Electronic Supplementary Information

Pseudo-kinetics Approach for Time-Series Metabolomics Investigation: More Reliable and Sensitive Biomarkers Revealed in Vincristine-induced Paralytic Ileus Rat†

Huimin Guo,[ab] Jiaqing Chen,[ab] Yin Huang,[ab] Wei Zhang,[c] Fengguo Xu,[*ab] Zunjian Zhang[*ab]

[a] *Key Laboratory of Drug Quality Control and Pharmacovigilance (China Pharmaceutical University), Ministry of Education, Nanjing 210009, China. E-mail: zunjianzhangcpu@hotmail.com, fengguoxu@gmail.com; Tel: +86-25-83271454, +86-25-83271021.*
[b] *State Key Laboratory of Natural Medicine, China Pharmaceutical University, Nanjing 210009, China*
[c] *State Key Laboratory of Quality Research in Chinese Medicines, Macau University of Science and Technology, Taipa, Macau , China*

† Electronic supplementary information (ESI) available: Gastrointestinal transit (GI) and Gastric emptying (GE) measurement. Table S-1. The stability and repeatability of the proposed method. Table S-2. Parameters of OPLS-DA models based on the different time points in GC-MS and LC-MS. Table S-3. Metabolites identified by GC-MS. Table S-4. Metabolites identified by LC-MS. Table S-5. Sensitivity and specificity of ROC curves based on four biomarkers. Figure S-1. Scheme and histology results of the animal experiment in the present study. Figure S-2. Gastric emptying, gastrointestinal transit, urea nitrogen/creatinine ratio and body weight profiles during the model periods. Figure S-3. Pearon's correlations of metabolites and ileus parameters. Figure S-4. Altered metabolic pathways during ileus.

GE and GI transit measurement

The animals were fasted for 24 h prior to the GE and GI transit measurements, but water was allowed *ad libitum* until 2 h before the measurements. We used a modification of the technique described by Reynell PC and Spray GH for GE and GI transit measurements.[15] First, 1.5 mL of the test meal containing a non-absorbable marker (0.5 mg/mL phenol red solution in gelatin) was given orally by gavage. After 30 min, the animals were killed by cervical dislocation. The stomach and small intestine were exposed and removed by laparotomy; gastrointestinal transit was calculated by the formula:

$$\mathrm{GI} = \frac{A}{B}$$

A: the distance traveled by the phenol red front; B: the total length of the small intestine.

Simultaneously, the stomach and its content were homogenized in 50 mL 0.1 M NaOH. The phenol red assay was essentially evaluated by UV-detector. In short, 10 mL of the homogenized mixture was centrifuged for 20 min and 5 mL of the supernatant was added to 0.5 mL 20% trichloroacetic acid to precipitate the proteins.[16] After vortexing and centrifugation (20 min, 2600 g) 4 mL of the supernatant was added to 2 mL 1 M NaOH to develop the maximum intensity of the color. The solution was colorimetric assayed with Tecan Infinite 200 PRO at 560 nm. Gastric emptying (%) was calculated according to the following formula:

$$\mathrm{GE} = 1 - \frac{amount\ of\ phenel\ red\ recorded\ after\ 20\ min}{amount\ of\ phenol\ red\ recorded\ after\ 0\ min}$$

Table S-1. The stability and repeatability of the proposed method

Mode	Metabolites	Stability (n=7)				Repeatability (n=7)			
		Rt (min)		Peak area		Rt (min)		Peak area	
		mean	CV	mean	CV	mean	CV	mean	CV
GC-MS	Butyric acid	8.23	0.04	53659	0.14	8.22	0.02	51987	0.12
	Isoleucine	10.16	0.03	32537	0.19	10.16	0.03	30531	0.20
	Serine	11.15	0.02	52669	0.13	11.15	0.02	49885	0.14
	Threonine	11.55	0.03	57586	0.17	11.55	0.03	53946	0.19
	Aspartic acid	12.68	0.05	10607	0.16	12.68	0.05	10246	0.13
	Pyroglutamic acid	13.39	0.01	44443	0.21	13.39	0.01	41704	0.25
	Glutamic acid	14.58	0.01	251319	0.25	14.58	0.01	224304	0.22
	Lysine	15.63	0.01	510968	0.18	15.63	0.01	472247	0.16
	Glutamine	16.42	0.01	34748	0.13	16.42	0.01	35057	0.14
	Citric acid	17.00	0.01	22360	0.31	17.00	0.01	20950	0.29
	Tyrosine	18.26	0.01	71492	0.20	18.26	0.01	66381	0.24
	Palmitelaidic acid	19.00	0.03	17985	0.24	19.00	0.03	16898	0.25
	Palmitic acid	19.18	0.02	25729	0.24	19.18	0.02	23689	0.20
	Uric acid	19.97	0.02	9630	0.17	19.97	0.02	9310	0.16
	Linoleic acid	20.81	0.02	18623	0.27	20.81	0.02	17561	0.29
	Oleic acid	20.84	0.01	52853	0.17	20.84	0.01	50719	0.19
	Tryptophan	21.09	0.01	24020	0.16	21.09	0.01	21436	0.28
ESI-	Propenoylcarnitine	7.37	0.04	61978	0.15	7.37	0.04	59678	0.18
	3-Hydroxy-octadecenoylcarnitine	8.21	0.03	73349	0.22	8.21	0.03	70120	0.27
	TCA(taurocholic acid)	8.27	0.02	65731	0.22	8.27	0.02	58485	0.15
	LysoPC(14:0)	11.93	0.03	186037	0.11	11.93	0.03	181154	0.10
	LysoPC(O-15:0/0:0)	12.66	0.04	276930	0.09	12.66	0.03	270376	0.10
	LysoPC(18:2)	12.90	0.01	1375000	0.05	12.90	0.01	1361376	0.05
	LysoPE(16:0)	13.30	0.01	416718	0.13	13.30	0.01	390815	0.13
	LysoPC(22:5/0:0)	13.32	0.02	34022	0.19	13.32	0.01	31168	0.20
	PE(P-16:0/0:0)	13.73	0.03	172151	0.19	13.73	0.01	152698	0.16
	LysoPC(O-16:0/0:0)	13.79	0.02	130633	0.09	13.79	0.01	126590	0.10
	LysoPC(17:0)	14.17	0.02	318891	0.09	14.17	0.01	310195	0.10
	LysoPC(17:1)	14.19	0.03	87092	0.15	14.19	0.03	84557	0.15
	HETE	14.21	0.02	1101109	0.18	14.21	0.02	965463	0.16
	LysoPE(18:0)	14.82	0.02	470593	0.19	14.82	0.02	417500	0.16
	Glycocholic acid	15.28	0.02	92791	0.19	15.28	0.02	85679	0.21
	SAM	16.92	0.01	124529	0.15	16.92	0.01	119790	0.18
	Docosahexaenoic acid	17.06	0.02	566890	0.23	17.06	0.01	487276	0.22
	MG(18:1(9Z)/0:0/0:0)	18.27	0.04	1790660	0.14	18.27	0.04	1724844	0.17
ESI+	L-Acetylcarnitine	0.75	0.03	185026	0.08	0.75	0.03	177021	0.08

LysoPC(14:0)	11.94	0.03	199552	0.09	11.94	0.02	194475	0.08
LysoPC(O-15:0/0:0)	12.66	0.03	260732	0.09	12.66	0.03	250047	0.10
LysoPC(18:2)	12.90	0.03	1207342	0.06	12.90	0.03	1189224	0.07
PG(20:1(11Z)/0:0)	12.90	0.01	74596	0.23	12.90	0.01	72662	0.24
LysoPE(16:1)	13.30	0.03	275443	0.12	13.30	0.01	255987	0.11
LysoPC(22:5/0:0)	13.33	0.03	42960	0.28	13.33	0.01	38690	0.23
PE(P-16:0/0:0)	13.74	0.01	123648	0.12	13.74	0.01	115536	0.10
LysoPC(O-16:0/0:0)	13.79	0.01	128484	0.10	13.79	0.01	123253	0.12
LysoPC(17:1)	14.20	0.05	136167	0.09	14.20	0.01	129670	0.09
LysoPE(18:0)	14.82	0.03	330433	0.17	14.82	0.03	298379	0.15
Glycocholic acid	15.29	0.03	77623	0.22	15.29	0.02	70665	0.24
LysoPC(19:0/0:0)	15.77	0.02	64325	0.29	15.77	0.02	54897	0.28

Table S-2. Parameters of OPLS-DA models based on the different time points in GC-MS and LC-MS

Models	R^2X	R^2Y	Q^2
GC-MS/4d	0.798	0.983	0.911
GC-MS/8d	0.855	0.943	0.789
GC-MS/11d	0.759	0.969	0.765
LC-MS ESI+/4d	0.522	0.892	0.685
LC-MS ESI+/8d	0.580	0.978	0.767
LC-MS ESI+/11d	0.253	0.731	0.366
LC-MS ESI-/4d	0.433	0.94	0.793
LC-MS ESI-/8d	0.640	0.979	0.840
LC-MS ESI-/11d	0.315	0.829	0.609

Table S-3. Metabolites identified by GC-MS

NO.	Ion Rt (min)	Similarity	Metabolites	M.W.
1	7.04	97	Alanine	89.09
2	7.32	85	Glycine	75.07
3	8.94	92	Valine	117.15
4	10.16	88	Isoleucine	131.17
5	10.21	94	Proline	115.13
6	11.15	94	Serine	105.09
7	11.55	93	Threonine	119.12
8	12.68	64	Aspartic acid	133.10
9	13.33	82	Methionine	149.21
10	13.39	94	Pyroglutamic acid	129.11
11	13.43	80	4-Hydroxy-L-proline	131.13
12	14.52	85	Ornithine	132.16
13	14.58	89	Glutamic acid	147.13
14	15.24	84	Asparagine	132.12
15	15.63	93	Lysine	146.19
16	16.42	87	Glutamine	146.14
17	18.26	89	Tyrosine	181.19
18	21.09	90	Tryptophan	204.23
19	19.00	80	Palmitelaidic acid	254.41
20	19.18	92	Palmitic acid	256.42
21	20.81	85	Linoleic acid	280.45
22	20.84	86	Oleic acid	282.46
23	22.27	86	Arachidonic acid	304.47
24	23.87	82	Docosahexaenoic acid	328.49
25	28.65	90	Cholesterol	386.65
26	17.79	83	Galactose	180.16
27	18.18	89	Mannose	180.16
28	10.37	93	Succinic acid	118.09
29	10.73	88	Propanoic acid	74.08
30	12.92	85	Malic acid	134.09
31	14.03	84	Oxoglutaric acid	146.10
32	15.49	80	Acetic acid	60.05
33	17.00	88	Citric acid	192.12
34	9.31	92	Urea	60.06
35	9.87	80	Phosphoric acid	98.00
36	10.83	88	Pyrimidine	80.09
37	13.86	84	Creatinine	113.12
38	19.97	81	Uric acid	168.11

Table S-4. Metabolites identified by LC-MS

No.	Metabolites	Ion Rt (min)	Model	Ion *m/z*	Ions
1	L-Acetylcarnitine	0.74	ESI+	204.12	$[M+H]^+$
2	Leucine	0.75	ESI+	132.10	$[M+H]^+$
3	Tryptophan	2.14	ESI+	205.10	$[M+H]^+$
4	Stearic acid	4.20	ESI-	283.08	$[M-H]^-$
5	Cholic acid	10.22	ESI-	407.28	$[M-H]^-$
		10.23	ESI+	373.27	Fragment
6	Oleoylcarnitine	10.23	ESI+	426.32	$[M+H]^+$
7	Uridine 5'-diphosphate	10.46	ESI+	405.24	$[M+H]^+$
8	Deoxycholic acid	12.19	ESI-	391.28	$]M-H]^-$
9	Tetradecanoylcarnitine	12.29	ESI+	372.31	$[M+H]^+$
10	Sphinganine	12.38	ESI+	302.31	$[M+H]^+$
11	LysoPC(16:1)	12.38	ESI-	538.31	$[M+HCOO]^-$
		12.39	ESI+	494.32	$[M+H]^+$
12	Stearic acid	12.39	ESI+	285.64	$[M+H]^+$
13	LysoPE(18:2)	12.81	ESI-	476.27	$[M-H]^-$
		12.82	ESI+	478.29	$[M+H]^+$
14	LysoPC(18:2)	12.90	ESI-	564.33	$[M+HCOO]^-$
		12.90	ESI+	520.34	$[M+H]^+$
15	PG(20:1(11Z)/0:0)	12.90	ESI+	774.31	$[M+H]^+$
16	Arachidonic acid	12.92	ESI-	303.65	$[M-H]^-$
17	LysoPC(20:4)	12.96	ESI-	588.32	$[M+HCOO]^-$
18	LysoPC(22:0)	12.96	ESI-	612.33	$[M+HCOO]^-$
		12.96	ESI+	568.34	$[M+H]^+$
19	LysoPE(16:0)	13.30	ESI-	452.27	$[M-H]^-$
		13.30	ESI+	454.29	$[M+H]^+$
20	LysoPC(22:5)	13.32	ESI-	614.34	$[M+HCOO]^-$
21	LysoPC(16:0)	13.41	ESI-	540.32	$[M+HCOO]^-$
22	Carnitine 16:0	13.67	ESI+	400.34	$[M+H]^+$
23	LysoPE(18:1)	13.71	ESI-	478.29	$[M-H]^-$
		13.72	ESI+	480.31	$[M+H]^+$
24	PE(P-16:0/0:0)	13.73	ESI-	436.28	$[M-H]^-$
		13.74	ESI+	438.30	$[M+H]^+$
25	LysoPC(O-16:0/0:0)	13.79	ESI+	482.36	$[M+H]^+$
		13.79	ESI-	526.35	$[M+HCOO]^-$
26	LysoPC(18:1)	13.82	ESI-	566.34	$[M+HCOO]^-$
		13.83	ESI+	522.35	$[M+H]^+$
27	LysoPE(0:0/18:0)	14.13	ESI-	462.29	$[M-H_2O-H]^-$
		14.14	ESI+	464.31	$[M-H_2O+H]^+$

28	LysoPC(17:0)	14.17	ESI-	554.34	$[M+HCOO]^-$
		14.19	ESI+	511.36	$[M+H]^+$
29	LysoPC(17:1)	14.19	ESI-	552.36	$[M+HCOO]^-$
		14.20	ESI+	508.37	$[M+H]^+$
30	HETE	14.25	ESI-	319.23	$[M-H]^-$
31	LysoPC(20:2/0:0)	14.28	ESI+	548.37	$[M+H]^+$
32	LysoPE(18:0)	14.82	ESI-	480.30	$[M-H]^-$
33	Ceramide(d18:1/12:0)	14.82	ESI+	482.32	$[M+H]^+$
34	LysoPC(18:0)	14.96	ESI-	568.36	$[M+HCOO]^-$
		14.96	ESI+	524.37	$[M+H]^+$
35	Glycocholic acid	15.28	ESI-	464.31	$[M-H]^-$
		15.29	ESI+	466.33	$[M+H]^+$
36	LysoPC(19:0/0:0)	15.77	ESI+	538.38	$[M+H]^+$
37	SAM	16.97	ESI-	353.14	$[M-H]^-$
38	Docosahexaenoic acid	17.06	ESI-	327.23	$[M-H]^-$
39	MG(18:1(9Z)/0:0/0:0)	18.27	ESI-	355.16	$[M-H]^-$

Table S-5. Sensitivity and specificity of ROC curves based on four biomarkers

Biomarkers	Sensitivity %	Specificity %	Area under the curve
Docosahexaenoic acid	87.2	91.3	0.948
L-glutamine	78.9	86.8	0.893
Glycocholic acid	79.3	89.3	0.851
L-Acetylcarnitine	89.7	92.3	0.972

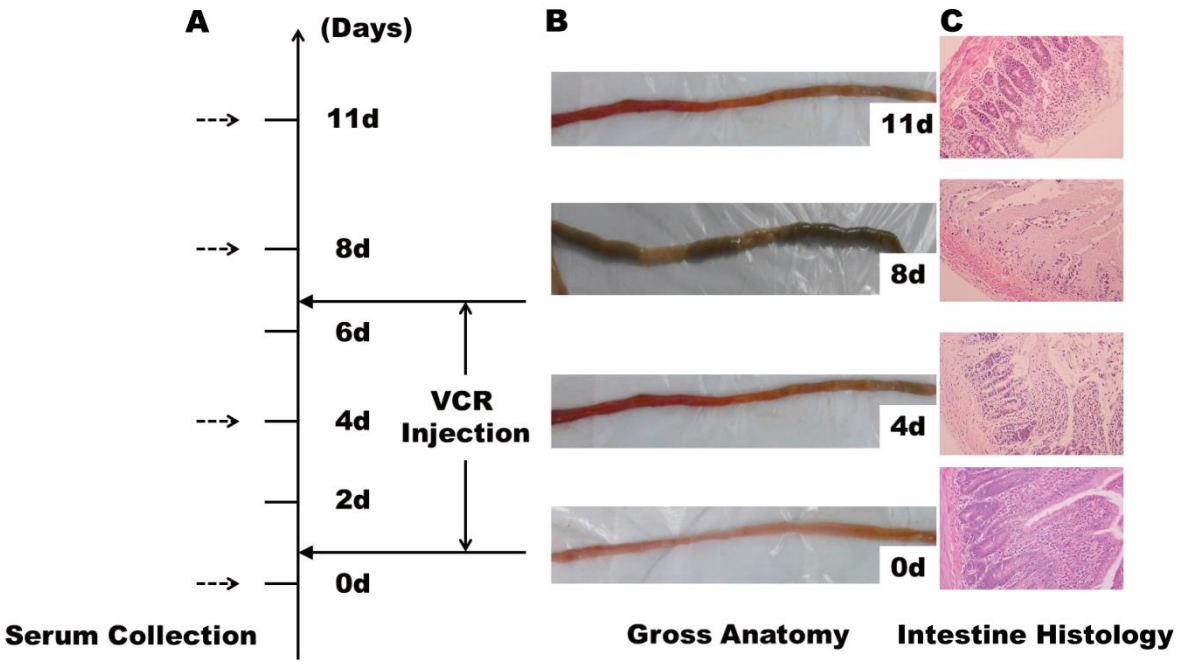

Figure S-1. Scheme and histology results of the animal experiment in the present study. Column A is the scheme of the animal experiment. The experimental beginning time was defined as 0 day; VCR was injected once every other day during day 1 to 7; Serum collection, intestine gross and histology examinations were conducted in the time points of 0, 4, 8 and 11 marked with dashed arrows. Column B presents the gross anatomy results and column C shows the pictures of corresponding intestine histopathology (H&E staining). In these examinations, ileus presented a stepwise development from day 0 to 8 and recovered after 11 days.

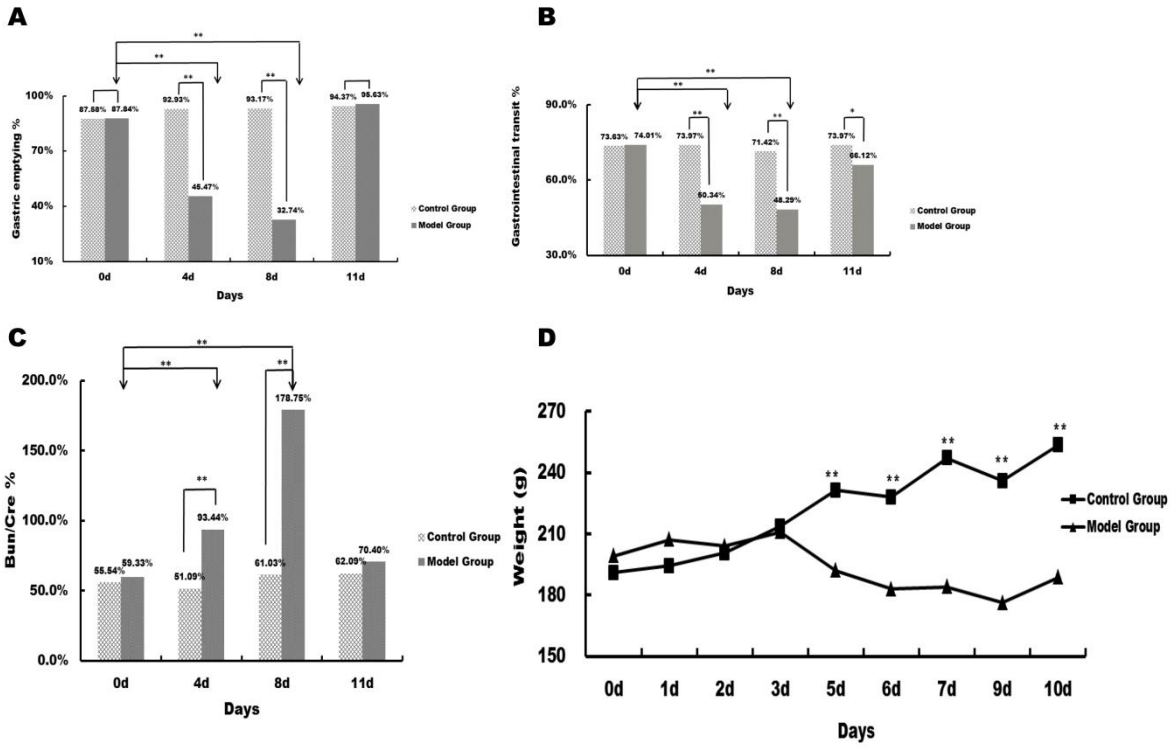

Figure S-2. Gastric emptying, gastrointestinal transit, urea nitrogen/creatinine ratio and body weight profiles during the model periods. (A-C) Gastric emptying (GE), gastrointestinal transit (GI) and serum urea nitrogen/creatinine ratio (Bun/Cre) variations between model and control group at day 0, 4, 8 and 11. (D) Body weight profiles during the experiment period. Box and triangle represents control and model group respectively, * and ** represent $p < 0.05$ and $p < 0.01$ models compared with corresponding controls.

Figure S-3. Pearson's correlations of metabolites and ileus parameters. GI is the abbreviation of gastrointestinal transit and GE is of gastric emptying. Metabolites with $|C_{ij}| > 0.7$ and $p < 0.05$ correlated to GI and GE were marked with double asterisk.

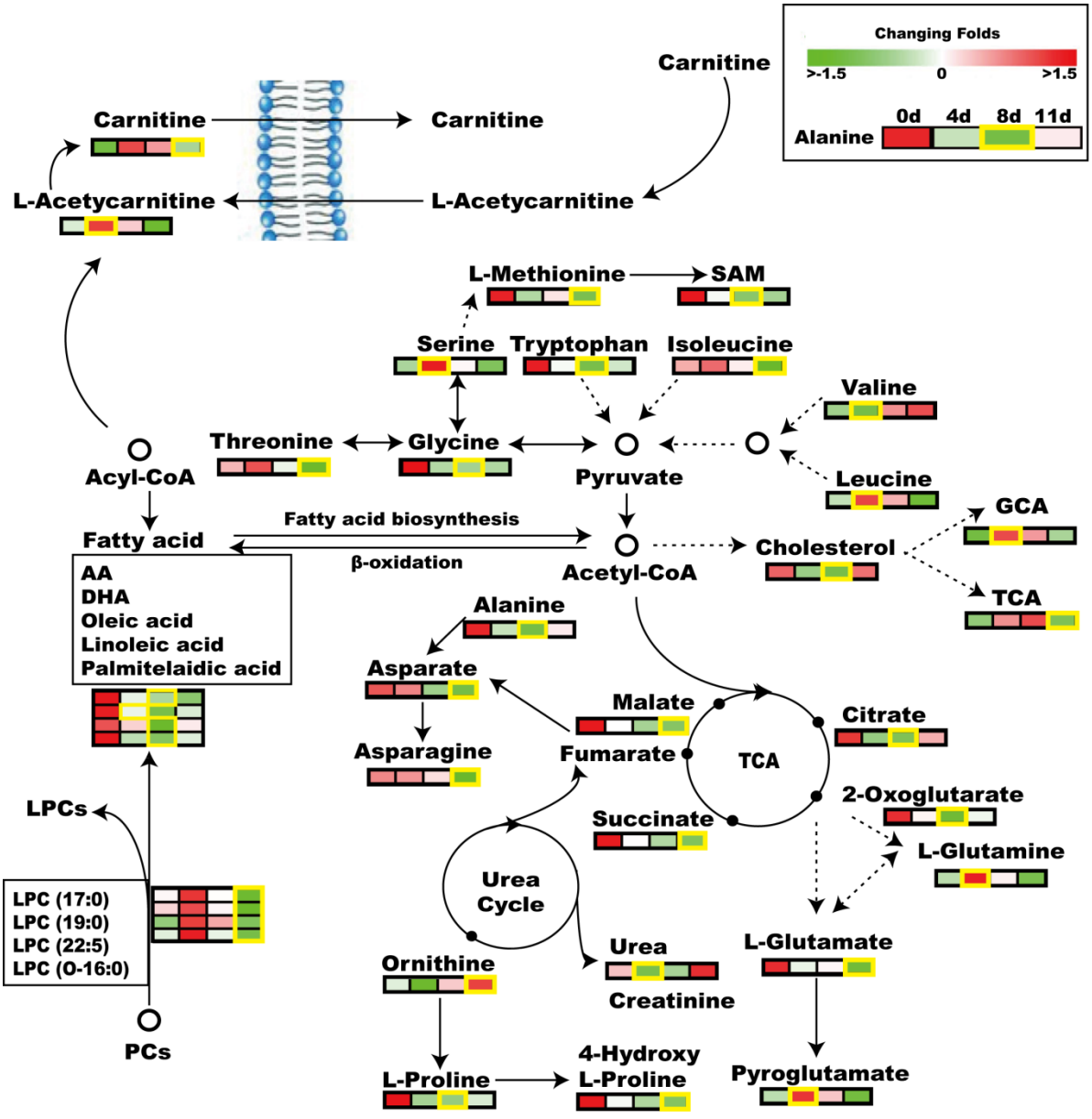

Figure S-4.Altered metabolic pathways during the ileus. Black arrows indicate a direct relationship between two metabolites; dotted arrows indicate indirect relationships between two metabolites. Heat-map segment is indicated with the degree of change in the models compared with the controls marked with colors. Green squares indicate down-regulation of metabolites in models compared with the controls; red squares indicate up-regulation of metabolites in models compared with the controls. The yellow wireframes mark the peak time of metabolites.