Electronic supplementary information (ESI)

Optimization of the quantitative protocol for the intermediate metabolites of the glycolysis pathway in human serum using gas chromatography-mass spectrometry

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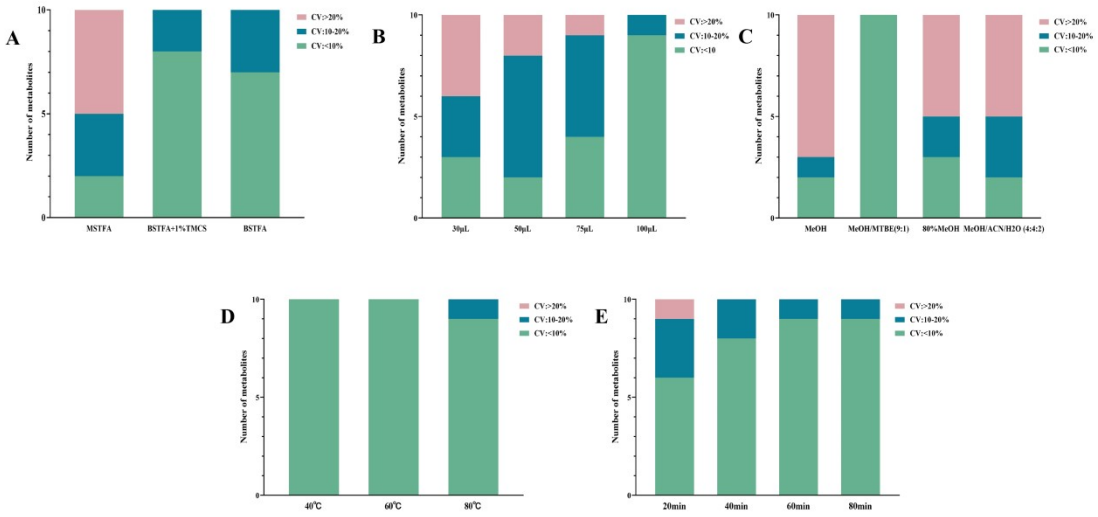

Fig. S1 CV% results of different conditions in the mixed solution of standard and BSA. Note: (A) The different derivatization reagents. (B) The derivatization solvent volume. (C) The extraction solvent. (D) The reaction temperature. (E) The reaction time.

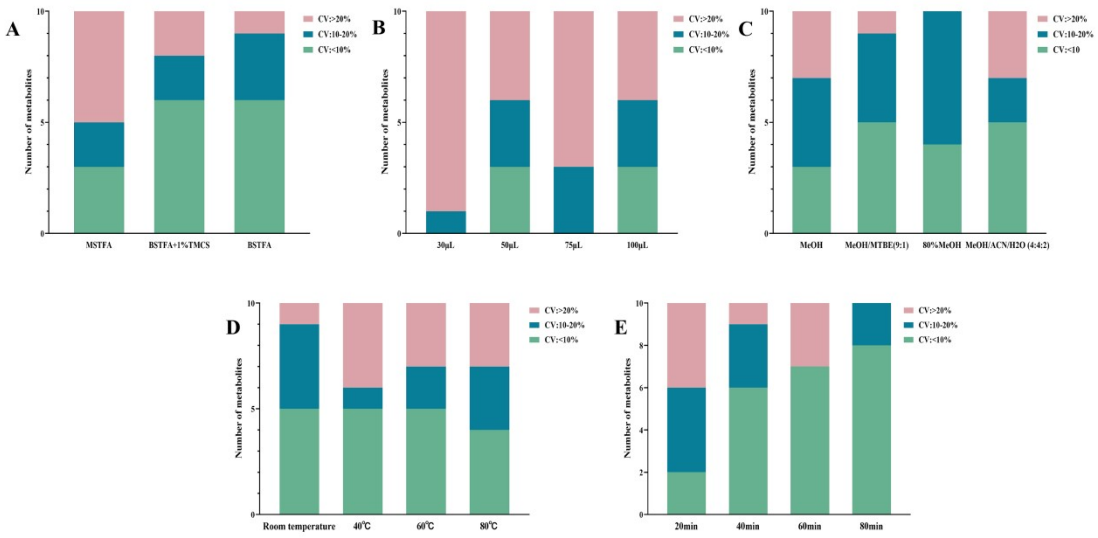

Fig. S2 CV% results of different conditions in serum sample. Note: (A) The different derivatization reagents. (B) The derivatization solvent volume. (C) The extraction solvent. (D) The reaction temperature. (E) The reaction time.

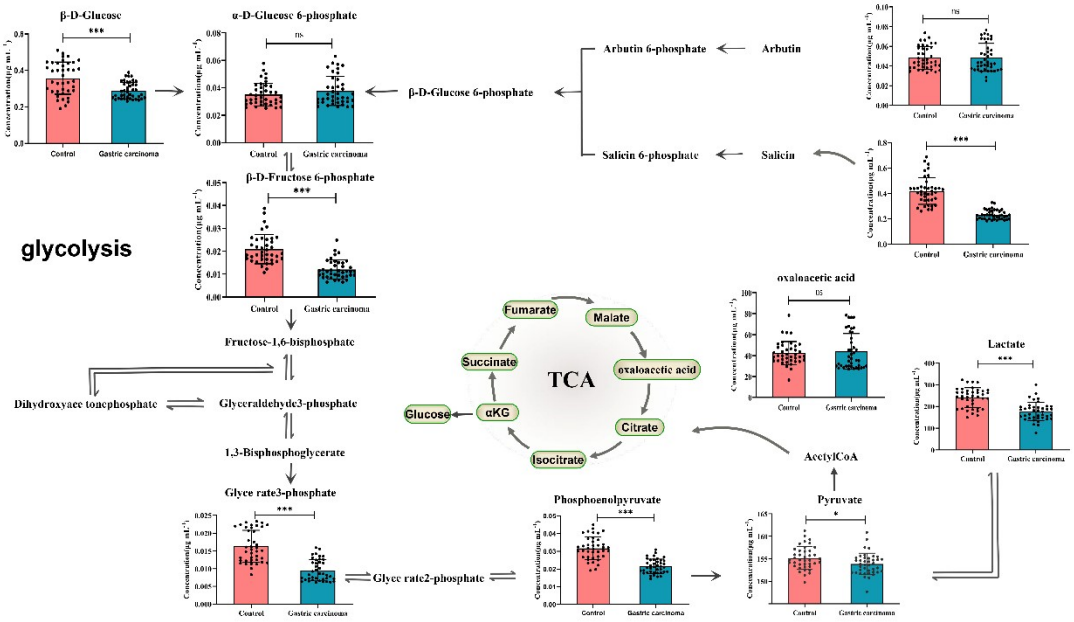

Fig. S3 Comparison of glycolysis metabolites in healthy controls and gastric cancer group. P-values were obtained from paired sample T test. nsP>0.05，*P < 0.05, **P < 0.01, and ***P < 0.001.

Table S1 The ion pairs of glycolysis metabolites for SRM mode.

Glycolysis metabolites	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	Collosion energy (eV)
Pyruvate	5.45	89	59	18
Lactate	5.61	191.1	147	24
Oxaloacetic acid	11.47	202.1	133	36
Phosphoenolpyruvate	13.96	369.1	147	16
β-D-Glucose	15.60	204.1	73	12
Glyce rate 3-phosphate	16.99	227	211	8
β-D-Fructose 6-phosphate	22.70	315.1	299	10
α-D-Glucose 6-phosphate	23.75	387.1	225	30
Salicin	25.65	169	73	12
Arbutin	26.22	254.1	239.1	14

Table S2 Experimental design, independent factors, levels of pretreatment process.

Run	A: The derivatization solvent volume (μL)	B: The reaction temperature (℃)	C: The reaction time (min)	D: The extraction solvent
1	50	80	60	80%MeOH
2	30	20	80	80%MeOH
3	75	40	60	MeOH:MTBE(v:v 9:1)
4	75	40	40	MeOH
5	75	80	20	MeOH:MTBE(v:v 9:1)
6	30	60	40	MeOH:MTBE(v:v 9:1)
7	75	60	40	MeOH:MTBE(v:v 9:1)
8	100	40	20	MeOH
9	50	20	20	MeOH:MTBE(v:v 9:1)
10	50	60	80	MeOH
11	100	20	40	80%MeOH
12	30	80	80	MeOH:MTBE(v:v 9:1)
13	100	80	20	80%MeOH
14	75	40	60	MeOH:MTBE(v:v 9:1)
15	50	20	20	MeOH:MTBE(v:v 9:1)
16	100	80	60	MeOH
17	100	20	80	MeOH
18	50	60	80	MeOH
19	100	60	80	80%MeOH
20	30	20	60	MeOH
21	30	80	20	MeOH
22	50	80	60	80%MeOH
23	75	40	60	MeOH:MTBE(v:v 9:1)
24	30	40	20	80%MeOH
25	30	60	40	MeOH:MTBE(v:v 9:1)

Table S3 Demographic and clinical chemistry characteristics of 80 subjects (Mean ± SD).

Parameters	Control (n=40)	Gastric carcinoma (n=40)
Age, years	61.60±9.00	58.30±9.51
Sex, F/M	15/25	10/30
Height, cm	165.51±8.00	167.78±8.50
Weight, kg	65.64±10.14	66.48±8.92
Waistline, cm	83.40±5.55	84.05±5.32
BMI, kg/m^2	23..83±2.37	23.56±2.20
DBP, mmHg	76.75±9.62	78.95±12.69
SBP, mmHg	128.75±16.53	128.35±20.53
ALT, U/L	14.65±9.74	9.93±7.17
AST, U/L	25.05±18.85	17.05±16.71
TBIL, μmol/L	16.27±3.58	14.45±3.68
BUN, mmol/L	6.37±1.39	6.22±1.33
CRE, μmol/l	71.05±21.85	78.70±20.78
TG, mmol/L	1.36±0.94	1.35±0.73
CHO, mmol/L	3.63±0.70	5.03±1.72
HDL-C, mmol/L	0.73±0.27	0.96±0.27
LDL-C, mmol/L	1.90±0.67	3.17±1.28
GLU, mmol/L	6.50±2.03	5.07±0.97

Note: SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, TG: triglycerides, AST: aspertate aminotransferase, ALT: cereal third transaminase, TBIL: total bilirubin, BUN: blood urea nitrogen, CRE: serum creatinine, CHO: total cholesterol, HDL-C: high density liptein cholesterol, LDL-C: low density lipoprotein cholesterol, GLU: blood glucose.

Table S4 The model coefficients computed based on radial basis functions with derivative reactions of the mixed solution of standard and BSA and serum sample.

The mixed solution of standard and BSA			Serum sample		
Glycolysis metabolites	R^2	Lack of fit	Glycolysis metabolites	R^2	Lack of fit
Pyruvate	0.9352	0.2219	Pyruvate	0.9403	0.2219
Lactate	0.9767	0.0075	Lactate	0.8315	0.0075
oxaloacetic acid	0.7647	0.2357	oxaloacetic acid	0.9471	0.2357
Phosphoenolpyruvate	0.9899	0.3926	Phosphoenolpyruvate	0.9437	0.3926
β-D-Glucose	0.9863	0.0546	β-D-Glucose	0.8564	0.0546
Glyce rate 3-phosphate	0.9737	0.5651	Glyce rate3-phosphate	0.8957	0.5651
β-D-Fructose 6-phosphate	0.9653	0.4136	β-D-Fructose 6-phosphate	0.7164	0.4136
α-D-Glucose 6-phosphate	0.9664	0.3248	α-D-Glucose 6-phosphate	0.9341	0.3248
Salicin	0.9484	0.2186	Salicin	0.8175	0.2186
Arbutin	0.9661	0.3585	Arbutin	0.7245	0.3585

Table S5 The precision of Intra-day and Inter-day of glycolysis metabolites derivatives.

Glycolysis metabolites （μg mL^{-1}）	Intra-day (n=6)		Inter-day (n=6)	
	Mean±SD	RSD(%)	Mean±SD	RSD(%)
Pyruvate	140.545±2.425	1.73	154.336±8.040	5.21
Lactate	114.466±4.688	4.10	117.725±5.083	4.32
Oxoacetic acid	25.885±0.226	0.87	25.176±0.511	2.03
Phosphoenolpyruvate	0.051±0.002	3.81	0.050±0.002	3.53
β-D-Glucose	0.133±0.002	1.20	0.140±0.006	4.40
Glyce rate3-phosphate	0.042±0.006	13.67	0.039±0.005	12.01
β-D-Fructose6-phosphate	0.053±0.004	6.80	0.053±0.003	4.88
α-D-Glucose6-phosphate	0.055±0.003	5.12	0.051±0.006	10.73
Salicin	2.183±0.096	4.41	2.107±0.112	5.32
Arbutin	0.064±0.005	7.74	0.057±0.004	7.29

Table S6 The stability of glycolysis metabolites derivatives at different temperature within 3 months.

Glycolysis metabolites ($\mu g\ mL^{-1}$)	-20℃ (n=3)		-40℃ (n=3)		-80℃ (n=3)	
	Mean±SD	RSD(%)	Mean±SD	RSD(%)	Mean±SD	RSD(%)
Pyruvate	176.971±23.196	13.11	177.867±35.810	20.13	168.573±14.440	8.57
Lactate	196.767±42.086	21.39	201.069±39.958	19.87	195.340±22.160	11.34
Oxoacetic acid	35.191±10.428	29.63	28.993±5.803	20.02	28.604±5.064	17.70
Phosphoenolpyruvate	0.054±0.019	34.71	0.069±0.010	14.64	0.063±0.009	14.93
β-D-Glucose	0.281±0.089	31.73	0.278±0.035	12.42	0.315±0.053	16.94
Glyce rate3-phosphate	0.039±0.013	34.72	0.041±0.015	37.77	0.057±0.007	13.03
β-D-Fructose6-phosphate	0.047±0.015	31.54	0.042±0.008	18.46	0.062±0.011	17.39
α-D-Glucose6-phosphate	0.103±0.065	63.25	0.088±0.036	40.33	0.092±0.019	20.50
Salicin	1.742±0.452	25.92	1.841±0.311	16.87	1.759±0.165	9.37
Arbutin	0.059±0.014	23.35	0.050±0.009	17.67	0.053±0.008	15.58

Table S7 The freeze-thaw stability test for glycolysis metabolites in human serum at -40℃.

Glycolysis metabolites ($\mu g\ mL^{-1}$)	Freeze–thaw (6 cycles)		Freeze–thaw (5 cycles)		Freeze–thaw (4 cycles)	
	Mean±SD	RSD(%)	Mean±SD	RSD(%)	Mean±SD	RSD(%)
Pyruvate	168.770±7.881	4.67	168.881±8.806	5.22	168.068±9.950	5.92
Lactate	191.293±5.374	2.81	191.814±5.836	3.04	191.269±6.591	3.45
Oxoacetic acid	36.113±9.833	27.23	34.056±9.442	27.73	30.196±4.417	14.63
Phosphoenolpyruvate	0.066±0.011	16.61	0.064±0.011	17.46	0.060±0.007	11.18
β-D-Glucose	0.213±0.062	29.11	0.202±0.061	30.49	0.174±0.003	1.85
Glyce rate3-phosphate	0.035±0.008	23.26	0.038±0.006	15.51	0.036±0.004	12.00
β-D-Fructose6-phosphate	0.037±0.003	9.33	0.036±0.003	9.50	0.036±0.004	10.69
α-D-Glucose6-phosphate	0.073±0.012	16.72	0.075±0.013	17.04	0.072±0.014	18.77
Salicin	1.325±0.260	19.61	1.252±0.212	16.91	1.337±0.111	8.33
Arbutin	0.064±0.007	10.89	0.065±0.007	10.17	0.064±0.007	11.57

Table S8 Matrix effect results of this protocol.

Glycolysis metabolites(μg mL^{-1})	Low	Medium	High
Pyruvate	102.30	97.10	100.73
Lactate	110.24	118.99	111.22
oxaloacetic acid	88.35	97.78	92.23
Phosphoenolpyruvate	117.76	116.89	94.32
β-D-Glucose	84.22	97.08	88.65
Glyce rate3-phosphate	85.18	109.40	128.38
β-D-Fructose6-phosphate	114.83	91.85	82.25
α-D-Glucose 6-phosphate	78.58	84.66	95.31
Salicin	89.44	94.00	83.54
Arbutin	87.52	102.33	84.63

Table S9 Quantitative analysis of glycolysis metabolites of the two groups (Mean ±SD).

Glycolysis metabolites(μg mL^{-1})	Control (n=40)	Gastric carcinoma (n=40)	P value
Pyruvate	155.187±2.495	153.857±2.410	0.017
Lactate	240.125±45.300	176.514±42.352	< 0.001
Oxaloacetic acid	42.427±11.067	44.023±17.043	0.621
Phosphoenolpyruvate	0.032±0.006	0.022±0.004	< 0.001
β-D-Glucose	0.358±0.088	0.290±0.045	< 0.001
Glyce rate 3-phosphate	0.016±0.005	0.010±0.003	< 0.001
β-D-Fructose 6-phosphate	0.021±0.006	0.012±0.004	< 0.001
α-D-Glucose 6-phosphate	0.035±0.008	0.037±0.011	0.212
Salicin	0.419±0.106	0.231±0.036	< 0.001
Arbutin	0.049±0.014	0.049±0.013	0.903