Supplemental Material

Data S1.

Supplemental Methods

HPLC-MS/MS analysis of KP in plasma

Tryptophan (TRP) and the KP metabolites kynurenine (KYN), kynurenic acid (KYNA) and 3-hydroxy-anthranilic acid (3-HAA) were quantified in plasma by HPLC-MS/MS adopting the analytical method previously used for human plasma samples (17). with slight modifications. Briefly, after addition of corresponding deuterated-internal standards (deuterated-IS), plasma samples were purified by protein precipitation with four volume of cold methanol, incubated at -20°C (1h) and centrifuged. Supernatants were collected, evaporated under nitrogen flow and the residues re-suspended in 0.1% HCOOH in water/ACN (98/2, v/v) and injected into the HPLC-MS/MS system. Separation was done on an Accucore PFP column with HCOOH 0.1% in water (mobile phase A, MP-A) and ACN (mobile phase B, MP-B) at 0.2 mL/min; Elution started with 1% mobile phase B held for 2 min, followed by a 18-min linear gradient to 60% MP-B, held for 1 min, and a 1-min linear-gradient to 90% of MP-B. TRP, KYN, KYNA and 3-HAA, and the deuterated-IS, were acquired in positive MRM mode monitoring the fragmentation products of the deprotonated pseudo-molecular ions (see supplemental files for ion transitions and MS parameters). Plasma samples were analysed in parallel with quality control samples (blank control plasma samples in triplicates) and with freshly prepared calibration curves (weighted $1/y^2$) linear in the ranges 0.5 - 20 µg TRP / mL, 0.025 – 1 µg KYN / mL, 1.2 – 48 ng KYNA / mL and 1.2 – 48 ng 3-HAA / mL plasma.

HPLC-MS/MS analysis of KP in plasma

Chemicals and reagents. Methanol (MeOH), acetonitrile (ACN), acetic acid (CH_3COOH) and formic acid (HCOOH) were from Sigma-Aldrich Co. (Milan, Italy); all solvents were of liquid chromatography-mass spectrometry (LC-MS) grade. LC-MS grade water was obtained in-house with

a Milli-Q system (Millipore, Bedford, MA, USA). L-Tryptophan (TRP), L-kynurenine (KYN), kynurenic acid (KYNA) and 3-hydroxyanthranilic acid (3-HAA) were from Sigma-Aldrich. TRP-d5 and KYNA-d5 were from CDN Isotopes (Chemical Research 2000, Rome, Italy); KYN-d4, and 3-HAA-d2 were from Buchem BV (Apeldoorn, The Netherlands).

Stock and working solutions. Stock solutions at 1 mg/mL were prepared in CH_3COOH 0.08 M (for TRP and KYN) and in H_2O (for KYNA and 3-HAA). Then, TRP and KYN were combined in CH_3COOH 0.08 M and serially diluted in the same solvent to have seven working solutions in the ranges 10 - 600 µg/mL (TRP) and 0.2 - 12 µg/mL (KYN). KYNA and 3-HAA stock solutions were combined in H_2O and serially diluted in the same solvent to have seven working solutions in the ranges 0.01 - 1.0 µg/mL (KYNA) and 0.02 - 2.0 µg/mL (3-HAA). Similarly to analytes, stock solutions of internal standards at 1 mg/mL were prepared in CH_3COOH 0.08 M (for TRP-d5 and KYN-d4) and in H_2O (for KYNA-d5 and 3-HAA-d2). Appropriate volume of the four stock solutions were combined in CH_3COOH 0.08M to obtain the final IS working solution containing 200 µg TRP-d5 / mL, 2 µg KYN-d4 / mL, 48 µg KYNA-d5 / mL and 48 µg 3-HAA-d2 / mL. Stock and working solutions were stored at -20°C for a maximum of 1 week.

Plasma sample preparation. Seven-point calibration curves were generated by spiking 100 µL of control pig plasma with 5 µL of the seven TRP/KYN and KYNA/3-HAA working solutions to have final plasma concentration in the ranges 0.5 - 20 µg TRP / mL, 0.025 – 1 µg KYN / mL, 1.2 – 48 ng KYNA / mL and 1.2 – 48 ng 3-HAA / mL. Unknown and quality control (QC, control blank plasma) samples were added with five µL of CH_3COOH 0.08M and five µL of H_2O before extraction. Briefly, 100 µL of plasma, were mixed with five µL of internal standards working solution, deproteinized by adding 400 µL of cold methanol and incubated for 1h at -20°C. After centrifugation for 10 min at 14.000x*g* (4°C), supernatant was transferred to eppedorf tubes and dried under nitrogen flow. The residue was dissolved in 100 µL of 1% acetonitrile in H_2O containing 0.1% formic acid, transferred to autosampler vial insert, and injected directly in the HPLC system (Alliance separation module

2695, Waters, Milford, MA, USA) coupled with Micromass Quattro Micro API triple-quadrupole (Waters, Milford, MA, USA).

HPLC-MS/MS method. The mass spectrometer Quattro Micro API triple quadrupole instrument (Waters Corp., Manchester, UK) was equipped with an electrospray ionization source (ESI) operating in positive ion mode and multiple reaction monitoring (MRM) mode, measuring the fragmentation products of the deprotonated pseudo-molecular ions of TRP, KYN, KYNA and 3-HAA, and the corresponding deuterated-IS as follow:

Analyte	**Parent ion (m/z)**	**Product ion (m/z)**	**Cone voltage (V)**	**Collision energy (eV)**
TRP	205.0	146.0	15	15
TRP-d5	210.0	192.4	20	8
KYN	209.1	146.1	12	15
KYN-d4	213.2	150.1	12	15
KYNA	190.0	144.2	25	15
KYNA-d5	195.1	149.3	30	15
3-HAA	154.0	136.2	15	8
3-HAA-d2	155.9	138.2	15	13

The optimized mass spectrometric parameters for capillary, extractor and RF lens voltages were respectively 3.5 kV, 2.0 V and 1.5 V. The source temperature and desolvation temperature were 100 and 350°C. The desolvation and cone gas flows were 600 and 60 L/h. Argon was used as collision gas.

Chromatographic separation was done on an Alliance 2695 (Waters Corp.) using an Accucore PFP column (150 × 2.1 mm; 2.6 μm particle size, Thermo-Scientific) at 30°C. The elution solvents used were 0.1% HCOOH in water (mobile phase A, MP-A) and acetonitrile (mobile phase B, MP-B). The

injection volume was 40 μL and the flow rate 200 μL/min. The auto-sampler temperature was maintained at 6°C.

Elution started with 99% MP-A for 2 min, followed by an 18-min linear gradient to 60% MP-B which was maintained for 1 min. Thereafter, a 1-min linear-gradient brought MP-B to 90%. After 2 min, a 1-min-linear gradient was set for returning to initial conditions (99% MP-A), which was maintained for 20 min to equilibrate the column. The total run time was 45 min. The HPLC-MS/MS system was controlled by the MassLynx® version 4.1 (Waters Corp.) and data were collected with the same software.

Plasma KP quantification. Concentration of TRP, KYN, KYNA and 3-HAA in plasma, were quantified by reference to a linear regression curve of calibration standards in which the responses - expressed as the peak area ratio of the analyte to corresponding deuterated-IS - were plotted against the spiked analyte concentration, and the data were fitted with a weighted ($1/y^2$) linear regression curve. Since KP metabolites are endogenously present in plasma, a control sample with the only addition of IS, was analysed and included in the calibration curve calculation as a sample corresponding to "zero" spiked concentration. Thus, KP metabolites concentrations were calculated dividing the response obtained in the unknown samples by the slope of the calibration curve. In each analytical session, unknown plasma samples run in parallel with a freshly prepared calibration curves and quality control samples (blank control plasma samples in triplicates). The r^2 of linear regression curves were always > 0.99 and the QCs showed a precision (coefficient of variation, CV %) and accuracy (relative error, RE %) always within the acceptance limits (<15%).

Table S1. Absolute Changes of Left Ventricle Ejection Fraction (LVEF), End-Diastolic Volume (EDV) and End-Systolic Volume (ESV).

	Control	Argon 50%	Argon 70%	Two-way ANOVA p value
LVEF, %				
PR 2 h - Baseline	-40.3 ± 6.0**	-23.2 ± 5.7**	-37.9 ± 5.4**	Group: p= 0.72
PR 4 h - Baseline	-39.5 ± 5.7**	-24.5 ± 6.4**	-33.4 ± 5.4**	Time: p< 0.0001
PR 96 h - Baseline	-21.0 ± 6.0**	-19.1 ± 5.7**	-4.9 ± 5.6	
PR 4 h - PR 2 h	0.9 ± 6.7	-1.2 ± 7.0	4.4 ± 5.7	
PR 96 h - PR 2 h	19.3 ± 6.9*	4.1 ± 6.4	32.9 ± 5.8**	
PR 96 h - PR 4 h	18.5 ± 6.7*	5.3 ± 7.0	28.5 ± 5.8**	
EDV, mL				
PR 2 h - Baseline	1.4 ± 4.5	-3.3 ± 4.1	-7.9 ± 4.2	Group: p= 0.10
PR 4 h - Baseline	8.5 ± 4.5	5.9 ± 4.8	-0.05± 4.1	Time: p= 0.03
PR 96 h - Baseline	7.2 ± 4.5	5.6 ± 4.3	-2.3 ± 4.2	
PR 4 h - PR 2 h	7.1 ± 5.2	9.2 ± 5.1	7.9 ± 4.4	
PR 96 h - PR 2 h	5.9 ± 5.5	8.9 ± 4.7	5.6 ± 4.5	
PR 96 h - PR 4 h	-1.3 ± 5.3	-0.3 ± 5.3	-2.3 ± 4.4	
ESV, mL				
PR 2 h - Baseline	14.7 ± 3.3**	5.0 ± 3.0	7.5 ± 3.1	Group: p= 0.30
PR 4 h - Baseline	18.3 ± 3.3**	12.0 ± 3.6**	10.2 ± 3.0**	Time: p< 0.0001
PR 96 h - Baseline	10.0 ± 3.3*	8.5 ± 3.2*	-0.5 ± 3.1	
PR 4 h - PR 2 h	3.6 ± 3.8	6.9 ± 3.8	2.6 ± 3.2	
PR 96 h - PR 2 h	-4.6 ± 3.8	3.5 ± 3.5	-8.0 ± 3.3	
PR 96 h - PR 4 h	-8.2 ± 3.8	-3.5 ± 3.9	-10.7 ± 3.2**	

PR, post-resuscitation. All data are reported as mean ± SEM. p value from two-way ANOVA with Tukey's multiple comparisons post-hoc test within each group.

* $p < 0.05$; ** $p < 0.01$.