, sweating, nausea and feeling of health represent the included items.

Table S5: Results of correlation analysis between TRP influx and TRP/CAA ratio for ATD Moja-De							
	Baseline T₀	T₁	T₂	T₃	T₄	T₅	T₆
r	0.859	0.936	0.911	0.904	0.924	0.912	0.843
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

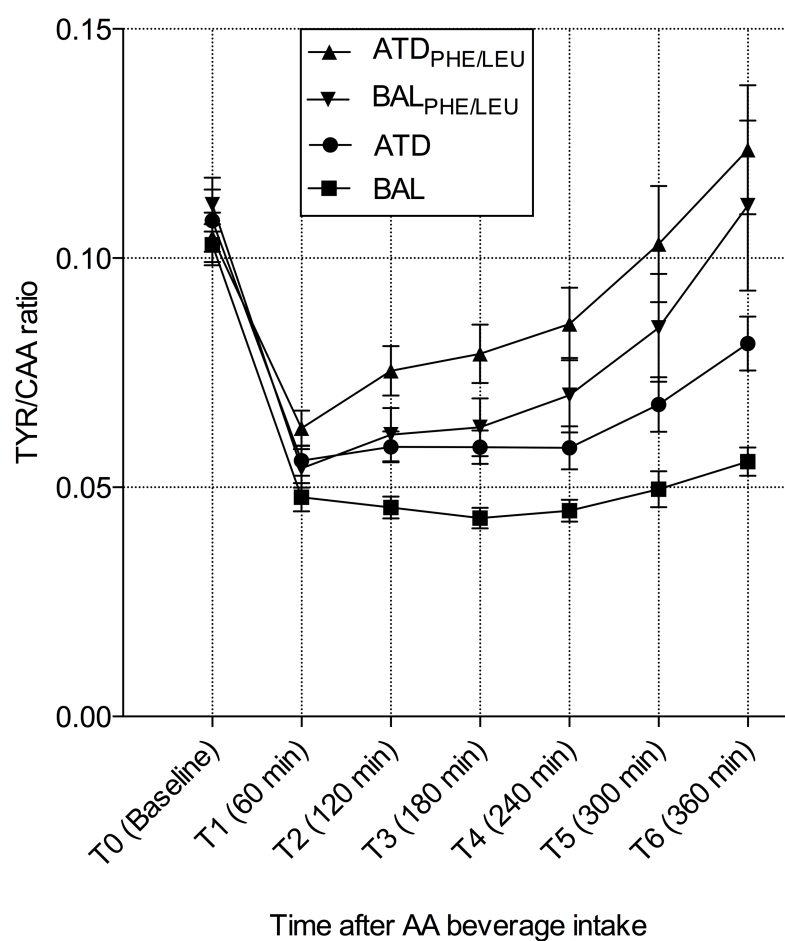
Legend to Tab. S5: Results of Pearson correlation analysis between tryptophan influx and TRP/CAA ratio for ATD Moja-De over time. Correlation coefficient (r) and statistical significance (p-value) are included for the seven time points of measurement (T₀-T₆).

Table S6: Results of correlation analysis between TRP influx and TRP/CAA ratio for ATD_{PHE/LEU}							
	Baseline T₀	T₁	T₂	T₃	T₄	T₅	T₆
r	0.827	0.928	0.962	0.970	0.976	0.979	0.980
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Legend to Tab. S6: Results of Pearson correlation analysis between tryptophan influx and TRP/CAA ratio for ATD_{PHE/LEU} (newly developed challenge condition) over time. Correlation coefficient (r) and statistical significance (p-value) are included for the seven time points of measurement (T₀-T₆).

Figure S1 *a,b*: Curve progression for calculated TYR/CAA and PHE/CAA ratios from baseline (T0) to time point T6 after the intake of the ATD Moja-De amino acid (AA) mixture, its balanced control condition (BAL), the newly developed tryptophan depletion protocol ATD_{PHE/LEU} and the corresponding control condition (BAL_{PHE/LEU}). Data are given as the mean values with bars representing the standard errors.

a)



b)

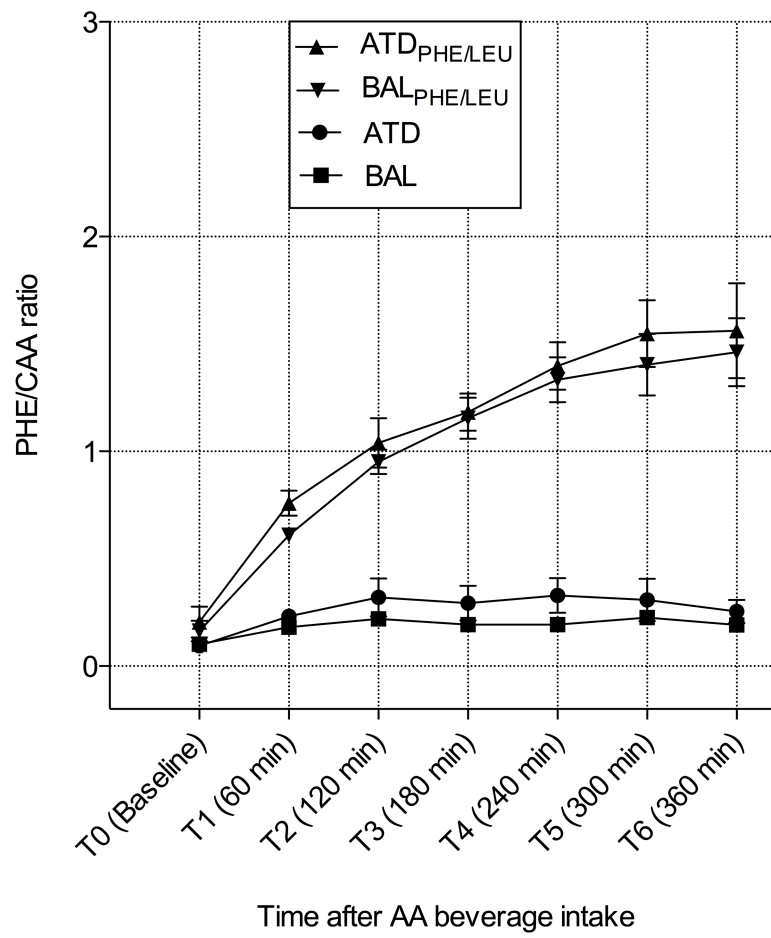
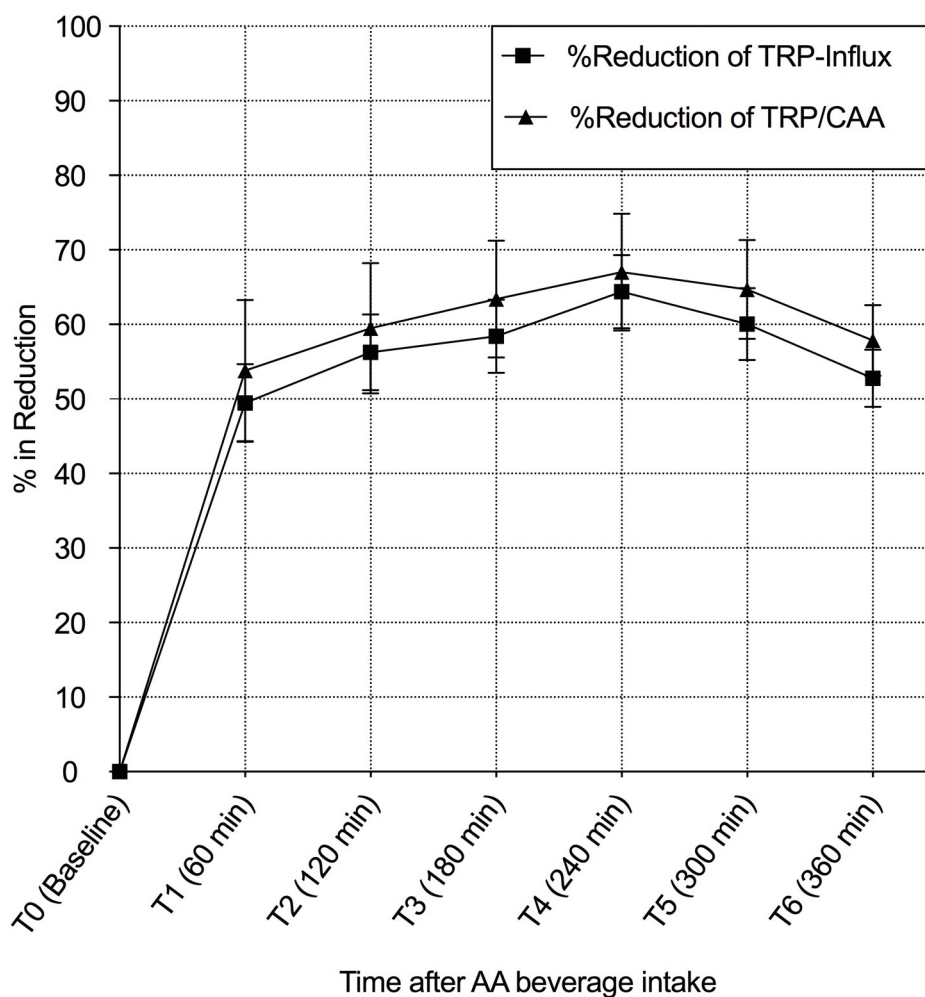
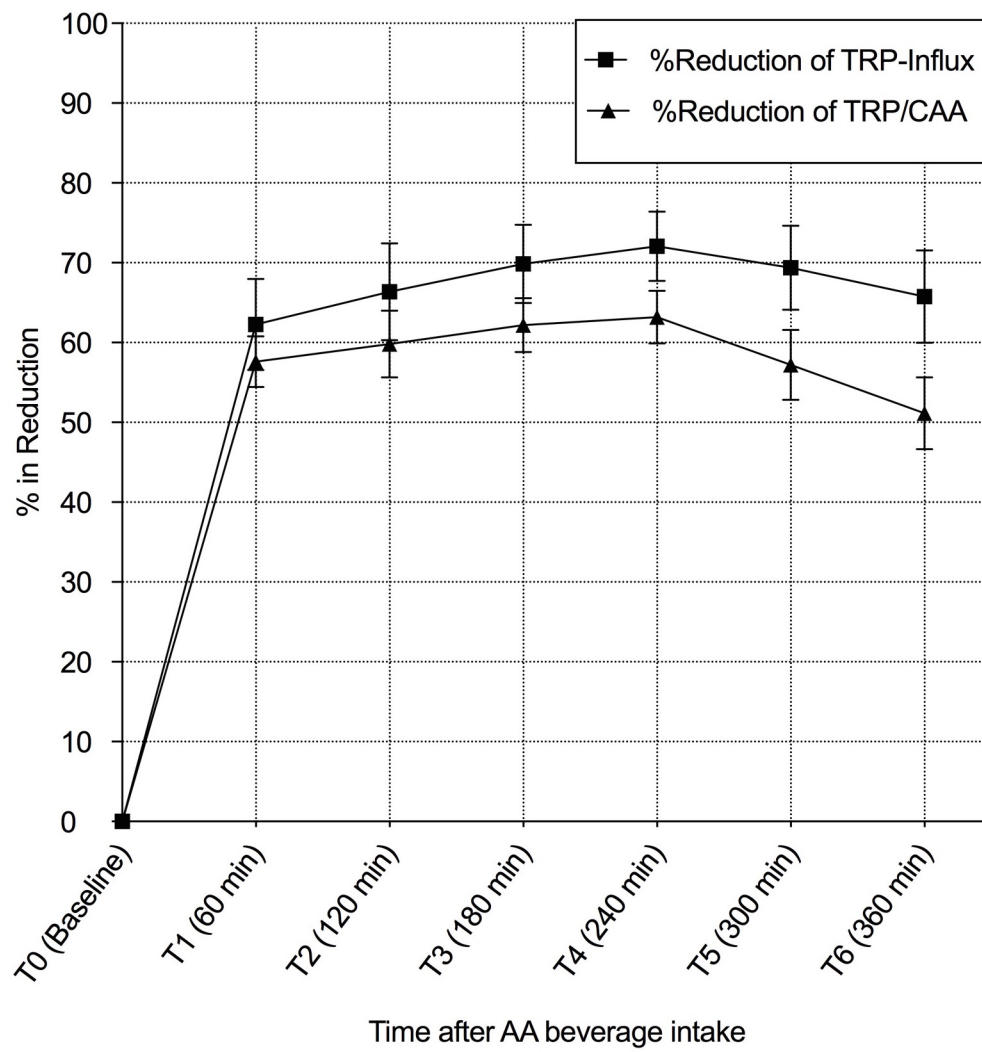


Figure S2 a,b: Curve progression for the percentage in reduction (%) calculated for the TRP influx and TRP/CAA ratio from baseline (T0) to time point T6 after the intake of the ATD Moja-De amino acid (AA) mixture and its balanced control condition (BAL, Figure S2 a), and for the newly developed tryptophan depletion protocol $ATD_{PHE/LEU}$ and the corresponding control condition ($BAL_{PHE/LEU}$, Figure S2 b). Data are given as the mean values with bars representing the standard errors.

a)



b)



Subject Code:

Time:

Questionnaire on Beverage Taste and Tolerance

Below, you find several statements that refer to the taste and tolerance of the amino acid beverage administered before. Please assess each statement as precise as possible by ticking the appropriate box most appropriate for you at the moment of evaluation:

- 0 disagree
- 1 mildly agree
- 2 agree
- 3 strongly agree

There are no correct or wrong answers. We are only interested in your personal opinion.

1.) Taste

	disagree	mildly agree	agree	strongly agree
1) It was possible to drink the amino acid mixture like a regular beverage (e.g., a milk shake).	0	1	2	3
2) The intake of the amino acid mixture was unpleasant for me.	0	1	2	3
3) The consistency of the beverage made it difficult to swallow.	0	1	2	3
4) The amino acid mixture tastes like sulfur.	0	1	2	3
5) The intake of the beverage triggered a feeling of disgust.	0	1	2	3
6) Usually, I do not have any problems regarding the oral administration of medicine (e.g., cough syrup, fizzy tablets).	0	1	2	3

2.) Tolerance

	disagree	mildly agree	agree	strongly agree
1) I feel healthy.	0	1	2	3
2) I have a headache at the moment.	0	1	2	3
3) The intensity of the headache would interfere with my daily activities.	0	1	2	3
4) I am suffering from severe nausea at the moment.	0	1	2	3
5) The intensity of the nausea would interfere with my daily activities.	0	1	2	3
6) The intensity of the nausea makes me feel the urge to vomit.	0	1	2	3
7) I feel dizzy at the moment.	0	1	2	3
8) I feel awake at the moment.	0	1	2	3
9) Usually I do not feel tired in this part of the day.	0	1	2	3
10) I am sweating much more than usual.	0	1	2	3

11) I am hungry.	0	1	2	3
12) My hunger is abnormally great for this part of the day.	0	1	2	3