

Supplementary Materials

D-Amino acids and classical neurotransmitters in healthy and type 2 diabetes affected human pancreatic islets of Langerhans

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Supplementary Materials and Methods:

Capillary zone electrophoresis (CZE)-LIF for chiral separation

Sample desalting

Homemade centrifuge extraction tips were prepared using a 200 μ L pipette tip packed with three layers of punched-out Empore SPE Disks (0.7-0.8 mg of polystyrene-divinylbenzene (SDB-XC)). The disk's SDB-XC material was conditioned with 15 μ L of ACN and then 45 μ L of water. The NDA-derivatized samples were dried in a SpeedVac, reconstituted in a minimum volume of water (typically 5 μ L), and loaded onto the tips. The tips were washed with 5 μ L of water aliquots twice. The analytes were eluted with 45 μ L of ACN. The resulting samples were dried in a SpeedVac and reconstituted in water for CE-LIF analysis.

Enzyme purification for confirmation of D-AA identification

Before the enzymatic treatment, 15 U/mL DAAO and 68 μ g/mL catalase, each in PBS 1X (Gibco, ThermoFisher), were prepared using the DAAO from porcine kidney (Catalog# A5222, Sigma-Aldrich) and catalase from bovine liver (Catalog# C30, Sigma-Aldrich). Both reagents underwent further purification, as the presence of interfering compound within the enzyme solution co-eluted with the analyte peak and prevented the confirmation by CE-LIF. 150 μ L of each prepared reagent was transferred to 3kDa MWCO filters (Amicon Ultra 0.5 mL centrifugal filters), conditioned with 500 μ L of PBS 1X. The reagents were centrifuged at $14,000 \times g$ for 15 min. 150 μ L of PBS 1X was added to the same filter and centrifuged again at $14,000 \times g$ for 15 min. The concentrated enzymes were recovered by centrifuging the filters upside down in tubes at $1,000 \times g$ for 2 min. The concentration of each purified enzyme was determined using a NanoDrop and further diluted in PBS 1X to make purified 15 U/mL DAAO and 68 μ g/mL catalase.

ZipChip-CZE-ESI-MS for neurotransmitters

Sample desalting

Aliquots of the extracted islet samples stored at -80°C were dried in a SpeedVac. The resulting samples were resuspended in a 50 μ L mixture of isopropanol and ACN in a 4:1 volume ratio and centrifuged at $15,000 \times g$ for 5 min at 4°C , solubilizing analytes and further cleaning up the samples. The supernatant was dried in a SpeedVac and stored at -80°C until analysis.

Table S1 Summary of biochemical characteristics of measured human pancreatic islets that varied by individual to individual in three groups: healthy, prediabetes, and type 2 diabetes (T2D)-affected.

Type	Subject No.	BMI	HbA1c (%)	D-Ser	D-Ser (%)	D-Asp	D-Asp (%)	Serotonin	GABA	Ach	Glutamate
Healthy (n = 9)	1	n/a	n/a	1.6E-01	4.8	1.7E-01	1.1	2.5E-02	1.1E+01	3.4E-02	1.0E+00
	2	n/a	n/a	8.3E-01	4.7	1.0E-01	0.5	4.7E-02	4.7E+01	1.6E-01	2.7E+01
	3	n/a	n/a	5.8E+00	6.6	1.5E+00	0.5	1.2E+00	7.2E+02	4.3E-01	7.7E+01
	4	n/a	n/a	4.5E-01	0.7	1.1E+00	0.5	1.9E+00	5.5E+01	1.9E-01	2.7E+01
	5	20.3	4.8	1.7E-01	1.5	2.1E-01	3.2*	5.7E-02	1.5E+02	3.3E-01	8.2E+00
	6	26.2	5.3	2.2E-01	2.2	3.2E-01	1.9	3.4E-01	1.9E+02	1.6E+00*	1.9E+01
	7	35	n/a	1.9E-01	1.7	8.2E-02	0.2	1.6E-02	5.2E+01	1.4E-01	4.1E+00
	8	28	n/a	2.1E-01	1.9	3.8E-01	0.4	3.8E+00*	6.1E+01	4.2E-01	6.5E+00
	9	30	5.4	1.1E-01	0.6	6.1E-01	1.2	1.3E-01	1.8E+01	1.1E-01	1.1E+01
Prediabetes (n = 4)	10	33.2	6.1	2.6E-01	0.6	1.2E-01	0.8	1.7E-02	1.1E+02	1.6E-01	3.4E+01
	11	27	5.9	2.1E-01	0.5	4.1E-02	0.5	1.4E-01	1.4E+01	6.3E-02	7.4E+00
	12	24	5.8	2.5E-01	0.9	8.0E-02	0.5	1.9E-01	2.1E+01	1.2E-01	6.9E+00
	13	27.5	5.7	1.6E-01	1.1	9.0E-02	1.3	1.1E-01	1.0E+01	5.7E-01	1.5E+01
T2D (n = 9)	14	n/a	n/a	2.1E-01	2.9	2.4E-01	0.7	1.8E-02	3.5E+01	3.7E-03	8.0E+00
	15	n/a	6.7	7.3E-01*	1.3	6.5E-01*	0.3	n.d.	1.6E+02	7.5E-01	7.4E+01
	16	38	6.3	1.2E-01	0.4	2.3E-01	0.9	n.d.	3.0E+01	3.1E-01	2.5E+01
	17	35.9	9.7	4.5E-02	0.8	2.0E-02	0.5	1.1E-02	9.5E+00	2.3E-02	1.9E+00
	18	35.8	n/a	3.9E-01	0.9	2.4E-01	1.4	n.d.	2.1E+00	8.7E-02	1.7E-01
	19	37.4	6.4	4.7E-01	0.6	1.9E-01	1.0	n.d.	1.6E+01	6.4E-01	6.6E+00
	20	26.7	6.0	2.0E-01	0.8	1.1E-01	0.9	n.d.	4.7E+01	1.6E-01	2.8E+02*
	21	28	n/a	2.5E-01	1.0	4.6E-02	0.9	n.d.	1.8E-01	8.4E-03	3.7E-01
	22	33	10.8	2.1E-01	0.5	6.2E-02	1.4	1.5E-02	3.1E+00	3.1E-02	3.5E+00

Table S1 (cont.)

Type	Subject No.	Dopamine	Tyramine	Synephrine	Norepinephrine	Epinephrine
Healthy (n = 9)	1	9.4E-03	1.9E-02	2.6E-02	n.d.	n.d.
	2	3.7E-02	n.d.	1.3E-01	n.d.	n.d.
	3	n.d.	n.d.	5.0E-01	n.d.	n.d.
	4	n.d.	8.4E-02	4.0E+00	n.d.	n.d.
	5	4.9E-01	n.d.	n.d.	n.d.	n.d.
	6	2.9E-01	1.8E-01	n.d.	3.2E-02	n.d.
	7	n.d.	n.d.	n.d.	n.d.	n.d.
	8	n.d.	n.d.	n.d.	n.d.	n.d.
	9	n.d.	1.2E-01	n.d.	6.0E-03	5.4E-03
Prediabetes (n = 4)	10	n.d.	1.3E-02	3.5E-03	n.d.	n.d.
	11	n.d.	5.3E-02	n.d.	n.d.	n.d.
	12	1.3E-01	1.2E-01	7.4E-03	7.4E-02	2.1E-03
	13	n.d.	1.8E-02	6.1E-03	0.0E+00	7.0E-03
T2D (n = 9)	14	1.6E-02	2.4E-02	2.7E-01	n.d.	n.d.
	15	n.d.	8.5E-02	n.d.	n.d.	n.d.
	16	n.d.	n.d.	n.d.	n.d.	n.d.
	17	3.6E-02	1.4E-02	n.d.	2.7E-01	n.d.
	18	n.d.	n.d.	n.d.	n.d.	n.d.
	19	n.d.	n.d.	n.d.	n.d.	n.d.
	20	n.d.	n.d.	n.d.	n.d.	n.d.
	21	2.8E-03	n.d.	n.d.	1.4E-01	n.d.
	22	n.d.	n.d.	n.d.	n.d.	n.d.

Ach, acetylcholine; n/a, not available; n.d., not detected; *, outlier excluded from correlation plot.

Values are shown in pmole/μg protein unless otherwise noted.

Tryptamine and L-DOPA are not included in this table since they were not detected in any of the measured samples.

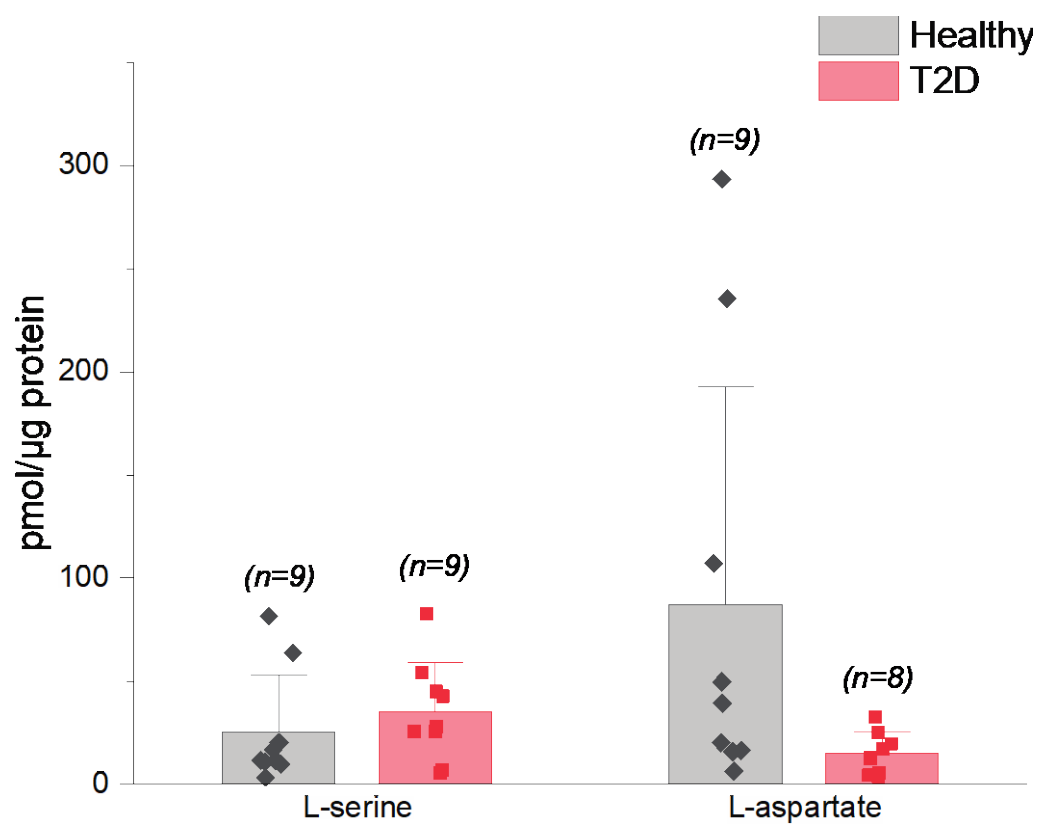


Figure S1. Levels of L-Ser and L-Asp in healthy versus type 2 diabetes (T2D)-affected human pancreatic islets. Values represent mean \pm SD of 8-9 human islet samples.

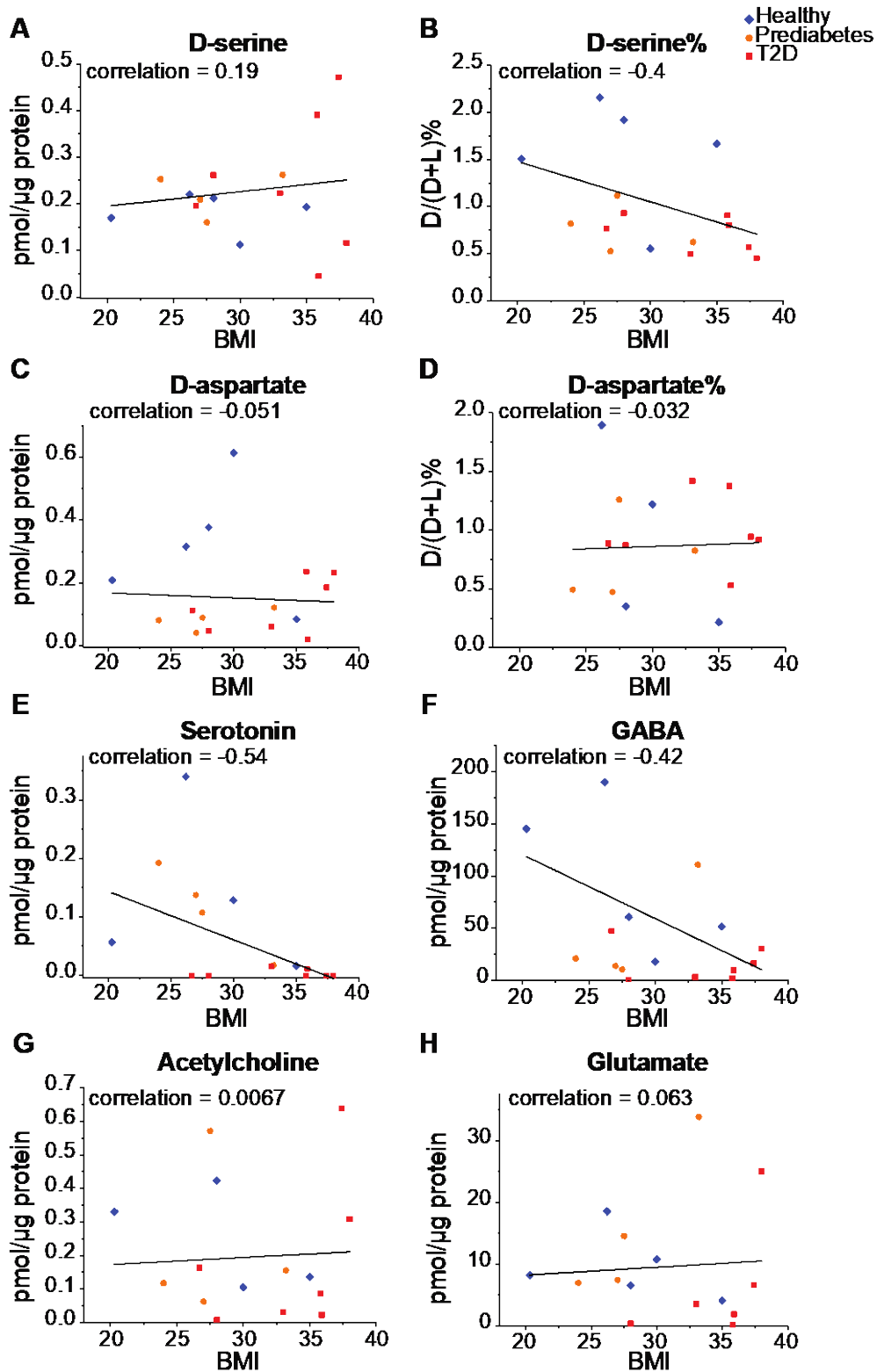


Figure S2. Scatter plots of BMI and each D-Ser, D-Asp, and classical neurotransmitters. The Pearson correlation coefficient is shown on each plot.

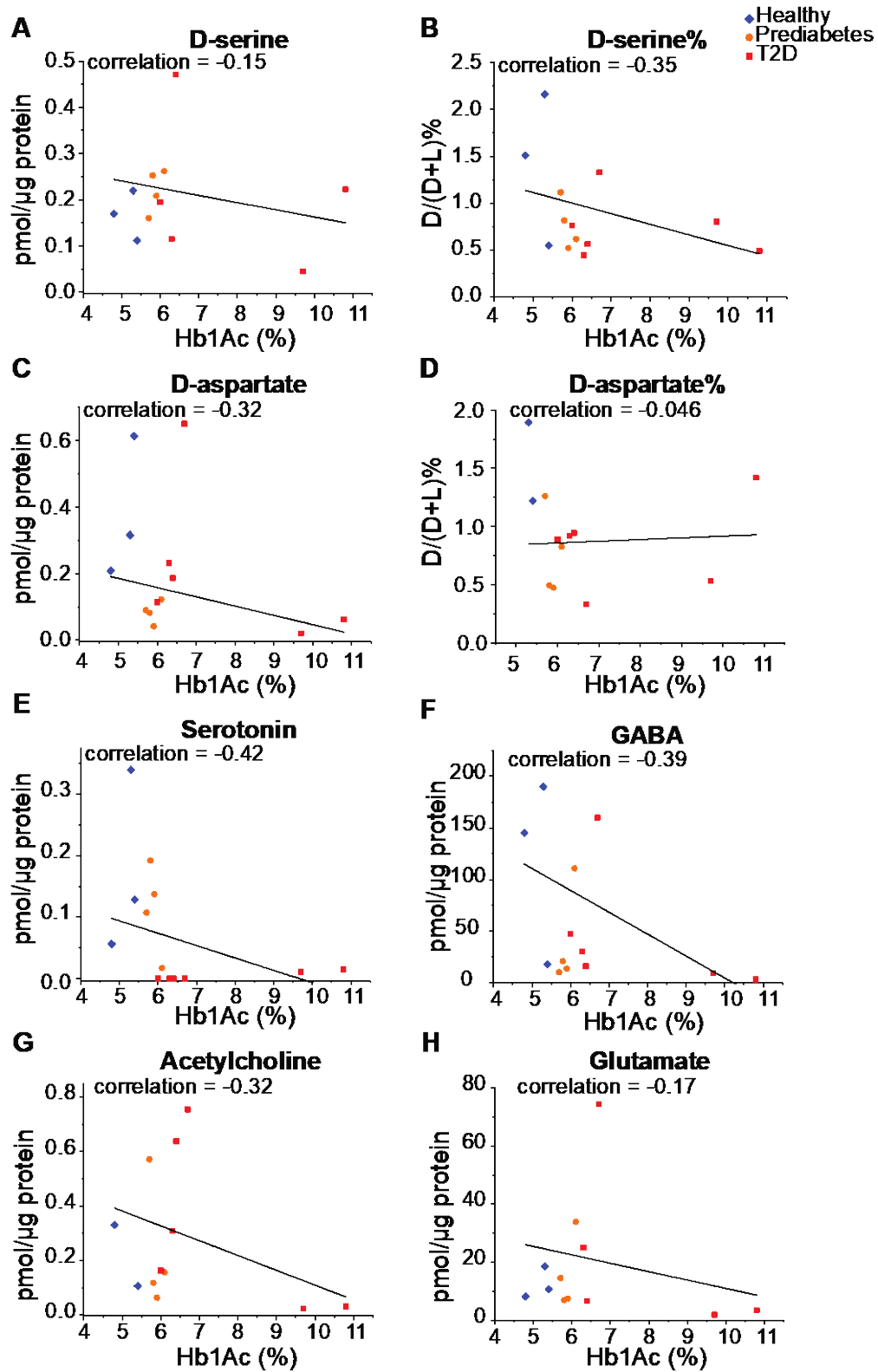


Figure S3. Scatter plots of HbA1c and each D-Ser, D-Asp, and classical neurotransmitters. The Pearson correlation coefficient is shown on each plot.