Supplementary Material 1

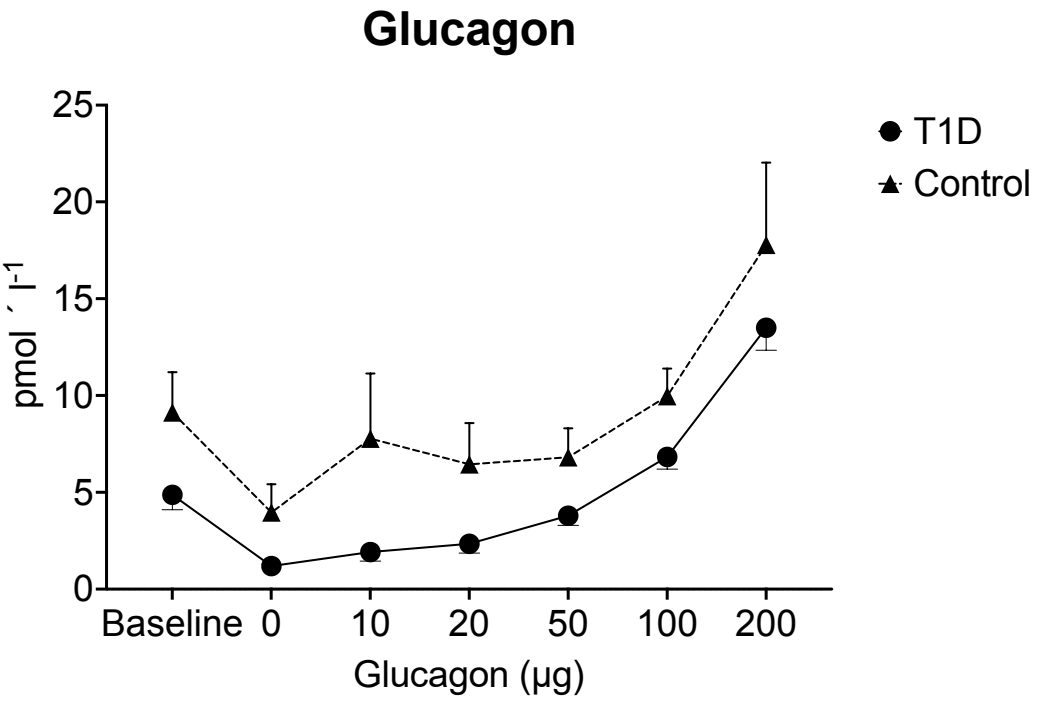
Table A: Effects of the stepwise glucagon intervention in the participants with type 1 diabetes and healthy controls.
Data are presented in the mean value and the standard error of the mean. Participants with type 1 diabetes and healthy controls were compared using a linear mixed effects model with repeated measurements and group-intervention interaction. Data with P-values are presented in the table.

Parameters, mean ± SEM	Unit	Group	Glucagon bolus injection, µg							P	Covariate P[1]
			Baseline	0	10	20	50	100	200		
Glucose (iAUC)	mmol × l^{-1} × 40 min	T1D			28 ± 7.6	24 ± 6.3	36 ± 7.5	33 ± 3.4	34 ± 6.7	0.37	0.59
		Control			-2.9 ± 8.9	28 ± 8.9	35 ± 5.0	25 ± 6.3	37 ± 10.		
Non-esterified fatty acids	µmol × l^{-1}	T1D	262 ± 39	50 ± 12	31 ± 7.4	22 ± 7.1	24 ± 8.3	31 ± 7.9	27 ± 6.4	0.68	0.48
		Control	303 ± 41	45 ± 7.5	32 ± 6.0	21 ± 5.0	19 ± 5.2	19 ± 5.3	18 ± 5.0		
Glycerol	µmol × l^{-1}	T1D	35 ± 5.4	19 ± 3.5	17 ± 3.3	15 ± 2.7	14 ± 2.9	15 ± 3.0	17 ± 3.7	0.55	0.44
		Control	41 ± 4.4	31 ± 6.3	28 ± 6.1	27 ± 5.7	25 ± 5.5	25 ± 5.7	25 ± 5.5		
Glucagon	pmol × l^{-1}	T1D	4.9 ± 0.8	1.2 ± 0.4	1.9 ± 0.5	2.3 ± 0.5	3.8 ± 0.5	6.8 ± 0.6	14 ± 0.6	0.74	0.59
		Control	9.1 ± 2.1	4.0 ± 1.5	7.8 ± 3.4	6.5 ± 2.1	6.8 ± 1.5	10 ± 1.4	18 ± 4.2		
Insulin	mU × l^{-1}	T1D	5.5 ± 3.5	39 ± 8.3	45 ± 8.3	44 ± 8.6	40 ± 8.0	43 ± 9.1	41 ± 8.3	0.008	0.017
		Control	7.1 ± 1.0	18 ± 3.7	16 ± 2.7	19 ± 4.1	22 ± 5.7	23 ± 5.7	28 ± 7.2		
Glucose Infusion Rate	mmol × kg^{-1} × min^{-1}	T1D			0.024 ± 0.004	0.022 ± 0.003	0.025 ± 0.003	0.026 ± 0.003	0.025 ± 0.003	0.22	0.20
		Control			0.037 ± 0.006	0.043 ± 0.005	0.047 ± 0.004	0.049 ± 0.004	0.049 ± 0.004		

[1]Posthoc covariate adjustment of the p-values using the M/I ratio as a measure of insulin sensitivity of each participants.

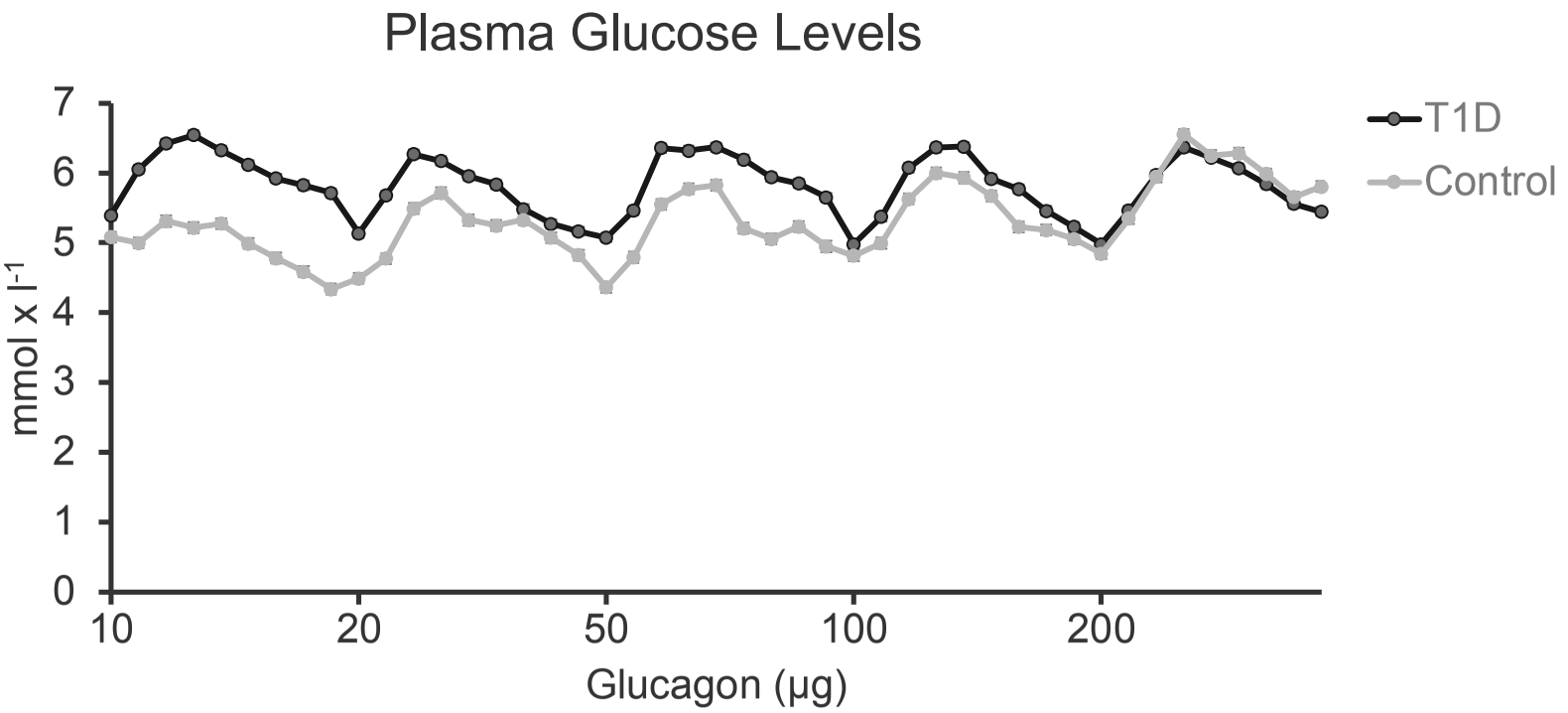
Supplementary Material 2

Figure: A significant positive correlation between the stepwise glucagon injection and plasma glucagon concentration was observed in T1D ($P < 0.001$). In controls, the trend was similar; however not significant ($P = 0.053$). Between-group analyses showed that while the glucagon levels in T1D were numerically lower than the controls throughout the study, the difference was insignificant ($P = 0.40$). Secondly, the plasma glucagon concentrations in T1D did not change differently to the glucagon injections compared to healthy controls ($P = 0.74$).



Supplementary Material 3

Figure: Plasma glucose levels. Plasma glucose was measured every five minutes during the stepwise glucagon injections.



Supplementary Material 4

Table: Data presents the differences in metabolite changes to glucagon injections in participants with type 1 diabetes compared to healthy controls using untargeted metabolomics profiling. Metabolomics data before and after the completion of the stepwise glucagon injections were assessed using a linear mixed-effects model with group-intervention interaction. Data are presented as a coefficient with a confidence interval. Raw P-values and the adjusted P-values using Benjamini-Hochberg are shown.

Metabolites	Coefficient	95% Confidence interval		P	P_{BH}
		Lower	Upper		
L-Histidine	-0.82	-1.52	-0.11	0.024	0.996
Phenylalanine	0.73	0.070	1.39	0.031	0.996
Glycerol	0.80	-0.04	1.65	0.061	0.996
Ornithine	-0.63	-1.33	0.064	0.075	0.996
Malonic acid	-0.67	-1.45	0.12	0.094	0.996
Isoleucine	0.54	-0.12	1.21	0.11	0.996
Phosphoric acid 2.3-dihydroxypropyl ester	0.56	-0.14	1.27	0.12	0.996
3-Hydroxyisovaleric acid	0.67	-0.19	1.53	0.13	0.996
Maltose	0.58	-0.18	1.33	0.13	0.996
Parabanic acid	0.58	-0.18	1.34	0.13	0.996
Hexadecanoic acid. methyl	0.51	-0.22	1.23	0.17	0.996
Hexadecanoic acid. butyl	0.57	-0.24	1.39	0.17	0.996
Leucine	0.47	-0.21	1.15	0.17	0.996
Glyoxylic acid	-0.51	-1.25	0.23	0.18	0.996
(??)-Tryptophan	-0.50	-1.26	0.25	0.19	0.996
Diethylene glycol	-0.50	-1.26	0.26	0.20	0.996
Methyl stearate	0.48	-0.26	1.22	0.20	0.996
3-Indoleacetic acid	-0.44	-1.14	0.25	0.21	0.996
Docosahexaenoic acid	0.46	-0.27	1.20	0.22	0.996
D-Glucose 6-phosphate	0.50	-0.30	1.30	0.22	0.996
Tetradecanoic acid	-0.50	-1.31	0.31	0.22	0.996
D-(+)-Trehalose	0.52	-0.33	1.37	0.23	0.996
Trehalose	-0.47	-1.24	0.30	0.23	0.996
Tyrosine	0.37	-0.25	0.99	0.24	0.996
1.5-Anhydrohexitol	0.40	-0.28	1.08	0.24	0.996
d-Galactose	-0.42	-1.15	0.30	0.25	0.996
D-(-)-Lyxofuranose	-0.46	-1.27	0.34	0.26	0.996
Palmitoleic Acid	-0.44	-1.23	0.35	0.28	0.996
L-Hydroxyproline	0.41	-0.34	1.16	0.29	0.996
Glycolic acid	0.41	-0.35	1.17	0.29	0.996
Valine	0.33	-0.29	0.95	0.29	0.996
α-Tocopherol	0.39	-0.34	1.12	0.29	0.996
Methionine	0.34	-0.30	0.97	0.30	0.996
Pyruvic acid	0.36	-0.34	1.05	0.31	0.996
Dodecanoic acid	-0.42	-1.24	0.41	0.32	0.996
L-2-Aminobutyric acid	-0.35	-1.04	0.34	0.32	0.996
Cholesterol	0.38	-0.37	1.12	0.32	0.996
Succinic acid	0.35	-0.37	1.07	0.33	0.996
Levoglucosan	0.39	-0.41	1.18	0.34	0.996
L-Threonic acid	0.34	-0.37	1.04	0.34	0.996
4-amino-3-hydroxyButanoic acid	0.35	-0.39	1.09	0.35	0.996
L-Norleucine	-0.36	-1.14	0.42	0.36	0.996
2-Methylglutaric acid	0.38	-0.46	1.21	0.37	0.996
L-Arginine	0.32	-0.39	1.03	0.38	0.996
Acetoacetic acid	-0.36	-1.15	0.44	0.38	0.996
2.5-di-tert-Butyl-1.4-benzoquinone	-0.36	-1.17	0.45	0.38	0.996

2-Desoxy-D-glycero-pentos-3-ulose	-0.31	-1.02	0.40	0.39	0.996
Phosphoric acid	-0.30	-1.05	0.44	0.42	0.996
N-Methyl-DL-Alanine	-0.27	-0.96	0.41	0.43	0.996
Hypoxanthine	0.33	-0.51	1.16	0.44	0.996
Phosphoric acid. monomethyl ester	-0.29	-1.02	0.45	0.44	0.996
Glutamine	-0.25	-0.90	0.40	0.45	0.996
Methyl linoleate	0.30	-0.48	1.07	0.45	0.996
Fumaric acid	-0.29	-1.04	0.47	0.45	0.996
d-Glucose	-0.29	-1.06	0.48	0.46	0.996
Arachidonic acid	0.27	-0.46	1.00	0.47	0.996
Linolenic acid	0.26	-0.46	0.99	0.47	0.996
Ethanolamine	-0.29	-1.08	0.51	0.47	0.996
meso-Erythritol	-0.26	-0.97	0.45	0.48	0.996
Eicosapentaenoic acid	0.26	-0.45	0.97	0.48	0.996
Putrescine	0.25	-0.47	0.97	0.49	0.996
L-Tryptophan	-0.27	-1.07	0.53	0.51	0.996
Melibiose	-0.24	-0.98	0.49	0.51	0.996
L-alpha-Aminobutyric acid	-0.23	-0.94	0.47	0.52	0.996
Myo-Inositol	-0.21	-0.85	0.43	0.52	0.996
Pseudo uridine	-0.23	-0.97	0.51	0.54	0.996
1.3-Propanediol	0.23	-0.53	0.99	0.55	0.996
n-Butylamine	-0.25	-1.06	0.57	0.55	0.996
Diethanolamine	0.23	-0.53	0.99	0.55	0.996
Alanine	0.20	-0.47	0.86	0.56	0.996
3-Hydroxyicosatetraenoyl-CoA	0.23	-0.54	0.99	0.56	0.996
1-Dodecanol	0.24	-0.59	1.08	0.56	0.996
α-D-(-)-Tagatopyranose	0.23	-0.55	1.01	0.57	0.996
β-D-Glucopyranose	-0.21	-0.94	0.53	0.58	0.996
Glyceryl-glycoside	0.19	-0.51	0.90	0.59	0.996
Sorbitol	0.21	-0.57	0.99	0.59	0.996
L-5-Oxoproline	-0.19	-0.88	0.50	0.59	0.996
L-Pipecolic acid	-0.19	-0.91	0.52	0.59	0.996
DL-Pyroglutamic acid	-0.19	-0.91	0.52	0.60	0.996
Acetylsalicylic acid	-0.21	-0.99	0.58	0.60	0.996
Triethylene glycol	0.21	-0.59	1.01	0.61	0.996
Phosphonoacetate	0.19	-0.55	0.94	0.61	0.996
Nonacosane	0.20	-0.57	0.96	0.61	0.996
3-Indolepropionic acid	0.19	-0.54	0.92	0.61	0.996
Isobutylamine	-0.21	-1.01	0.60	0.61	0.996
2-Palmitoylglycerol	-0.19	-0.92	0.55	0.61	0.996
Glutamic acid	-0.18	-0.88	0.52	0.61	0.996
2-Hydroxybutyric acid	-0.19	-0.94	0.57	0.63	0.996
D-Glucarate	-0.18	-0.90	0.54	0.63	0.996
Aspartic acid	0.18	-0.56	0.91	0.64	0.996
D-Gluconic acid	0.18	-0.57	0.93	0.64	0.996
L-(-)-Sorbose	0.17	-0.57	0.91	0.65	0.996
Ketoisoleucine	-0.18	-0.93	0.58	0.65	0.996
Glyceric acid	0.17	-0.56	0.89	0.65	0.996
Nonanoic acid	0.16	-0.57	0.89	0.67	0.996
(2E)-Decenoyl-ACP	0.15	-0.54	0.83	0.67	0.996
Hydroquinone	-0.16	-0.88	0.57	0.67	0.996
Malic acid	-0.16	-0.92	0.60	0.68	0.996
Linoleic acid	0.16	-0.58	0.89	0.68	0.996
Ribitol	0.16	-0.59	0.90	0.68	0.996
2-Ketoisocaproic acid	-0.17	-1.00	0.66	0.68	0.996
Tetradecanol	0.17	-0.66	0.99	0.69	0.996
(R*.R*)-2.3-Dihydroxybutanoic acid	0.14	-0.58	0.86	0.70	0.996

1.6-Anhydroglucose	-0.14	-0.88	0.60	0.71	0.996
Quininic acid	-0.13	-0.80	0.55	0.72	0.996
Threonine	0.12	-0.53	0.76	0.72	0.996
Lysine	0.12	-0.53	0.76	0.72	0.996
β-Tocopherol	-0.14	-0.90	0.63	0.72	0.996
Benzoic acid	-0.14	-0.95	0.66	0.72	0.996
L-Norvaline	-0.13	-0.83	0.58	0.73	0.996
1-Monopalmitin	0.13	-0.59	0.84	0.73	0.996
Ibuprofen	0.13	-0.63	0.88	0.74	0.996
Methylmalonic acid	0.11	-0.59	0.81	0.75	0.996
D-(-)-Rhamnose	-0.13	-0.94	0.68	0.75	0.996
p-Cresol	0.12	-0.71	0.96	0.77	0.996
Cysteine	-0.11	-0.86	0.64	0.77	0.996
Taurine	0.11	-0.66	0.88	0.77	0.996
Proline	0.088	-0.58	0.76	0.80	0.996
Glutamic acid-d5	0.094	-0.64	0.83	0.80	0.996
Valine-d8	0.089	-0.62	0.80	0.81	0.996
N-Acetylneuraminate	0.086	-0.61	0.78	0.81	0.996
D-Arabitol	0.084	-0.61	0.78	0.81	0.996
d-Galactose	-0.078	-0.81	0.65	0.83	0.996
D-(+)-Galacturonic acid	-0.076	-0.81	0.66	0.84	0.996
Octanoic acid	-0.077	-0.89	0.73	0.85	0.996
D-(+)-Xylose	0.068	-0.65	0.79	0.85	0.996
Serine	0.056	-0.59	0.70	0.86	0.996
Decanoic acid	-0.071	-0.90	0.76	0.87	0.996
Aminomalonic acid	0.060	-0.66	0.78	0.87	0.996
Succinic acid-d4	0.055	-0.65	0.76	0.88	0.996
Pyrophosphate	0.053	-0.66	0.76	0.88	0.996
Glycine	-0.049	-0.71	0.61	0.88	0.996
2-Hydroxypyridine	0.054	-0.70	0.81	0.89	0.996
4-Methylvaleric acid	-0.046	-0.77	0.68	0.90	0.996
4.4'-Dibromooctafluorobiphenyl	0.046	-0.67	0.76	0.90	0.996
2-Methyl-6-tert-butylphenol	-0.050	-0.86	0.76	0.90	0.996
Urea	-0.046	-0.82	0.73	0.91	0.996
Ribonic acid	0.037	-0.66	0.73	0.92	0.996
β-Alanine	-0.038	-0.76	0.68	0.92	0.996
2-hydroxypropanoic acid	0.039	-0.70	0.78	0.92	0.996
Heptadecanoic acid	-0.035	-0.76	0.69	0.92	0.996
Ketovaline	0.036	-0.71	0.78	0.92	0.996
α-Ketoglutaric acid	-0.032	-0.75	0.69	0.93	0.996
Stearic acid	-0.031	-0.80	0.74	0.94	0.996
Oxalic acid	0.025	-0.75	0.80	0.95	0.996
Dihydrouracil	0.024	-0.72	0.77	0.95	0.996
Oleic acid	-0.021	-0.77	0.73	0.96	0.996
D-Fucitol	0.021	-0.71	0.76	0.96	0.996
Palmitic acid	-0.021	-0.80	0.75	0.96	0.996
O-Phosphoethanolamine	0.019	-0.70	0.74	0.96	0.996
n-Propylamine	0.019	-0.73	0.77	0.96	0.996
Phenoxyacetic acid	0.017	-0.69	0.72	0.96	0.996
Uric acid	0.018	-0.75	0.79	0.96	0.996
Citric acid	-0.015	-0.68	0.65	0.96	0.996
Heptadecanoate	0.019	-0.82	0.85	0.96	0.996
D-(-)-Tagatofuranose	0.017	-0.79	0.82	0.97	0.996
3-Pyridinol	0.015	-0.75	0.78	0.97	0.996
Pentanoic acid. 2-keto-. -3-methyl	-0.011	-0.85	0.82	0.98	0.996
3-Hydroxybutyric acid	0.010	-0.72	0.74	0.98	0.996
Heptanoic acid	-0.0069	-0.81	0.80	0.99	0.996
L-(-)-Arabitol	-0.0020	-0.69	0.69	1.00	0.996

N-Acetyl-D-glucosamine	0.0022	-0.82	0.82	1.00	0.996

Supplementary Material 5

Table A: Impact of hypoglycemic exposure using time below range level 1. Metabolomics data before and after the completion of the glucagon injections of the participants with type 1 diabetes were assessed using a linear mixed-effects model and TBR level 1 as a fixed factor. Data are presented as a coefficient with a confidence interval. Raw P-values and the adjusted P-values using Benjamini-Hochberg are shown. Only metabolites with raw P-values <0.05 are presented. The complete dataset is available from the corresponding author upon request.

Metabolites	Coefficient	95% Confidence interval		P	P_{BH}
		Lower	Upper		
2-Ketoisocaproic acid	-12.90	-21.50	-4.23	0.004	0.66
Melibiose	9.46	2.44	16.50	0.009	0.73
L-Norleucine	-8.73	-15.80	-1.64	0.02	0.84
Fumaric acid	-9.66	-17.80	-1.51	0.02	0.84
L-Tryptophan	-8.92	-17.30	-0.50	0.04	0.99
Isoleucine	-7.41	-14.60	-0.26	0.04	0.99
1.5-Anhydrohexitol	8.82	0.29	17.40	0.04	0.99
Threonine	-6.60	-13.30	0.13	0.05	0.99

Table B: Impact of hypoglycemic exposure using time below range level 2. Metabolomics data before and after the completion of the glucagon injections of the participants with type 1 diabetes were assessed using a linear mixed-effects model and TBR level 2 as a fixed factor. Data are presented as a coefficient with a confidence interval. Raw P-values and the adjusted P-values using Benjamini-Hochberg are shown. Only metabolites with raw P-values <0.05 are presented. The complete dataset is available from the corresponding author upon request.

Metabolites	Coefficient	95% Confidence interval		P	P_{BH}
		Lower	Upper		
2-Ketoisocaproic acid	-82.30	-127.00	-37.90	0.00	0.06
L-Tryptophan	-49.90	-94.20	-5.55	0.03	0.98
Fumaric acid	-45.70	-89.20	-2.15	0.04	0.98
Methyl linoleate	-42.80	-86.00	0.41	0.05	0.98

Table C: Impact of hypoglycemic exposure using hypoglycemic events per week level 1. Metabolomics data before and after the completion of the glucagon injections of the participants with type 1 diabetes were assessed using a linear mixed-effects model and HEPW level 1 as a fixed factor. Data are presented as a coefficient with a confidence interval. Raw P-values and the adjusted P-values using Benjamini-Hochberg are shown. Only metabolites with raw P-values <0.05 are presented. The complete dataset is available from the corresponding author upon request.

Metabolites	Coefficient	95% Confidence interval		P	P_{BH}
		Lower	Upper		
Fumaric acid	-0.073	-0.013	-0.012	0.02	0.98
L-5-Oxoproline	-0.052	-0.10	-0.003	0.04	0.98
Threonine	-0.052	-0.10	-0.001	0.04	0.98
L-alpha-Aminobutyric acid	-0.055	-0.11	-0.001	0.05	0.98
Melibiose	0.054	-0.001	0.108	0.05	0.98

Table D: Impact of hypoglycemic exposure using hypoglycemic events per week level 2. Metabolomics data before and after the completion of the glucagon injections of the participants with type 1 diabetes were assessed using a linear mixed-effects model and HEPW level 2 as a fixed factor. Data are presented as a coefficient with a confidence interval. Raw P-values and the adjusted P-values using Benjamini-Hochberg are shown. Only metabolites with raw P-values <0.05 are presented. The complete dataset is available from the corresponding author upon request.

Metabolites	Coefficient	95% Confidence interval		P	P_{BH}
		Lower	Upper		
Fumaric acid	-0.37	-0.60	-0.14	0.002	0.39
2-Ketoisocaproic acid	-0.27	-0.53	-0.014	0.04	0.88
L-Tryptophan	-0.25	-0.50	-0.005	0.05	0.88
L-5-Oxoproline	-0.20	-0.39	0.002	0.05	0.88