Supplementary Information

Chronic toxicity of crude ricinine in rats assessed by ^{1}H NMR metabolomics analysis

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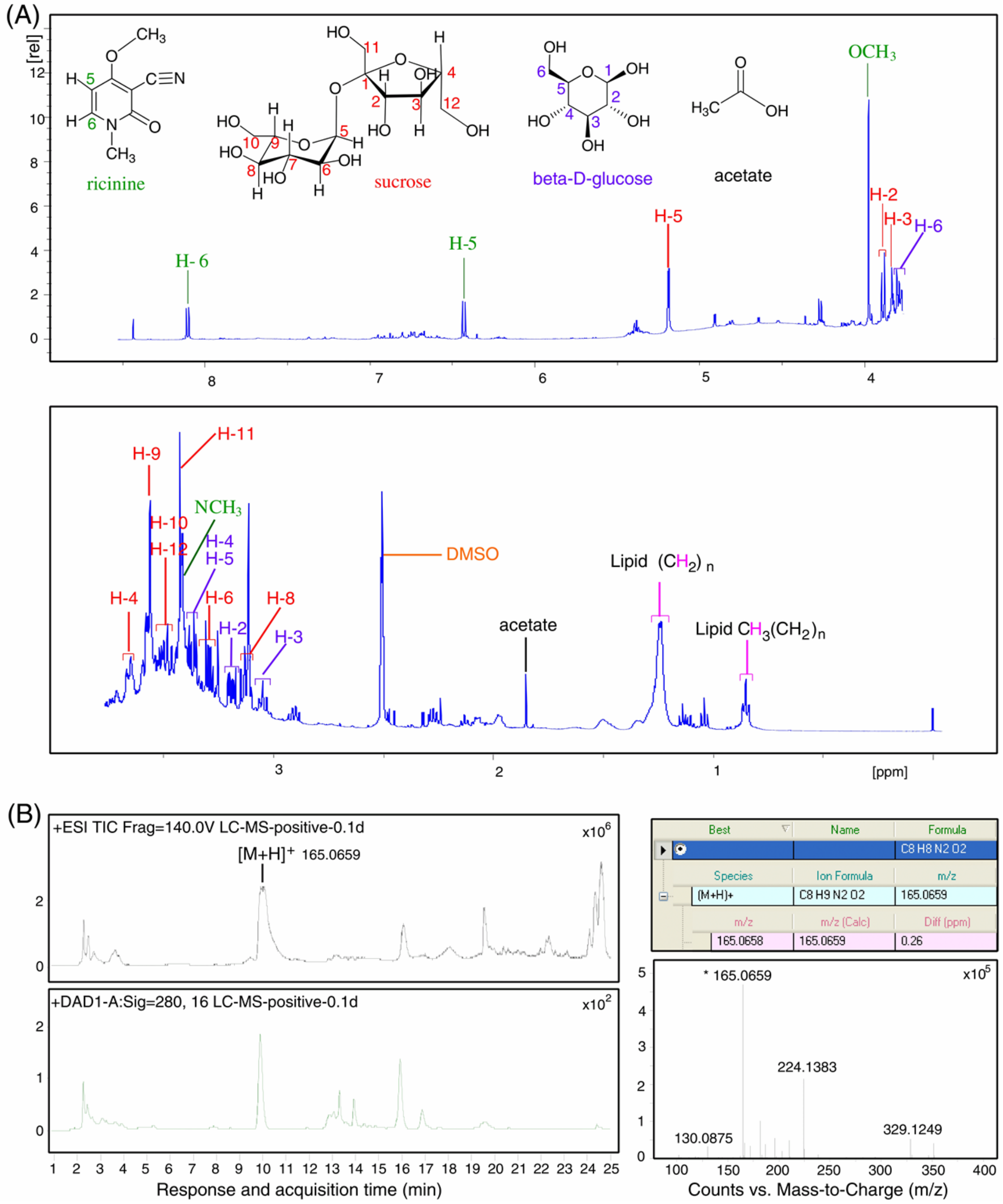

Best	Name	Formula
		C8 H8 N2 O2

Species	Ion Formula	m/z
(M+H)+	C8 H9 N2 O2	165.0659

m/z	m/z (Calc)	Diff (ppm)
165.0658	165.0659	0.26

Fig.S1 ^{1}H NMR (500 MHz, DMSO) spectrum (A) and LC-MS spectrum (B) of ricinine from CBS extract.

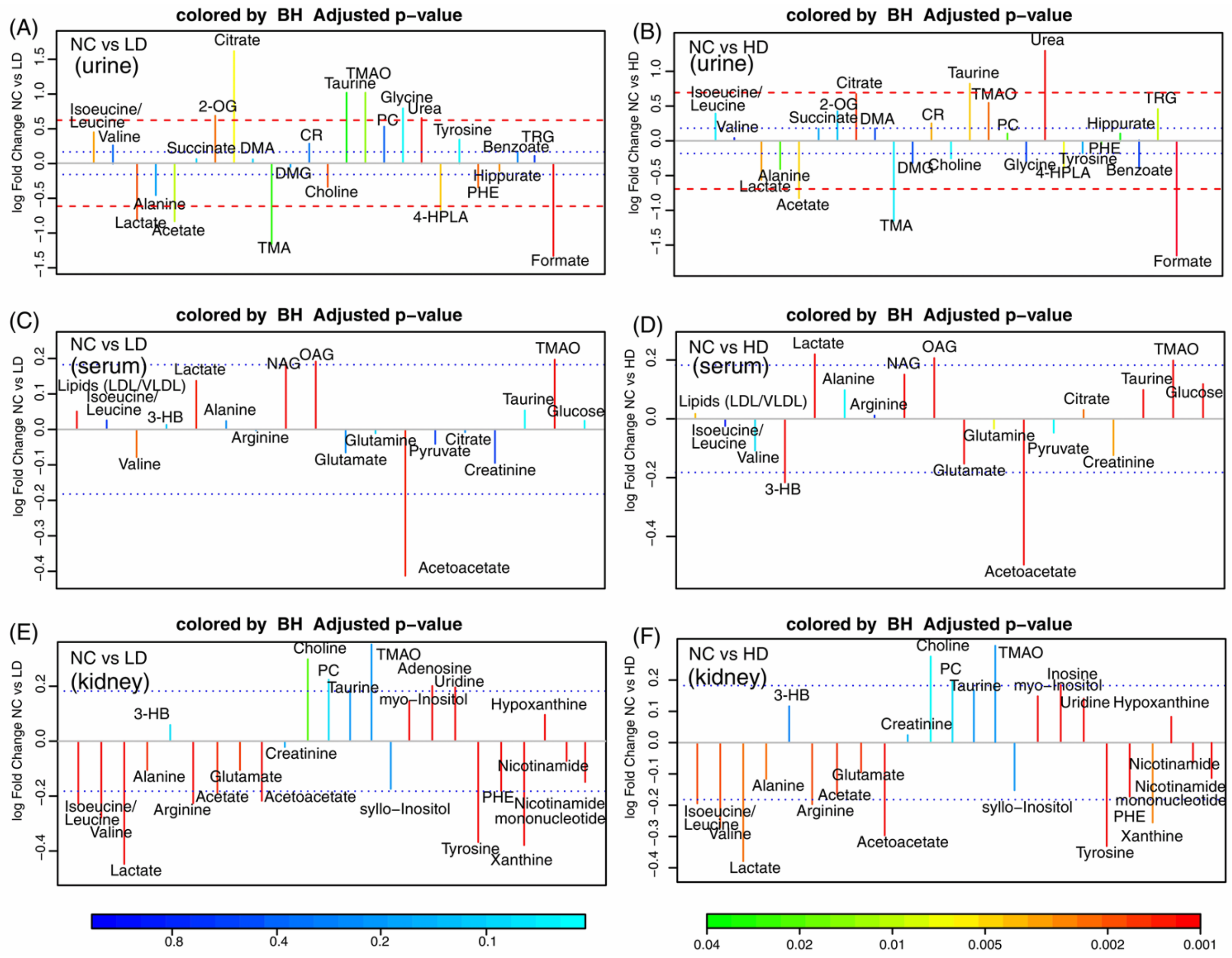

Fig.S2 Color-coded fold change plots adjusted by Benjamini-Hochberg method indicating significance of altered metabolites in LD rats (A, C, E) and HD rats (B, D, F). The blue dotted lines and red dashed lines representing an increase or decrease of 20% and 100%, respectively.

Table S1 Results of altered pathways in urine of HD group compared with NC

	Total	Expected	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact
Phenylalanine, tyrosine and tryptophan biosynthesis	4	0.05	2	8.19E-04	7.11E+00	6.63E-02	6.03E-02	1.00
Citrate cycle (TCA cycle)	20	0.24	3	1.49E-03	6.51E+00	1.19E-01	6.03E-02	0.15
Alanine, aspartate and glutamate metabolism	24	0.29	3	2.56E-03	5.97E+00	2.03E-01	6.92E-02	0.06
Methane metabolism	9	0.11	2	4.74E-03	5.35E+00	3.70E-01	7.68E-02	0.00
Phenylalanine metabolism	9	0.11	2	4.74E-03	5.35E+00	3.70E-01	7.68E-02	0.41
Aminoacyl-tRNA biosynthesis	67	0.81	4	7.08E-03	4.95E+00	5.38E-01	9.56E-02	0.00
Glyoxylate and dicarboxylate metabolism	16	0.19	2	1.50E-02	4.20E+00	1.00E+00	1.74E-01	0.41
Butanoate metabolism	20	0.24	2	2.31E-02	3.77E+00	1.00E+00	2.34E-01	0.00
Pyruvate metabolism	22	0.27	2	2.77E-02	3.59E+00	1.00E+00	2.50E-01	0.06
Ubiquinone and other terpenoid-quinone biosynthesis	3	0.04	1	3.60E-02	3.33E+00	1.00E+00	2.79E-01	0.00
Glycolysis or Gluconeogenesis	26	0.32	2	3.79E-02	3.27E+00	1.00E+00	2.79E-01	0.03
D-Glutamine and D-glutamate metabolism	5	0.06	1	5.93E-02	2.83E+00	1.00E+00	4.00E-01	0.00
Cyanoamino acid metabolism	6	0.07	1	7.07E-02	2.65E+00	1.00E+00	4.41E-01	0.00
Taurine and hypotaurine metabolism	8	0.10	1	9.32E-02	2.37E+00	1.00E+00	5.31E-01	0.43
Nitrogen metabolism	9	0.11	1	1.04E-01	2.26E+00	1.00E+00	5.31E-01	0.00
Primary bile acid biosynthesis	46	0.56	2	1.05E-01	2.26E+00	1.00E+00	5.31E-01	0.06
Selenoamino acid metabolism	15	0.18	1	1.68E-01	1.78E+00	1.00E+00	8.00E-01	0.00
Propanoate metabolism	20	0.24	1	2.18E-01	1.52E+00	1.00E+00	9.80E-01	0.00
Glutathione metabolism	26	0.32	1	2.74E-01	1.29E+00	1.00E+00	1.00E+00	0.01
Porphyrin and chlorophyll metabolism	27	0.33	1	2.83E-01	1.26E+00	1.00E+00	1.00E+00	0.00
Glycine, serine and threonine metabolism	32	0.39	1	3.26E-01	1.12E+00	1.00E+00	1.00E+00	0.29
Tyrosine metabolism	42	0.51	1	4.06E-01	9.03E-01	1.00E+00	1.00E+00	0.14
Arginine and proline metabolism	44	0.53	1	4.20E-01	8.67E-01	1.00E+00	1.00E+00	0.00
Purine metabolism	68	0.82	1	5.73E-01	5.57E-01	1.00E+00	1.00E+00	0.00

Table S2 Results of altered pathways in serum of HD group compared with NC

	Total	Expected	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact
Valine, leucine and isoleucine biosynthesis	11	0.13	4	3.57E-06	1.25E+01	2.89E-04	2.89E-04	1.00
Aminoacyl-tRNA biosynthesis	67	0.76	6	5.23E-05	9.86E+00	4.18E-03	2.12E-03	0.00
Valine, leucine and isoleucine degradation	38	0.43	4	6.62E-04	7.32E+00	5.23E-02	1.67E-02	0.00
D-Glutamine and D-glutamate metabolism	5	0.06	2	1.20E-03	6.73E+00	9.34E-02	1.67E-02	0.00
Synthesis and degradation of ketone bodies	5	0.06	2	1.20E-03	6.73E+00	9.34E-02	1.67E-02	0.60
Butanoate metabolism	20	0.23	3	1.24E-03	6.70E+00	9.40E-02	1.67E-02	0.10
Alanine, aspartate and glutamate metabolism	24	0.27	3	2.14E-03	6.15E+00	1.60E-01	2.47E-02	0.15
Citrate cycle (TCA cycle)	20	0.23	2	2.06E-02	3.88E+00	1.00E+00	2.08E-01	0.13
Pyruvate metabolism	22	0.25	2	2.47E-02	3.70E+00	1.00E+00	2.22E-01	0.19
Glycolysis or Gluconeogenesis	26	0.30	2	3.38E-02	3.39E+00	1.00E+00	2.74E-01	0.10
Arginine and proline metabolism	44	0.50	2	8.74E-02	2.44E+00	1.00E+00	5.94E-01	0.08
Taurine and hypotaurine metabolism	8	0.09	1	8.79E-02	2.43E+00	1.00E+00	5.94E-01	0.43
Nitrogen metabolism	9	0.10	1	9.84E-02	2.32E+00	1.00E+00	6.13E-01	0.00
Selenoamino acid metabolism	15	0.17	1	1.59E-01	1.84E+00	1.00E+00	8.54E-01	0.00
Pantothenate and CoA biosynthesis	15	0.17	1	1.59E-01	1.84E+00	1.00E+00	8.54E-01	0.00
Glyoxylate and dicarboxylate metabolism	16	0.18	1	1.69E-01	1.78E+00	1.00E+00	8.54E-01	0.30
Starch and sucrose metabolism	23	0.26	1	2.34E-01	1.45E+00	1.00E+00	1.00E+00	0.04
Galactose metabolism	26	0.30	1	2.60E-01	1.35E+00	1.00E+00	1.00E+00	0.04
Cysteine and methionine metabolism	28	0.32	1	2.77E-01	1.28E+00	1.00E+00	1.00E+00	0.02
Glycine, serine and threonine metabolism	32	0.37	1	3.10E-01	1.17E+00	1.00E+00	1.00E+00	0.00
Pyrimidine metabolism	41	0.47	1	3.80E-01	9.68E-01	1.00E+00	1.00E+00	0.00
Tyrosine metabolism	42	0.48	1	3.87E-01	9.49E-01	1.00E+00	1.00E+00	0.00
Primary bile acid biosynthesis	46	0.52	1	4.15E-01	8.79E-01	1.00E+00	1.00E+00	0.03
Purine metabolism	68	0.78	1	5.51E-01	5.97E-01	1.00E+00	1.00E+00	0.00

Table S3 Results of altered pathways in kidney of HD group compared with NC

	Total	Expected	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact
Aminoacyl-tRNA biosynthesis	67	0.67	6	2.12E-05	1.08E+01	1.71E-03	1.71E-03	0.00
Valine, leucine and isoleucine biosynthesis	11	0.11	3	1.25E-04	8.99E+00	1.00E-02	5.06E-03	1.00
Phenylalanine, tyrosine and tryptophan biosynthesis	4	0.04	2	5.50E-04	7.51E+00	4.34E-02	1.48E-02	1.00
Phenylalanine metabolism	9	0.09	2	3.20E-03	5.74E+00	2.50E-01	6.49E-02	0.41
Valine, leucine and isoleucine degradation	38	0.38	3	5.44E-03	5.21E+00	4.19E-01	8.82E-02	0.00
Purine metabolism	68	0.68	3	2.70E-02	3.61E+00	1.00E+00	3.43E-01	0.04
Ubiquinone and other terpenoid-quinone biosynthesis	3	0.03	1	2.97E-02	3.52E+00	1.00E+00	3.43E-01	0.00
Ascorbate and aldarate metabolism	9	0.09	1	8.66E-02	2.45E+00	1.00E+00	8.77E-01	0.00
Pantothenate and CoA biosynthesis	15	0.15	1	1.40E-01	1.96E+00	1.00E+00	1.00E+00	0.00
Pyruvate metabolism	22	0.22	1	1.99E-01	1.61E+00	1.00E+00	1.00E+00	0.00
Glycolysis or Gluconeogenesis	26	0.26	1	2.31E-01	1.46E+00	1.00E+00	1.00E+00	0.00
Galactose metabolism	26	0.26	1	2.31E-01	1.46E+00	1.00E+00	1.00E+00	0.00
Inositol phosphate metabolism	26	0.26	1	2.31E-01	1.46E+00	1.00E+00	1.00E+00	0.14
Glycerophospholipid metabolism	30	0.30	1	2.62E-01	1.34E+00	1.00E+00	1.00E+00	0.02
Glycine, serine and threonine metabolism	32	0.32	1	2.77E-01	1.28E+00	1.00E+00	1.00E+00	0.00
Pyrimidine metabolism	41	0.41	1	3.41E-01	1.07E+00	1.00E+00	1.00E+00	0.01
Tyrosine metabolism	42	0.42	1	3.48E-01	1.06E+00	1.00E+00	1.00E+00	0.14
Arginine and proline metabolism	44	0.44	1	3.61E-01	1.02E+00	1.00E+00	1.00E+00	0.08

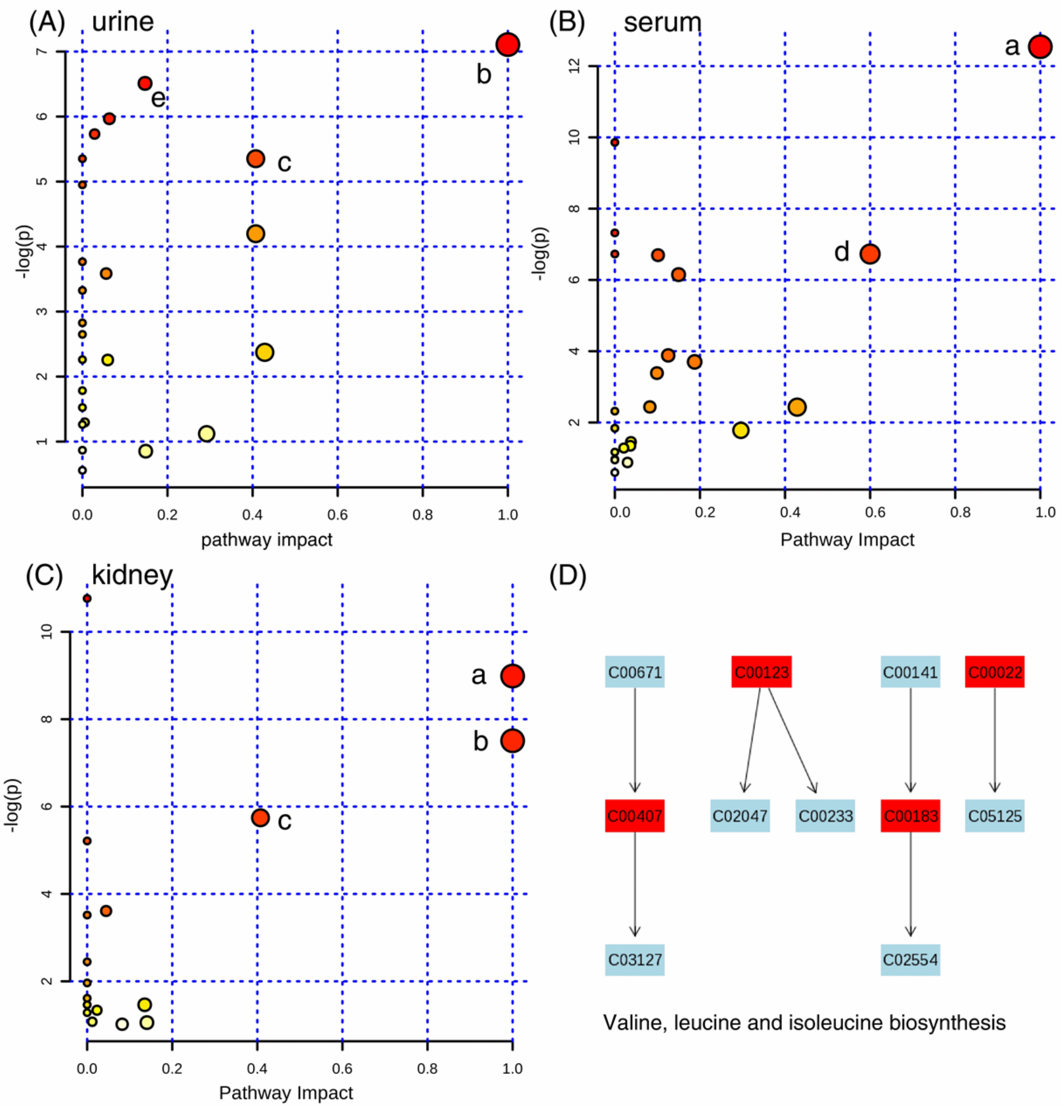

Fig. S3 Overview of altered metabolic pathways in urine (A), serum (B), kidney (C) and the pathway flowchart of most impacted valine, leucine and isoleucine biosynthesis (D) of HD rats compared with NC rats as visualized by bubble plots. Bubble area is proportional to the impact of each pathway, with color denoting the significance from highest in red to lowest in white. ((a) Valine, leucine and isoleucine biosynthesis; (b)Phenylalanine, tyrosine and tryptophan biosynthesis; (c) Phenylalanine metabolism; (d) Synthesis and degradation of ketone bodies; (e) TCA cycle).