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Supplemental information

Model selection reveals selective regulation of blood amino acid and lipid metabolism by insulin in humans

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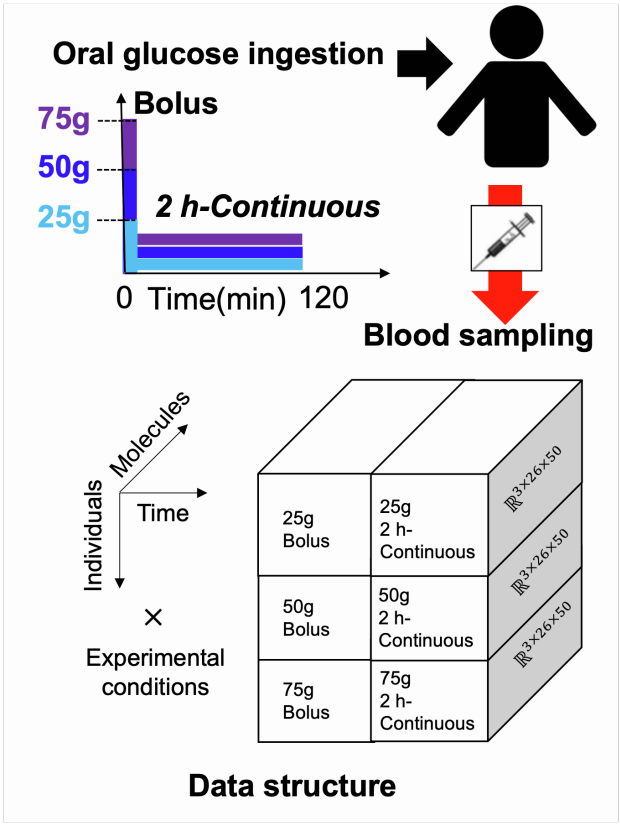
Supplementary Figures

a



b

$$x_{ijkm} = \sum_{l_1=1}^{3}\sum_{l_2=1}^{26}\sum_{l_3=1}^{6}\sum_{l_4=1}^{50} G(l_1 l_2 l_3 l_4) u_{l_1 i} v_{l_2 j} w_{l_3 k} y_{l_4 m}. \quad \text{(Eq.1)}$$

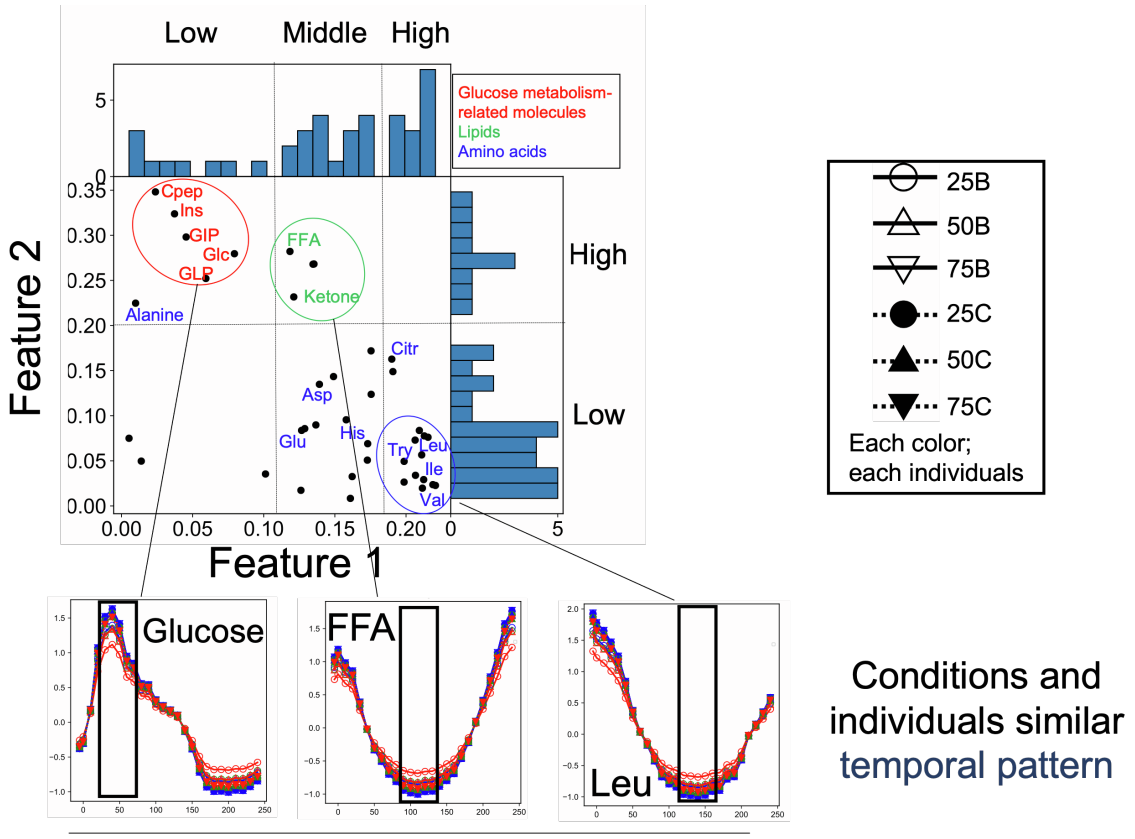


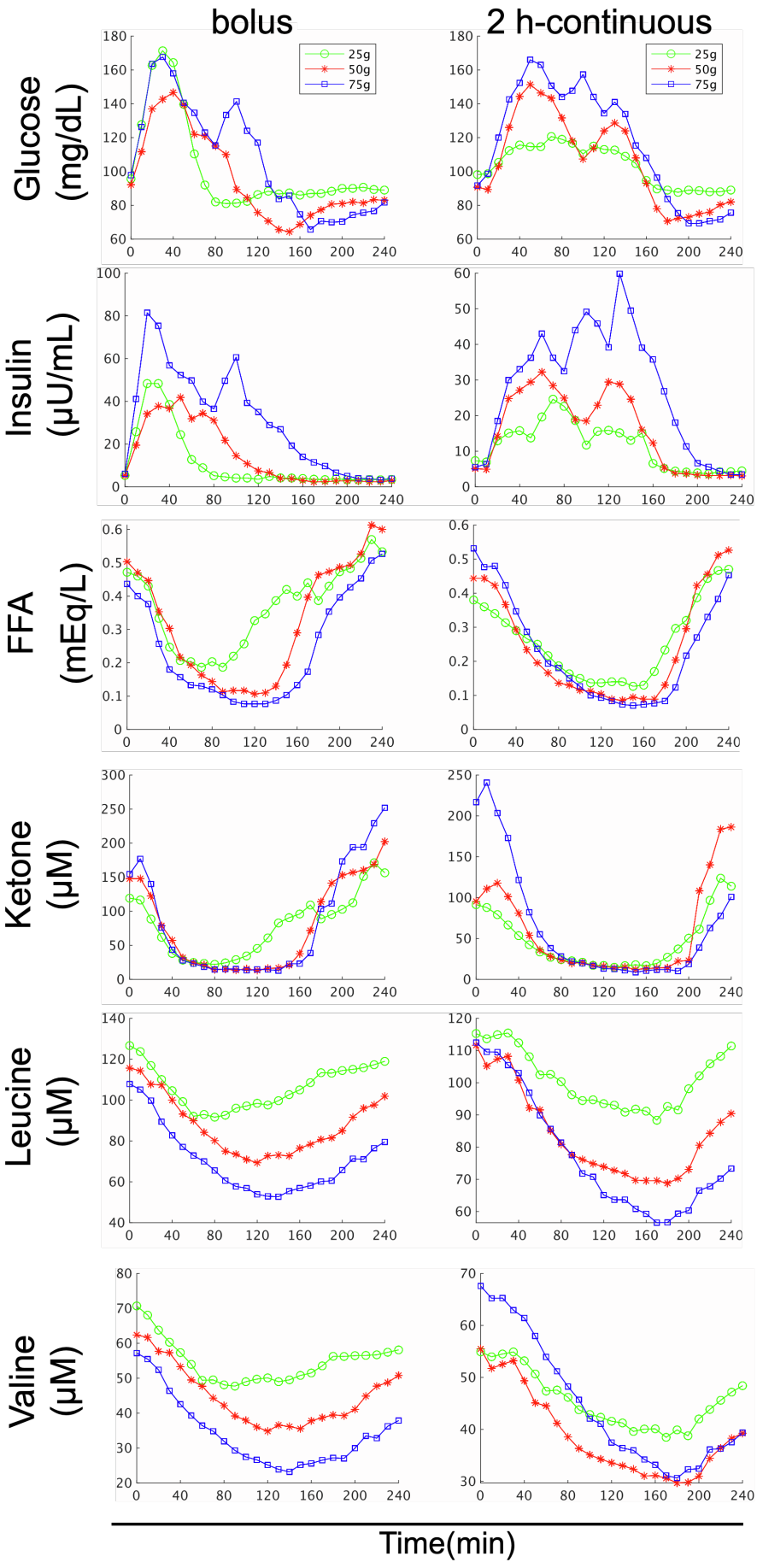
Figure. S1 Data set for model construction. Related to Figure 1.

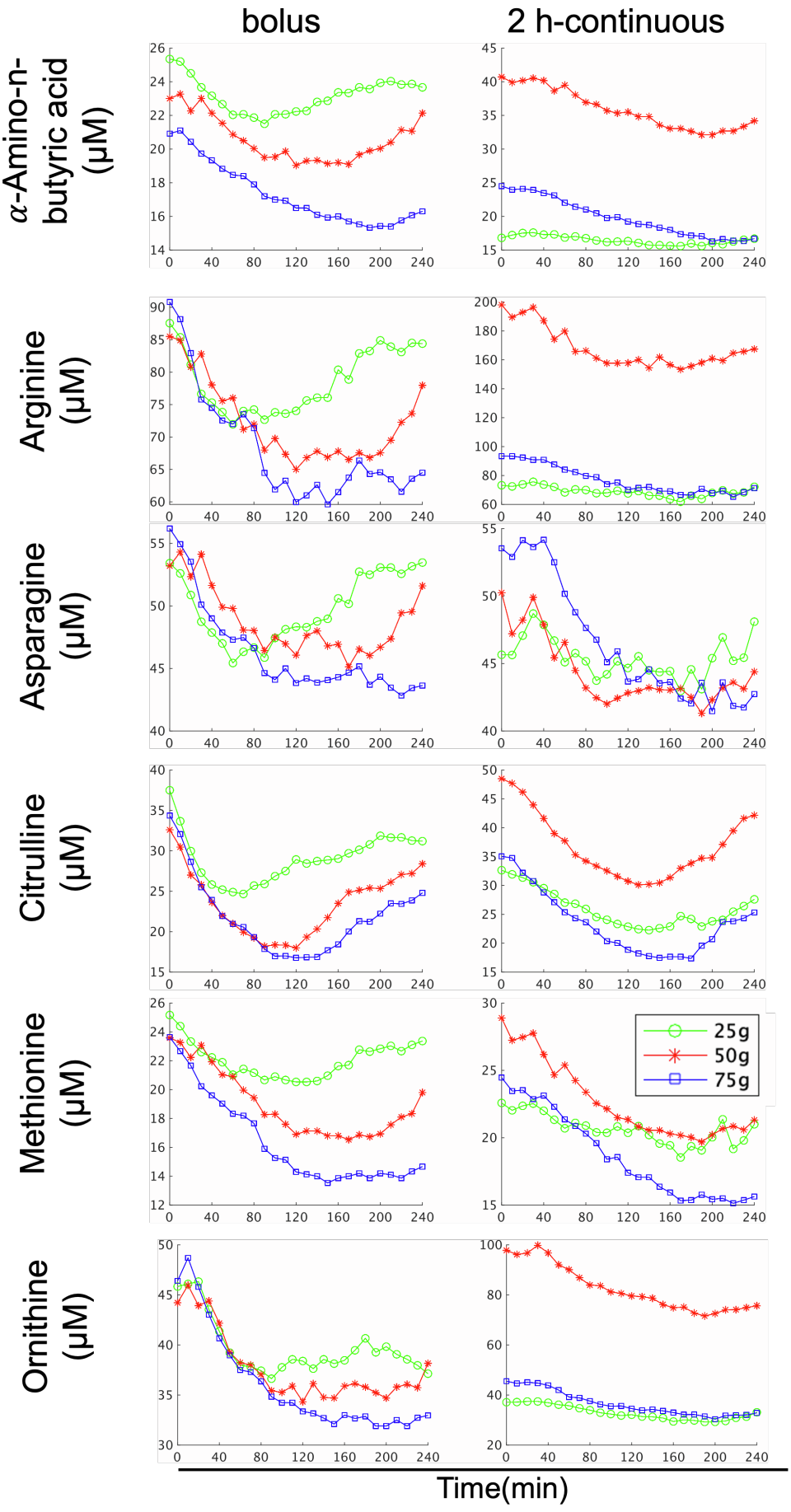
a The experimental dataset for model selection. Three individuals orally ingested glucose with three doses 75, 50, and 25 g in two durations of bolus and 2 h continuous ingestion. The data structure has four axes: individual × time × experimental condition × molecule. The data represent the concentration changes at 26 time points (-5, 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240 minutes) from 5 min before fasting to 240 min after glucose ingestion for 50 molecules in three healthy subjects, in six different experimental conditions.

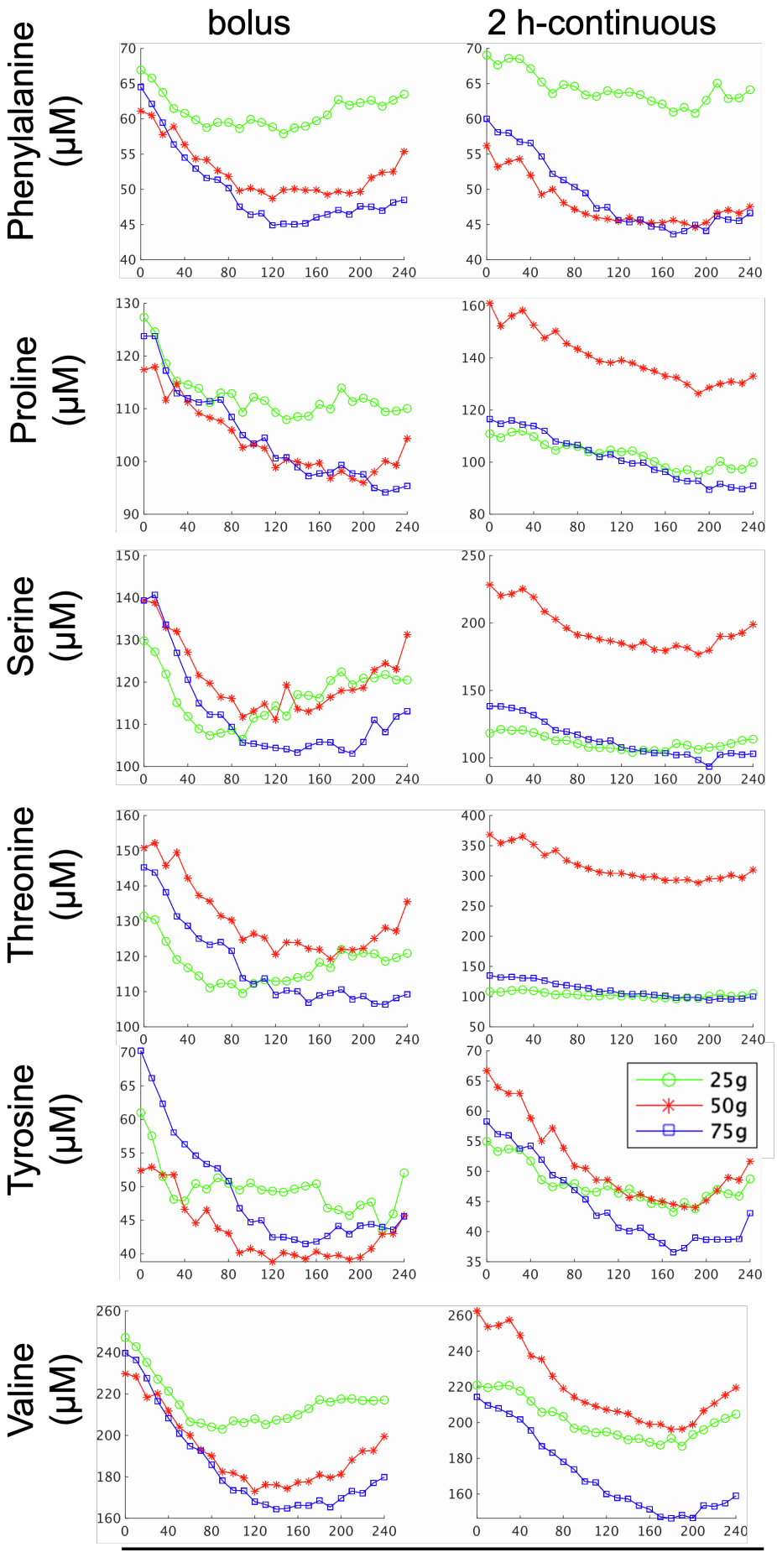
b Distribution of features extracted by tensor decomposition. Time series indicate similar temporal patterns among experimental conditions (‘Conditions similar’) and among individuals (‘Individuals similar’) for representative molecules[28]. The dashed lines show the values of Feature 1 divided into three, and Feature 2 divided into two, based on the shape of the distribution.The black lines in the time series indicate the time point of the peak. See our previous study for more details[28]. The color of each line indicates each condition. Solid lines indicate bolus ingestion, and dahed lines indicate 2 h continuous ingestion. Circles, triangles, and lower triangles indicate the three doses, 25, 50, and 75 g, respectively. Abbreviations for the representative molecules are as follows: Asp aspartic acid; Cit, citrulline; CRP, C-reactive peptide; FFA, free fatty acid; 3-OH, 3-hydroxybutyric acid; Ketone, Total ketone body; GIP, gastric inhibitory polypeptide (active); Glc, glucose; GLP, glucagon-like peptide-1; Glu,

glutamic acid; His, histidine; Ile, isoleucine; Ins, insulin; Leu, leucine; Tyr, tyrosine; Val, valine.

The label colors correspond to the metabolic group list (inset).


bolus
2 h-continuous
25g
50g
75g
Glucose (mg/dL)
Insulin (μU/mL)
FFA (mEq/L)
Ketone (μM)
Leucine (μM)
Valine (μM)
Time(min)


bolus
2 h-continuous
α-Amino-n-butyric acid (μM)
Arginine (μM)
Asparagine (μM)
Citrulline (μM)
Methionine (μM)
Ornithine (μM)
25g
50g
75g
Time(min)


bolus
2 h-continuous
Phenylalanine (μM)
Proline (μM)
Serine (μM)
Threonine (μM)
Tyrosine (μM)
Valine (μM)
25g
50g
75g

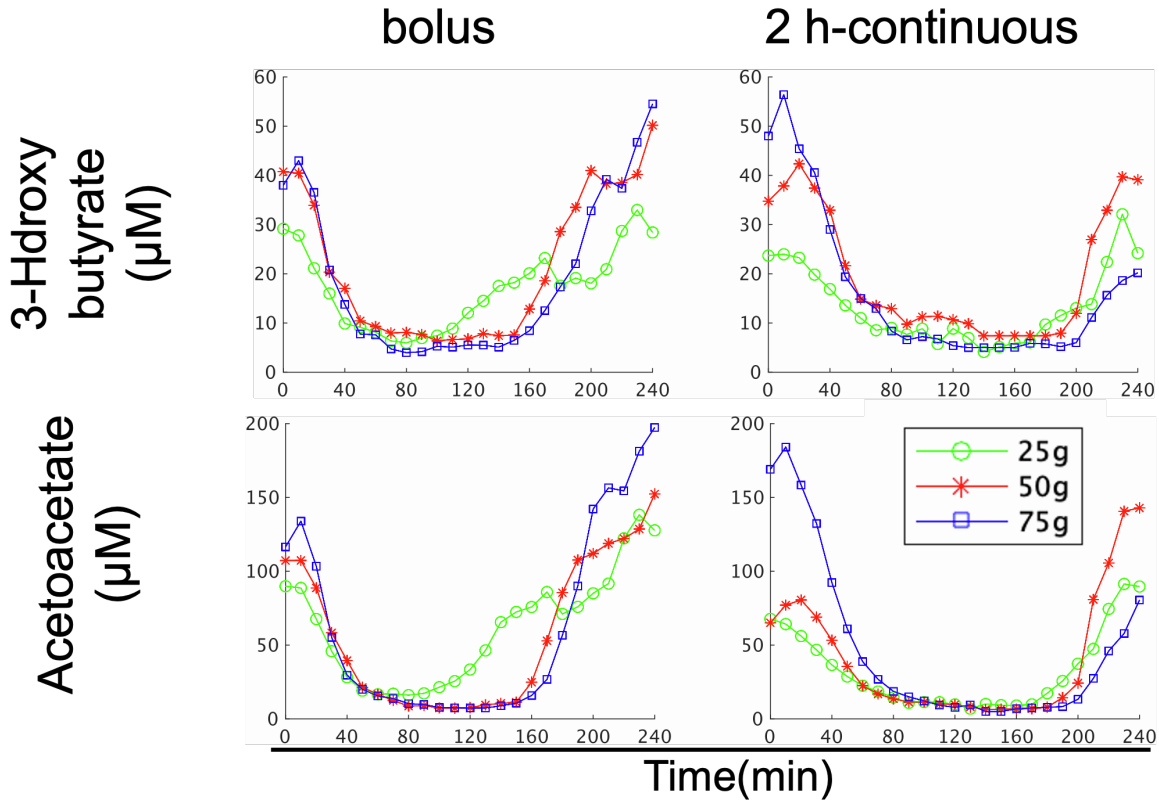


Figure. S2 Time course data of blood insulin and blood metabolites in three individuals by glucose ingestion. Related to Figure 1.

The dose and ingestion pattern are indicated at the top. Green, red, and blue indicate three different doses: 25 g, 50 g, and 75 g, respectively.

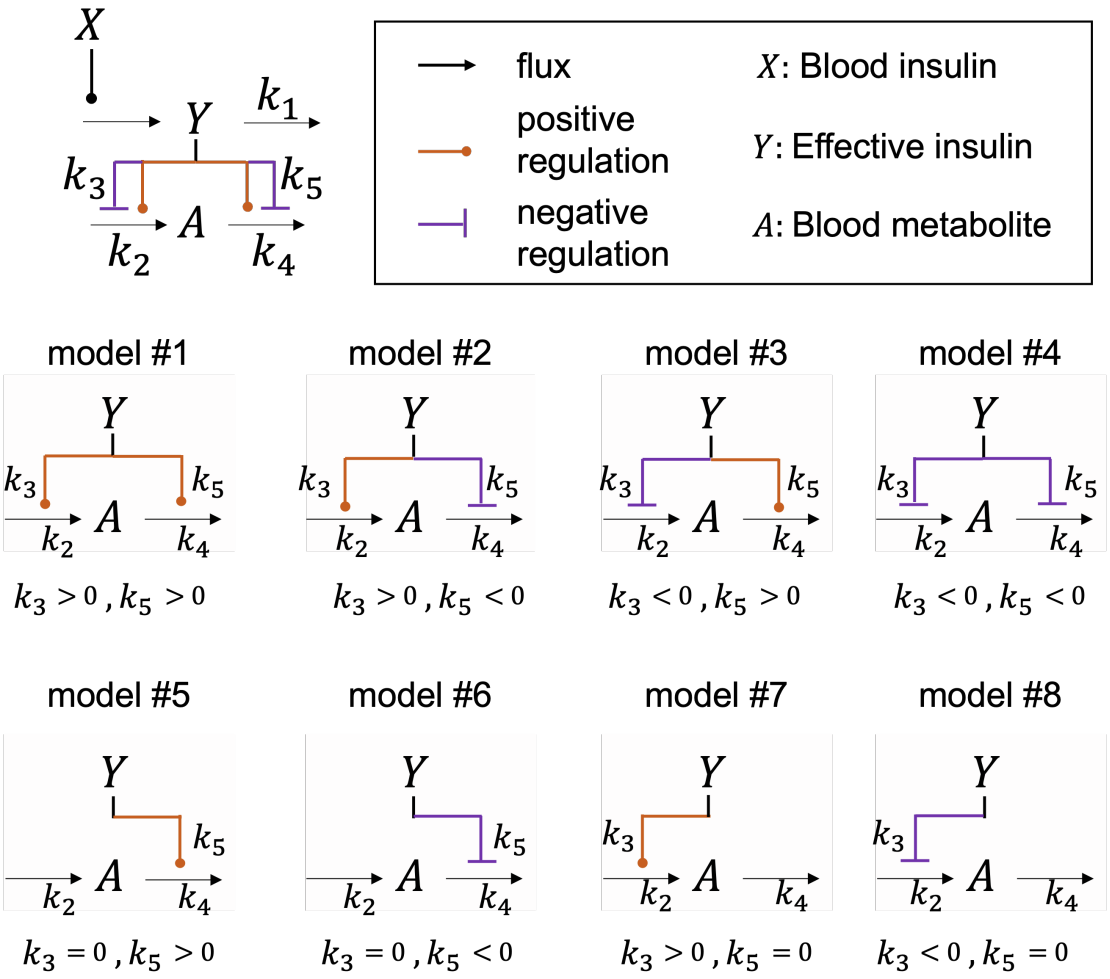
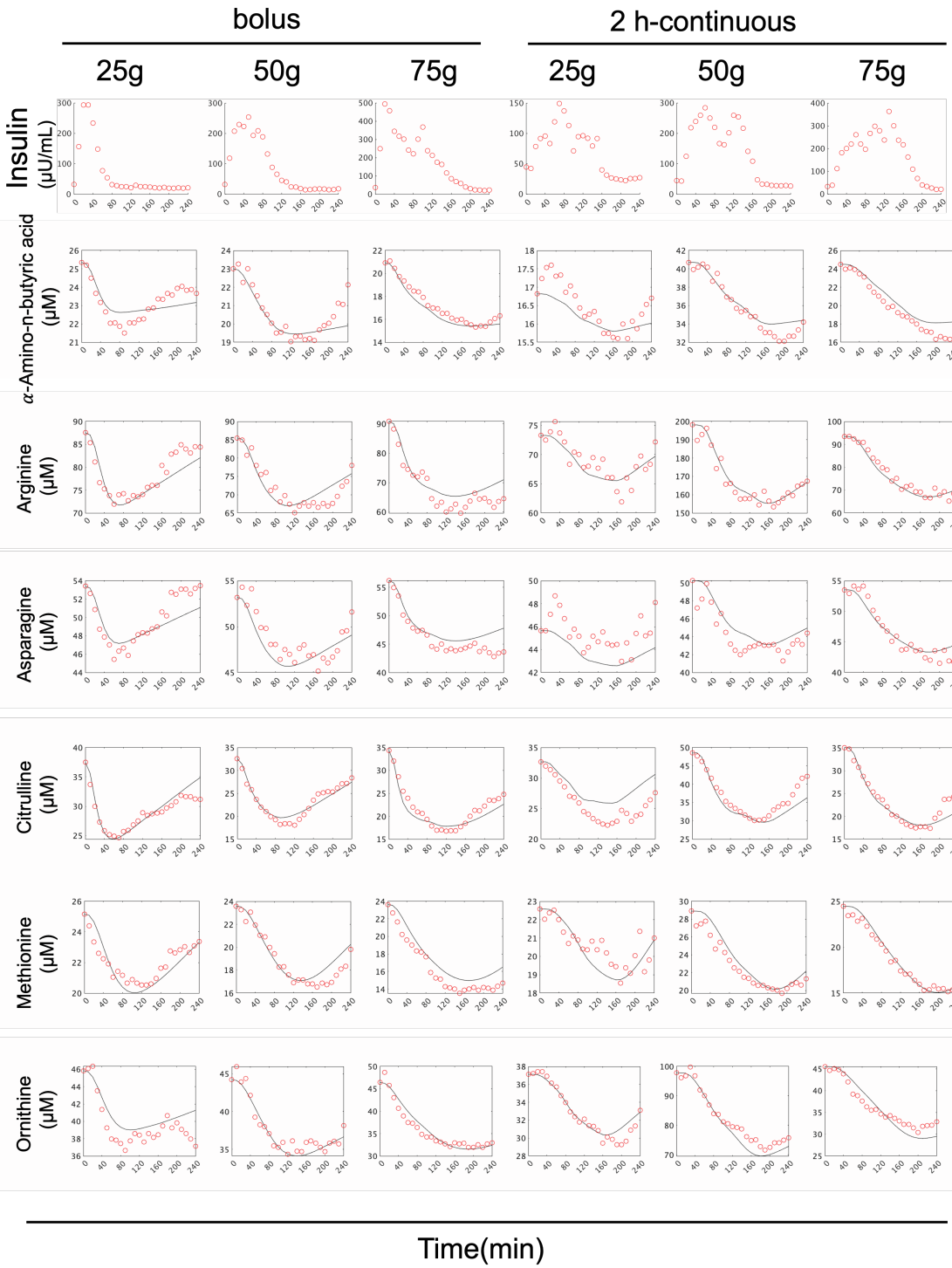
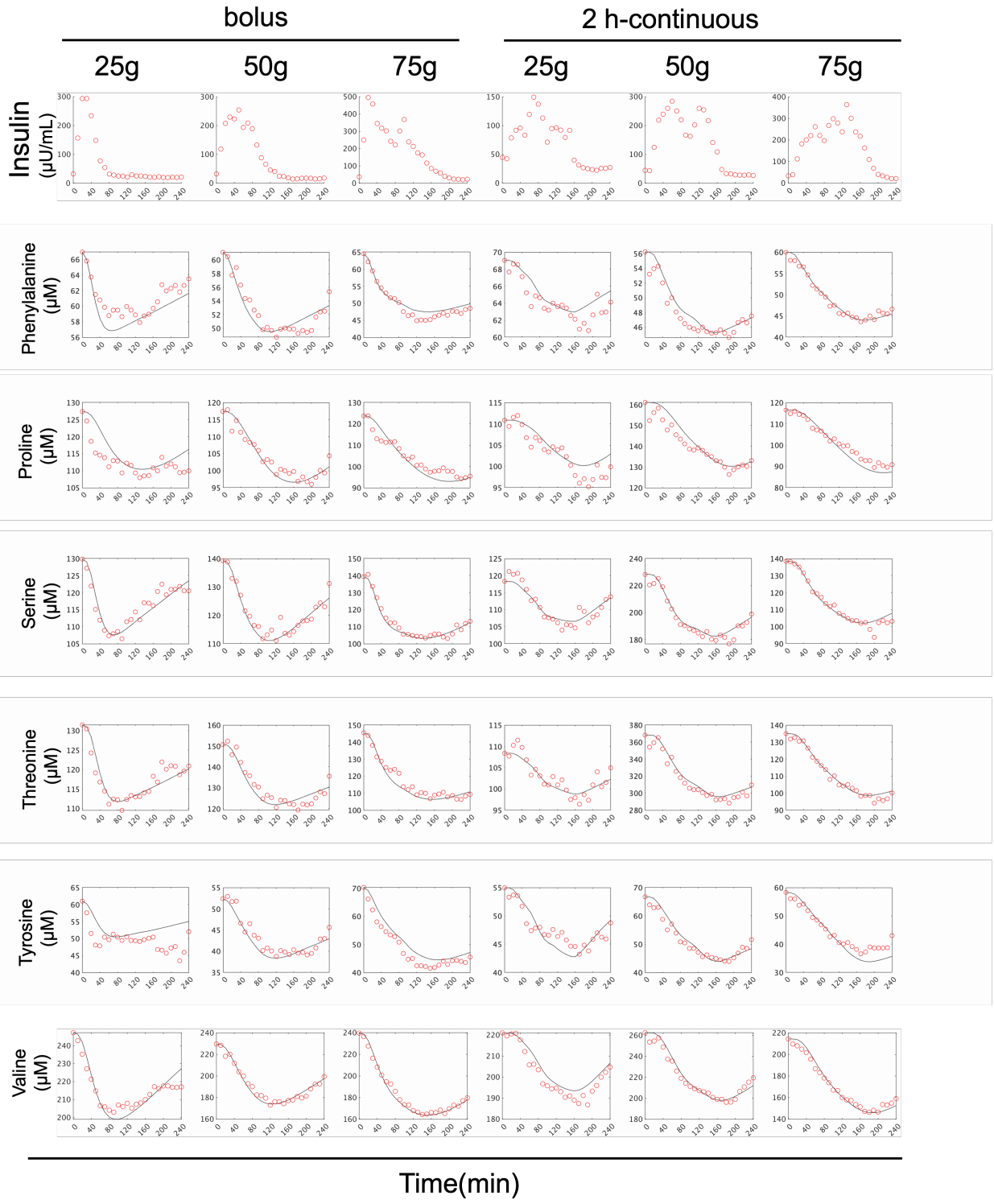


Figure. S3 Model diagram. Related to Figure 1.

FigS5


bolus
2 h-continuous
25g
50g
75g
25g
50g
75g
Insulin (μU/mL)
α-Amino-n-butyric acid (μM)
Arginine (μM)
Asparagine (μM)
Citrulline (μM)
Methionine (μM)
Ornithine (μM)
Time(min)


bolus
2 h-continuous
25g
50g
75g
25g
50g
75g
Insulin (μU/mL)
Phenylalanine (μM)
Proline (μM)
Serine (μM)
Threonine (μM)
Tyrosine (μM)
Valine (μM)
Time(min)

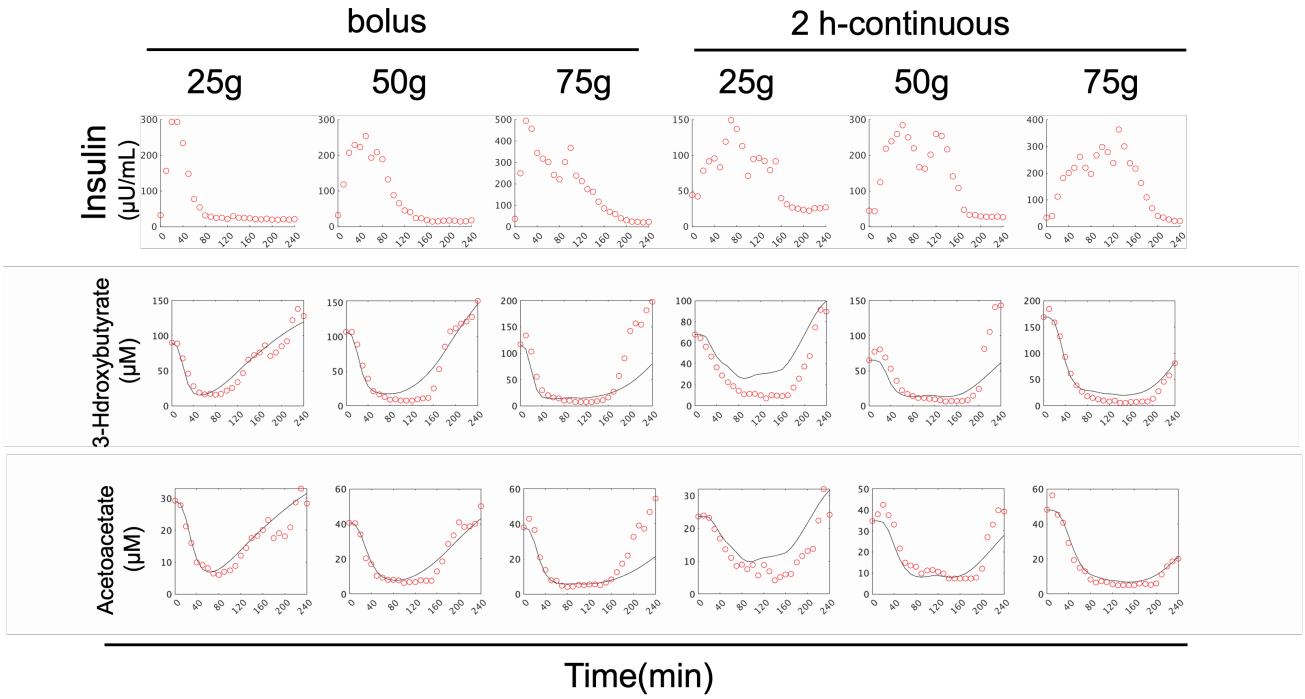
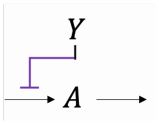
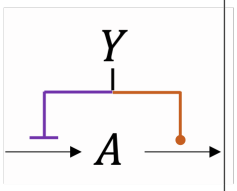


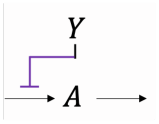
Figure. S4 Time course data on the mean values of blood insulin and blood metabolites in three individuals by glucose ingestion. Related to Figure 1.

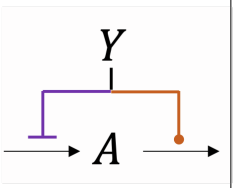
The doses and ingestion patterns are indicated at the top. The black lines indicate the temporal patterns of simulations, and the red circles indicate the time course data of experiments. The temporal patterns of the simulations were caluculated using the best fit model.

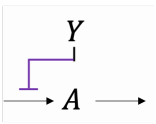
Previous knowledge

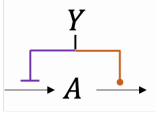






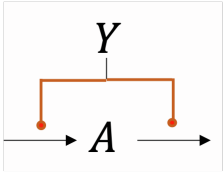


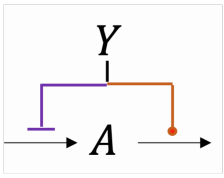


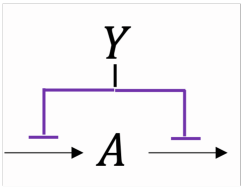


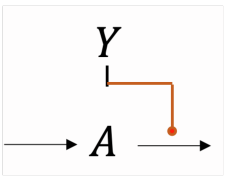
Metabolites	Model #
Ornithine *1	Model #1
Tyrosine *1	Model #1
3-Hydroxybutyrate *2,3	Model #3
Free fatty acid *4,5,6	Model #3
Acetoacetic acid *2,3	Model #3
Total ketone body *2,3	Model #3
Arginine *1	Model #4
Proline	Model #4
Isoleucine *1,7	Model #5
Leucine *1,7	Model #5
Phenylalanine *1,7	Model #5
Valine *1,7	Model #5
α-Amino-n-butyric acid	Model #5
Asparagine	Model #8
Citrulline *1	Model #8
Methionine *1	Model #8
Serine	Model #8
Threonine *8	Model #8

This study









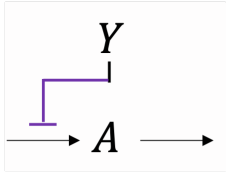


Figure. S5 The model structures of of previous knowledge and the selected model of this study. Related to Figure 2.

Metabolites, numbers of selected models, model structures selected for this study and the regulatory structure of previous knowledge $*1^{4}$, $*2^{23}$, $*3^{26}$, $*4^{40}$, $*5^{41}$, $*6^{5}$, $*7^{3}$, $*8^{19}$.

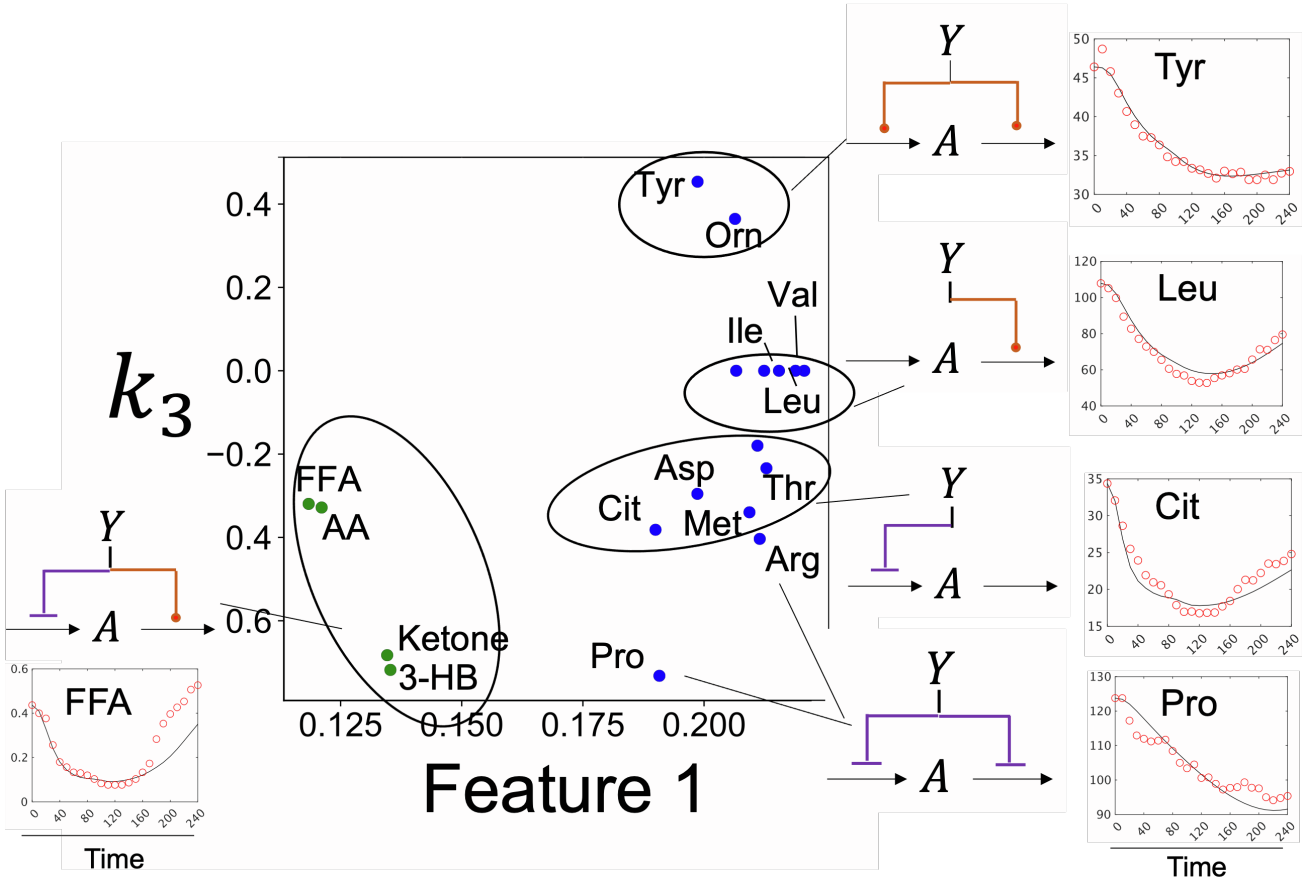
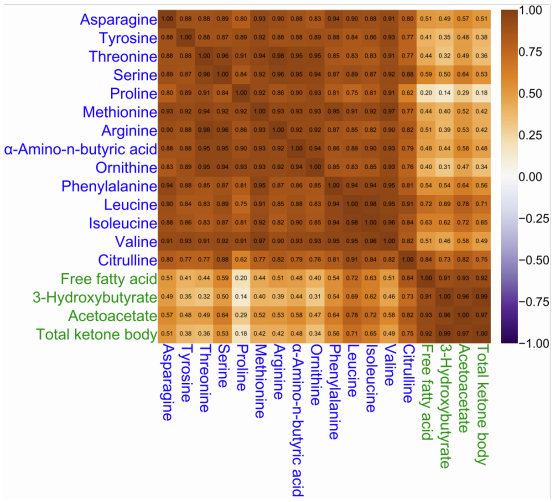

k_3
Feature 1
Tyr
Orn
Val
Ile
Leu
Asp
Thr
Cit
Met
Arg
FFA
AA
Ketone
3-HB
Pro
Y
A
Tyr
Leu
Cit
Pro
FFA
Time
Time

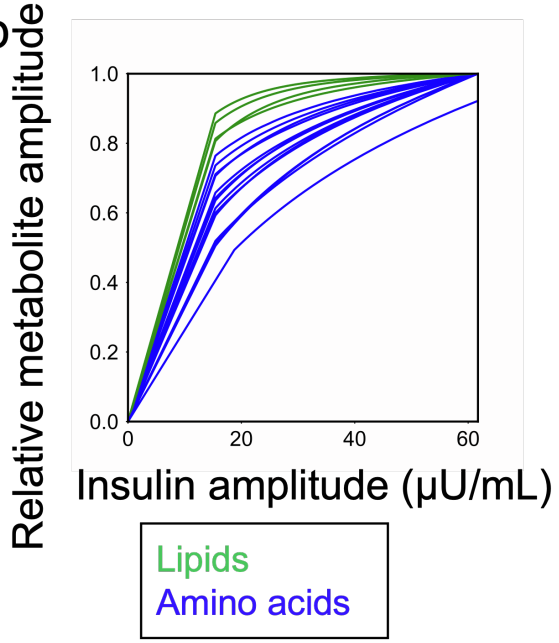
Figure. S6 The features extracted by tensor decomposition. Related to Figure 3.

a Distribution of model parameters and Feature 1 (Fig. 3b). Circles and lines represent metabolites for which the same model structure was selected (Fig. 2). Time series of experimental and simulated values for a representative molecule for each selected model are shown (75 g bolus).

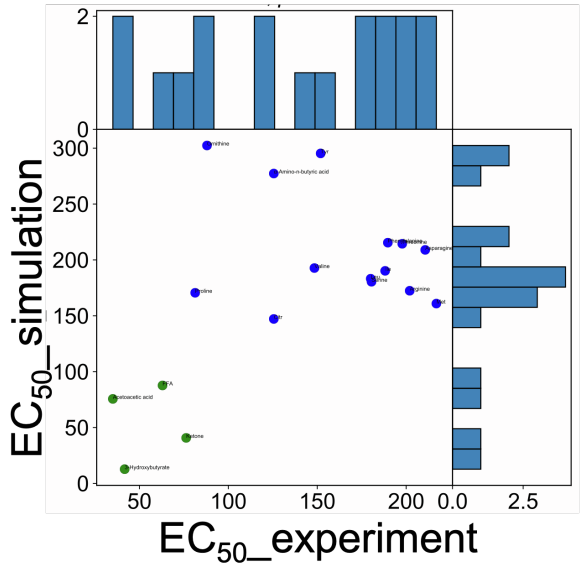
a


Asparagine
Tyrosine
Threonine
Serine
Proline
Methionine
Arginine
α-Amino-n-butyric acid
Ornithine
Phenylalanine
Leucine
Isoleucine
Valine
Citrulline
Free fatty acid
3-Hydroxybutyrate
Acetoacetate
Total ketone body
1.00
0.75
0.50
0.25
0.00
−0.25
−0.50
−0.75
−1.00

b


Relative metabolite amplitude
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
Insulin amplitude (μU/mL)
Lipids
Amino acids

c


EC50_simulation
EC50_experiment
2
0
300
250
200
150
100
50
0
50
100
150
200
0.0
2.5

d

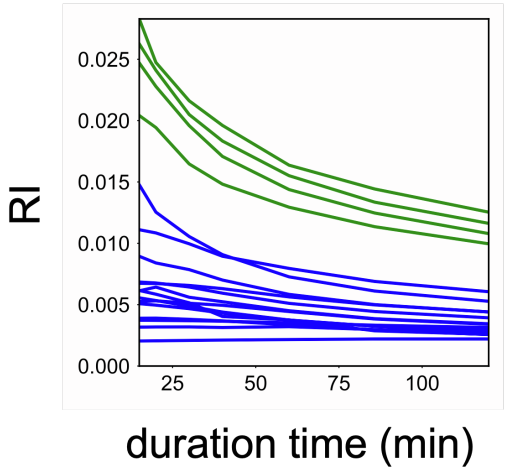

RI
0.025
0.020
0.015
0.010
0.005
0.000
25
50
75
100
duration time (min)

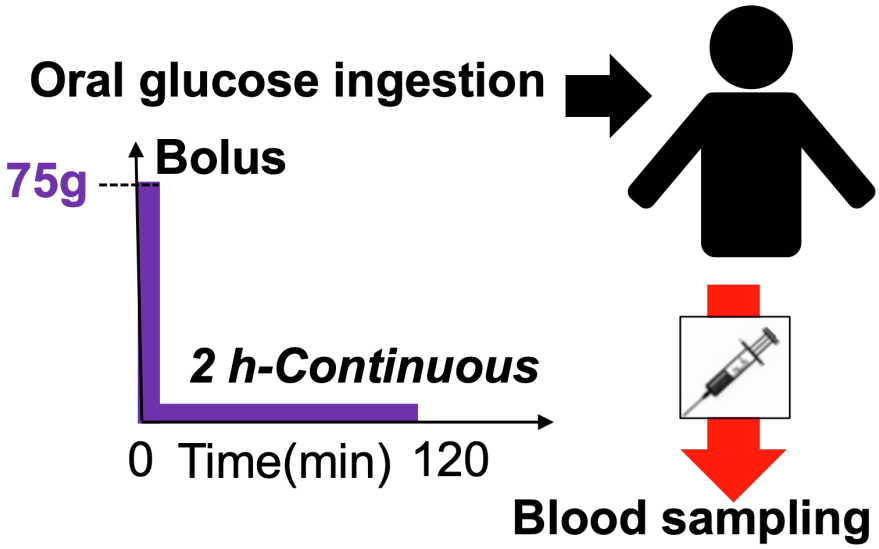
Figure. S7 The regulation of the amplitude of amino acids and lipids by amplitude of insulin. Related to Figure 4.

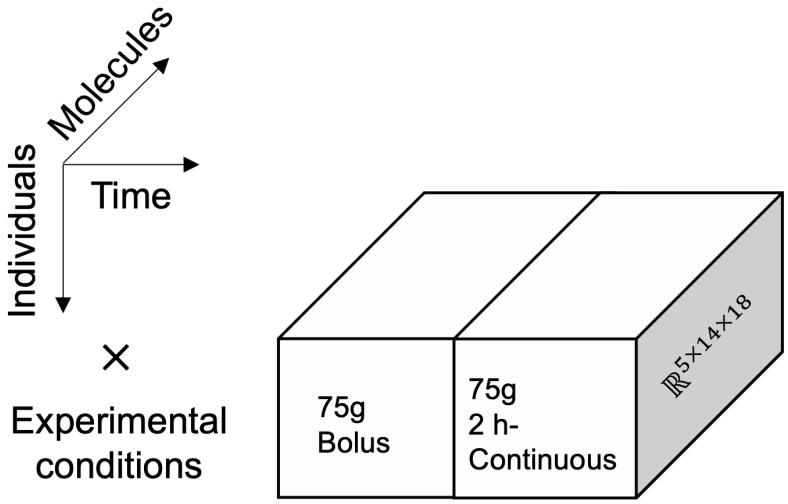
a Heat map showing the temporal pattern similarity among metabolites among all metabolites(see Methods). The color of the letters indicates metabolic group (blue: amino acids, green: lipids)

b The peaks of all metabolite against the amplitude concentration of insulin in the simulation. The color of the line indicates the metabolic group.

c Distributions of experimental EC_{50} value and simulated EC_{50} value of metabolite. As with the simulated EC_{50} value, nonlinear fitting was performed to calculate the experimental EC_{50} value using the maximum value of insulin in the time series and the log2 Fold change relative to the metabolite fasting value.

d RTs for the metabolite against the duration time(inverse of rate of response) of insulin.

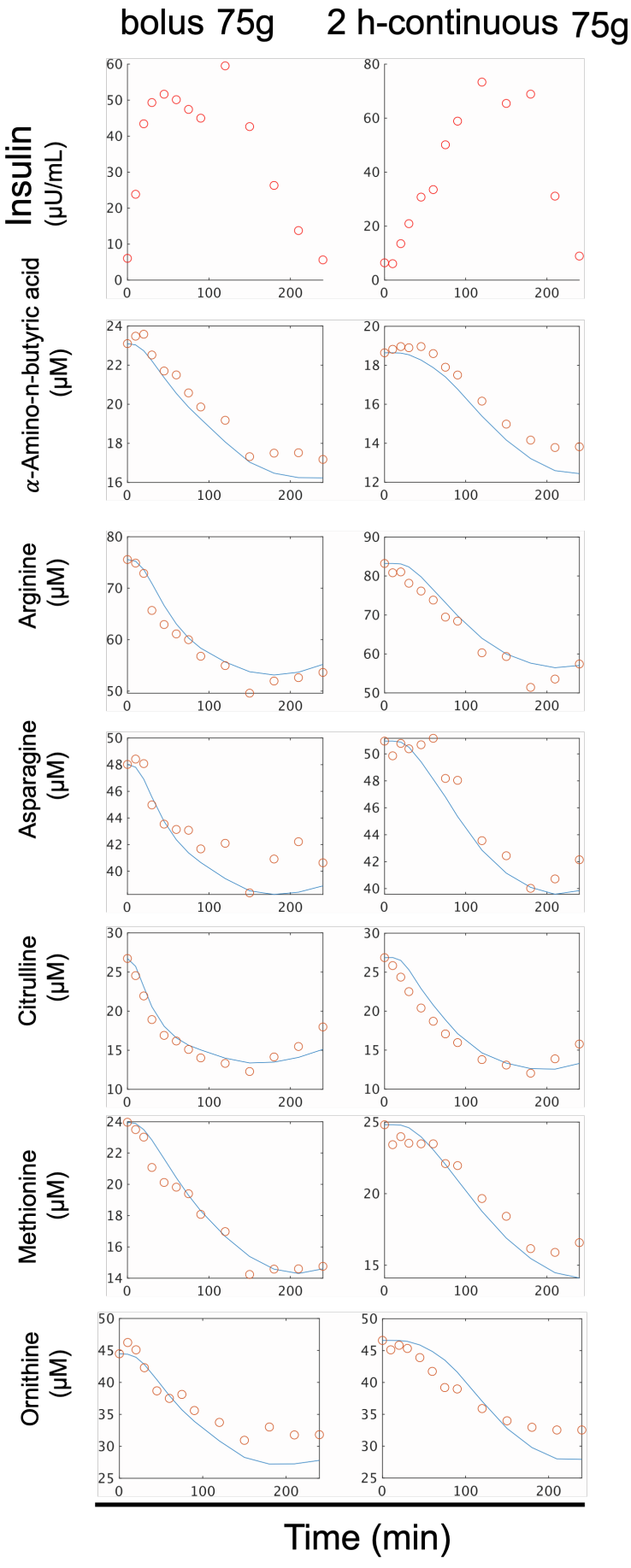


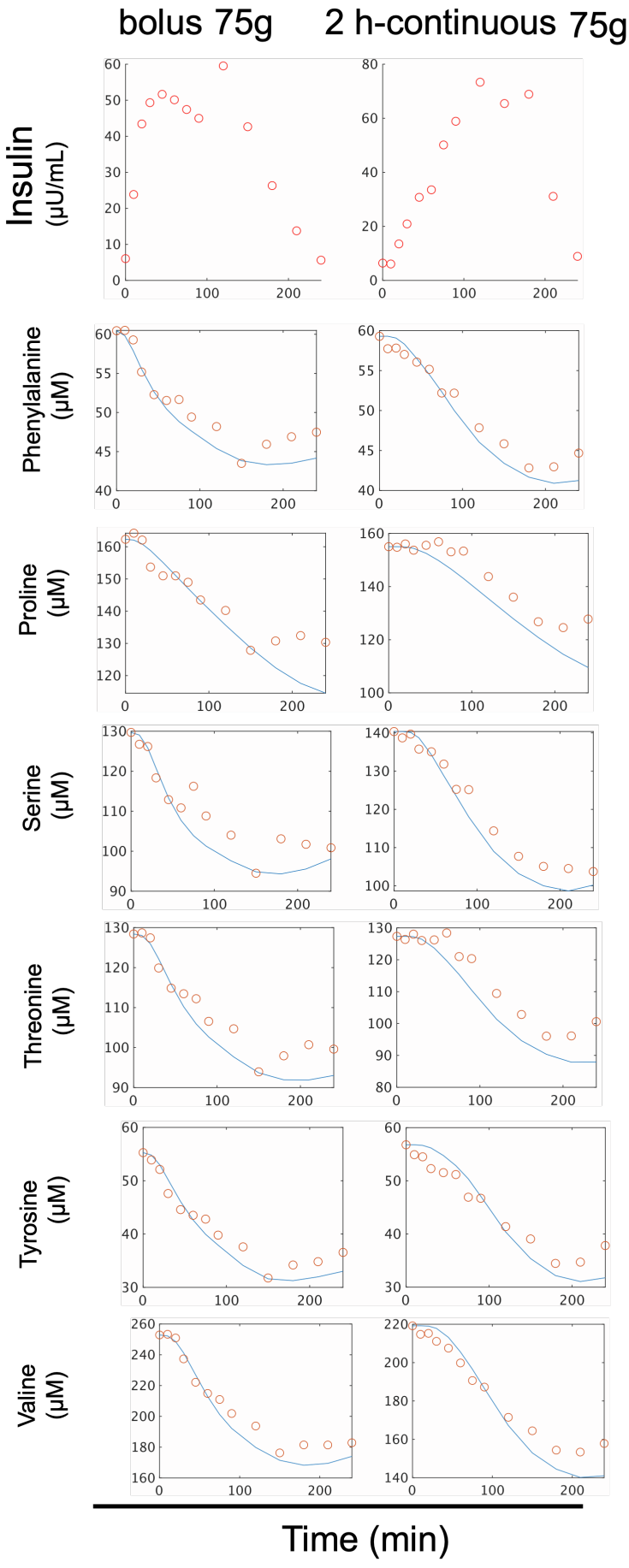


Data structure

Figure. S8 Data set for model validation. Related to Figure 6.

Experimental dataset for validation. Five individuals orally ingested glucose with three doses 75g in two durations of bolus and 2 h continuous ingestion. The data structure has four axes: individual × time × experimental condition × molecule. The data represent the concentration changes at 14 time points (-5, 0, 10, 20, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240 minutes) from 5 min before fasting to 240 min after glucose ingestion for 20 molecules in five healthy subjects, in two different experimental conditions.


bolus 75g
2 h-continuous 75g
Insulin (μU/mL)
α-Amino-n-butyric acid (μM)
Arginine (μM)
Asparagine (μM)
Citrulline (μM)
Methionine (μM)
Ornithine (μM)
Time (min)


bolus 75g
2 h-continuous 75g
Insulin (μU/mL)
Phenylalanine (μM)
Proline (μM)
Serine (μM)
Threonine (μM)
Tyrosine (μM)
Valine (μM)
Time (min)

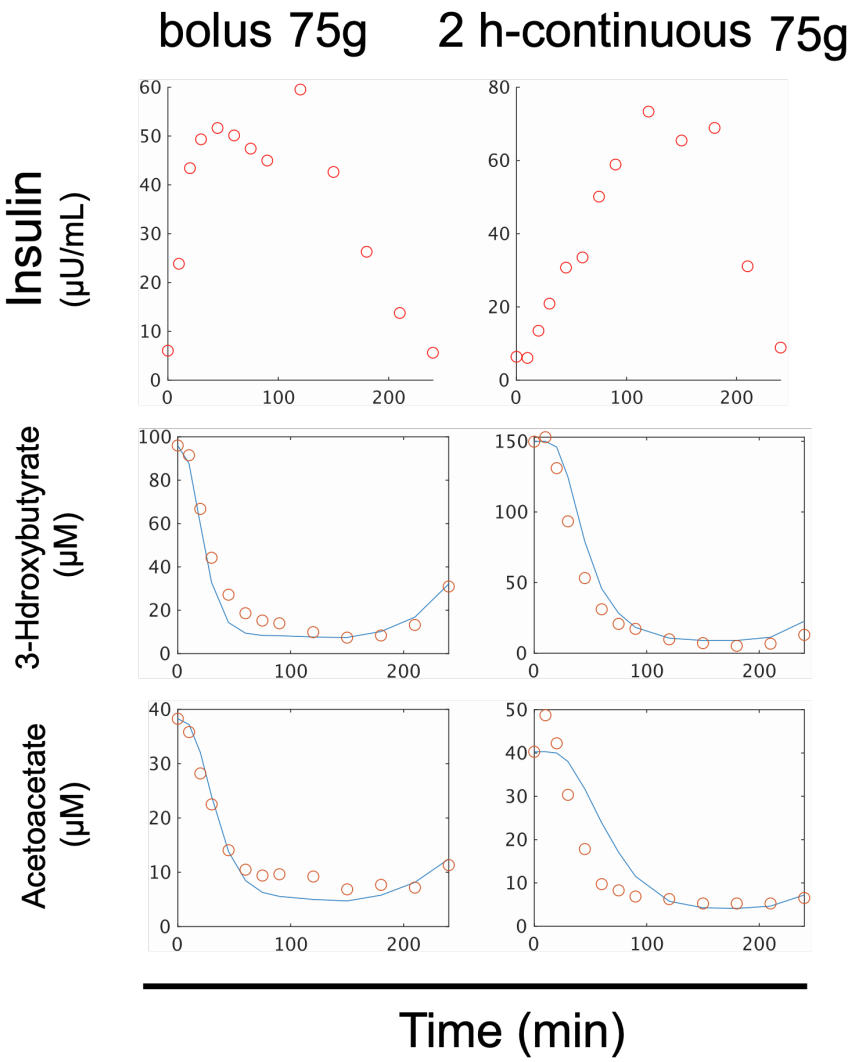
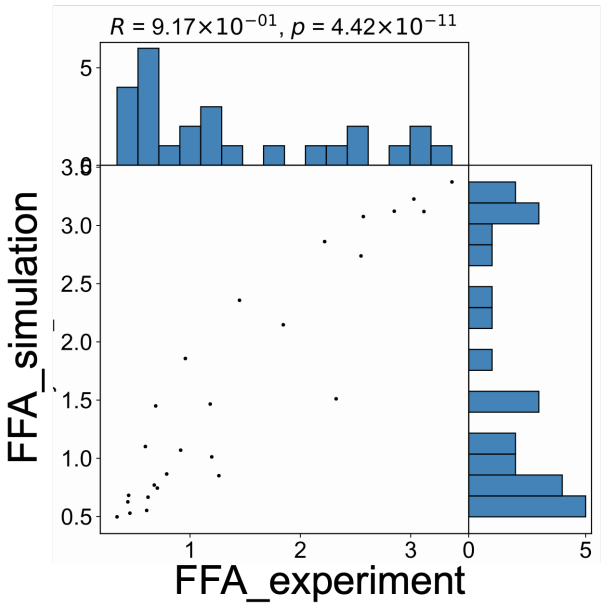
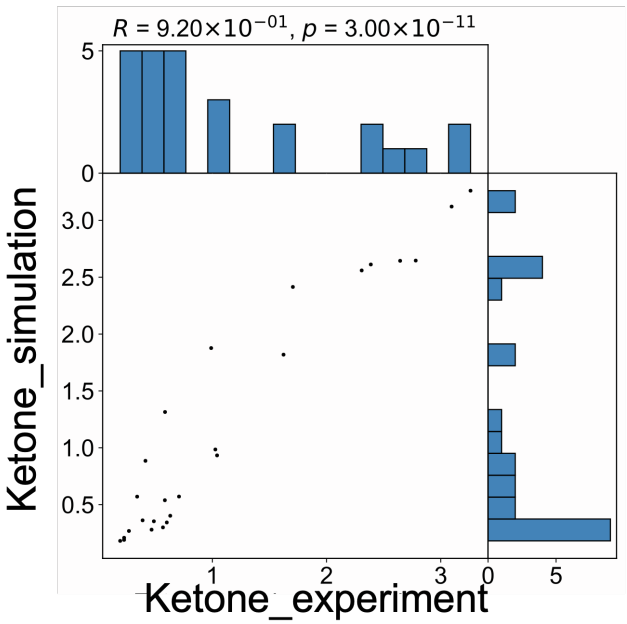
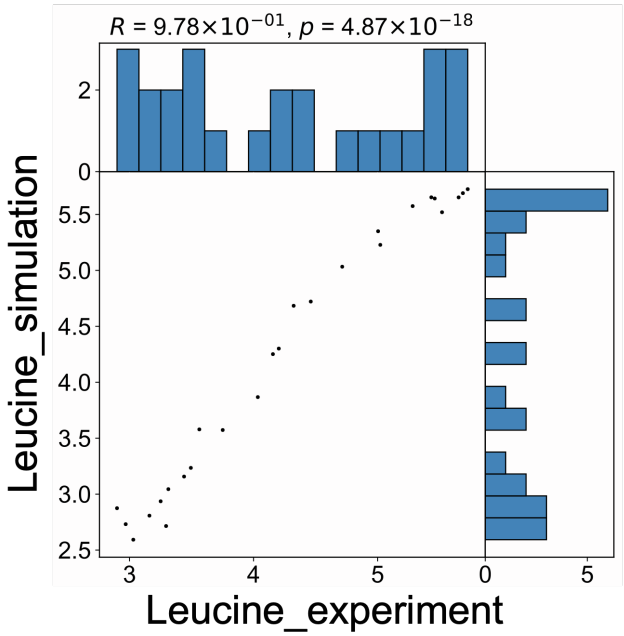

bolus 75g
2 h-continuous 75g
Insulin
(μU/mL)
3-Hdroxybutyrate
(μM)
Acetoacetate
(μM)
Time (min)

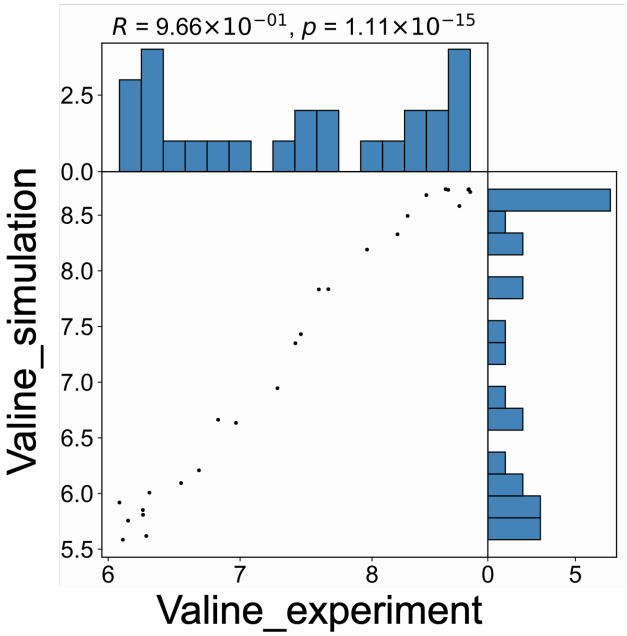
Figure. S9 Time course data on the mean values of blood insulin and blood metabolites in five individuals by glucose ingestion for model validation. Related to Figure 6.

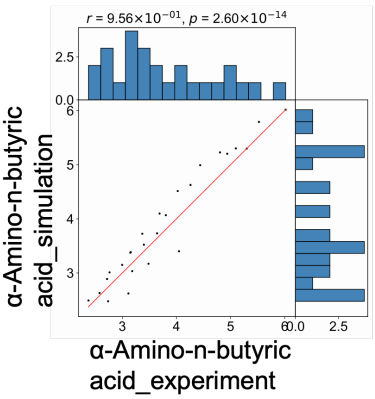
The dose and ingestion pattern are indicated at the top. The bluelines indicate the temporal patterns of simulations, and the red circles indicate the time course data of experiments.

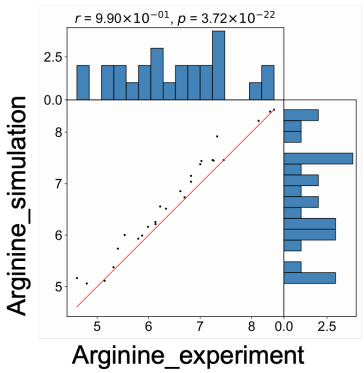

$R = 9.17\times10^{-01}$, $p = 4.42\times10^{-11}$
FFA_simulation
FFA_experiment

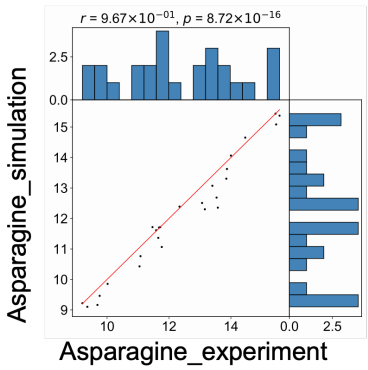

$R = 9.20\times10^{-01}$, $p = 3.00\times10^{-11}$
Ketone_simulation
Ketone_experiment

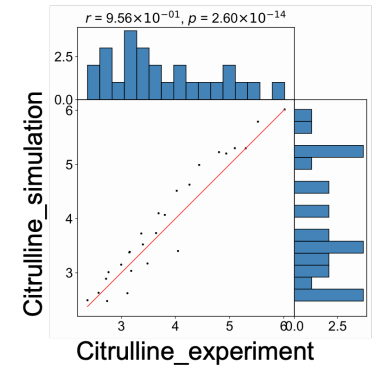

$R = 9.78\times10^{-01}$, $p = 4.87\times10^{-18}$
Leucine_simulation
Leucine_experiment

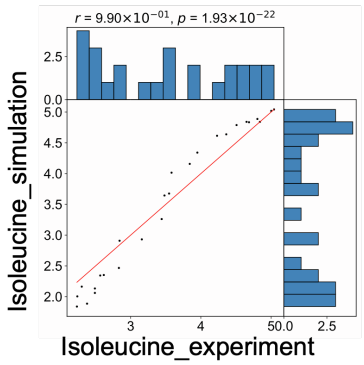

$R = 9.66\times10^{-01}$, $p = 1.11\times10^{-15}$
Valine_simulation
Valine_experiment

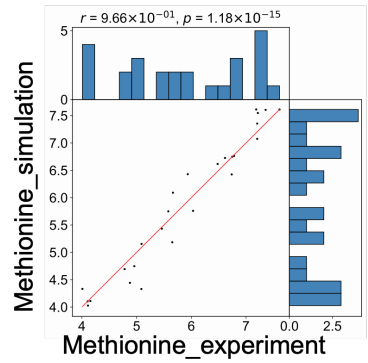

r = 9.56×10−01, p = 2.60×10−14
α-Amino-n-butyric acid_simulation
α-Amino-n-butyric acid_experiment

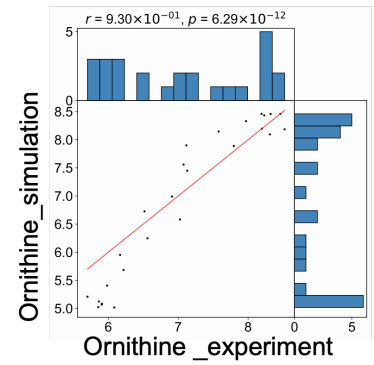

r = 9.90×10−01, p = 3.72×10−22
Arginine_simulation
Arginine_experiment

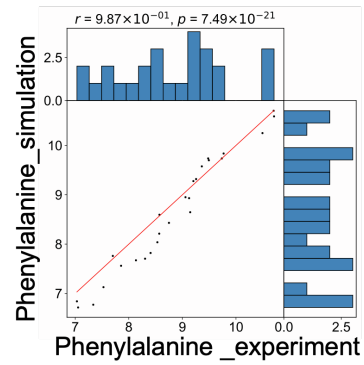

r = 9.67×10−01, p = 8.72×10−16
Asparagine_simulation
Asparagine_experiment

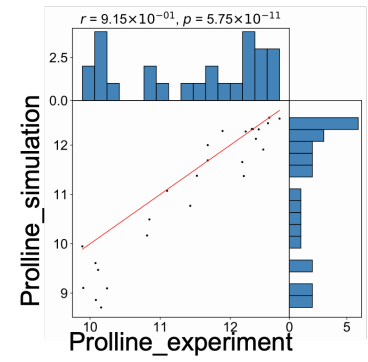

r = 9.56×10−01, p = 2.60×10−14
Citrulline_simulation
Citrulline_experiment

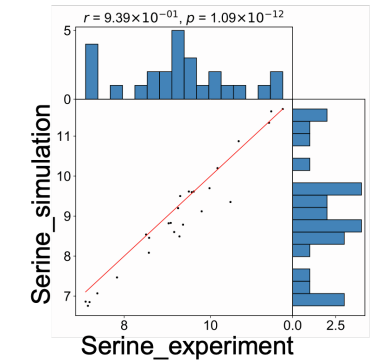

r = 9.90×10−01, p = 1.93×10−22
Isoleucine_simulation
Isoleucine_experiment

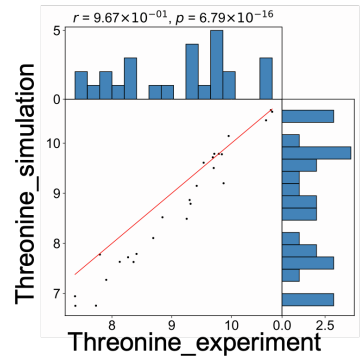

r = 9.66×10−01, p = 1.18×10−15
Methionine_simulation
Methionine_experiment

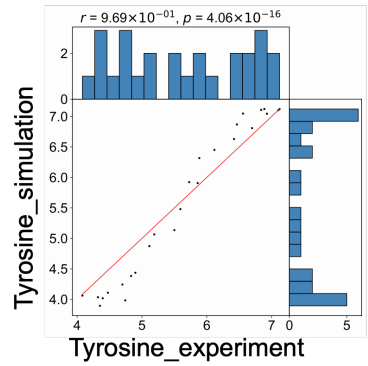

r = 9.30×10−01, p = 6.29×10−12
Ornithine_simulation
Ornithine _experiment

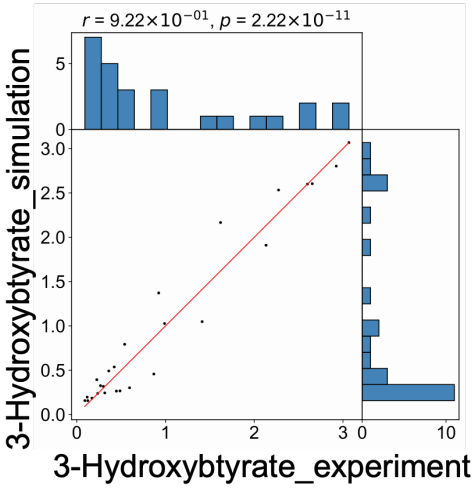

r = 9.87×10−01, p = 7.49×10−21
Phenylalanine_simulation
Phenylalanine _experiment


r = 9.15×10−01, p = 5.75×10−11
Prolline_simulation
Prolline_experiment


r = 9.39×10−01, p = 1.09×10−12
Serine_simulation
Serine_experiment


r = 9.67×10−01, p = 6.79×10−16
Threonine_simulation
Threonine_experiment


r = 9.69×10−01, p = 4.06×10−16
Tyrosine_simulation
Tyrosine_experiment


r = 9.22×10−01, p = 2.22×10−11
3-Hydroxybtyrate_simulation
3-Hydroxybtyrate_experiment

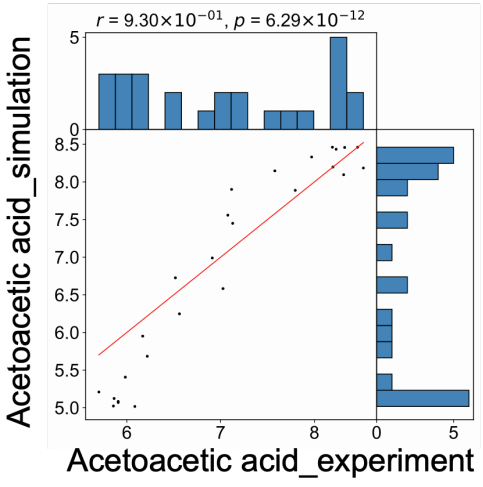

r = 9.30×10−01, p = 6.29×10−12
Acetoacetic acid_simulation
Acetoacetic acid_experiment

Figure. S10 Distributions of experimental value and simulated value of metabolite. Related to Figure 6.

In the upper right, *r* and *p* indicate the correlation coefficient and *p* value, respectively. The red line indicates y=x. Experimental and simulated values are shown normalized by the standard deviation for each experimental condition.

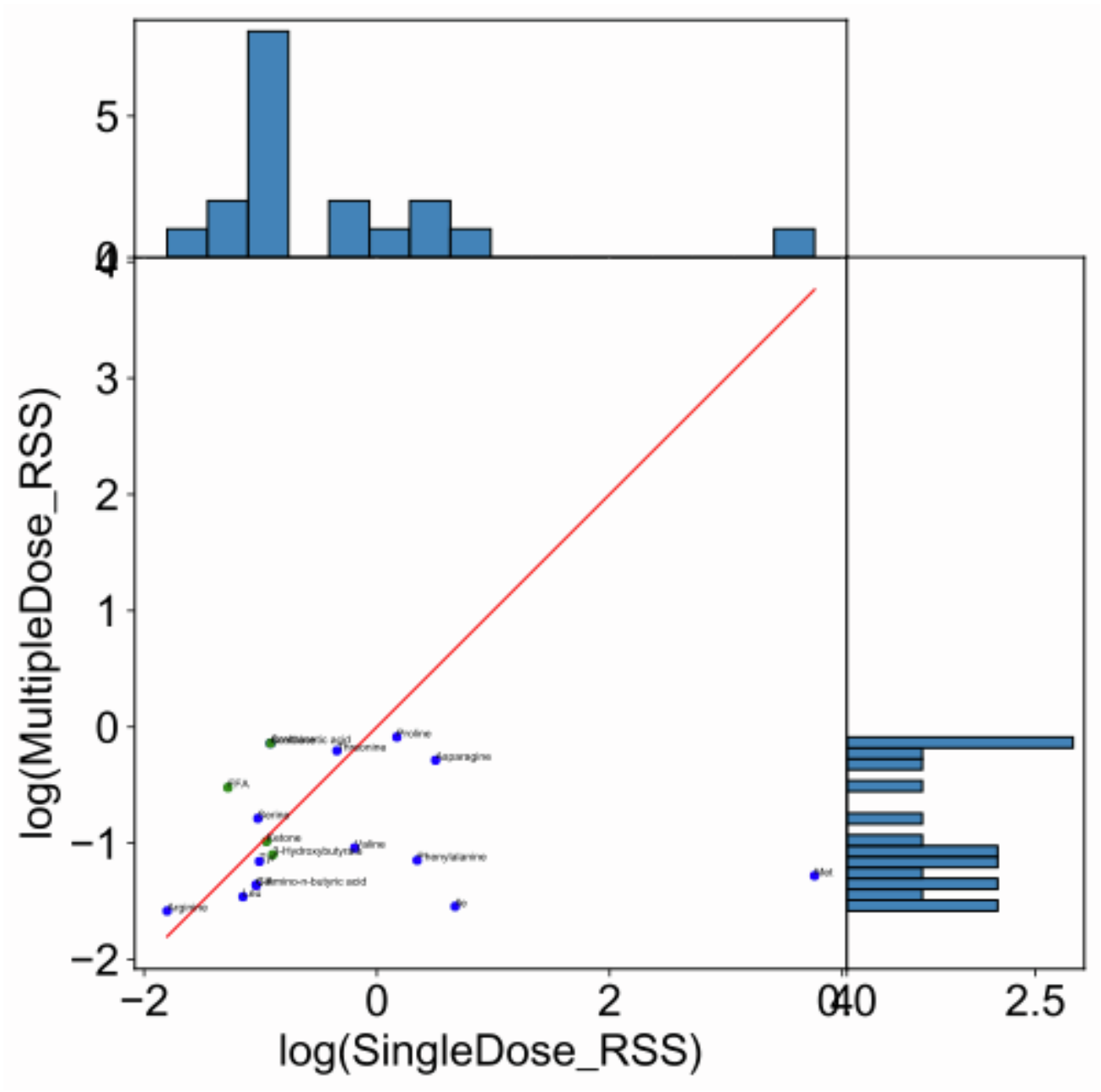

log(MultipleDose_RSS)
log(SingleDose_RSS)
5
4
3
2
1
0
−1
−2
−2
0
2
4
2.5
Proline
Asparagine
PFA
Phenylalanine

Figure. S11 Influence of learning data on prediction. Related to Figure 1.

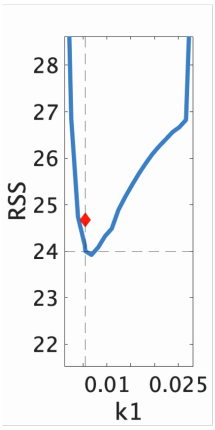
The rss between simulations with parameters estimated from experimental data for 75 g, 50 g, and 25 g bolus ingestion and 2 h-continuous ingestion (all experiments, y axis) and 75 g bolus ingestion only (75 g bolus only, x axis), and experimental values for all metabolites.The red line indicates y=x. The dot's colors correspond to the metabolic group (blue: amino acids, green: lipids).

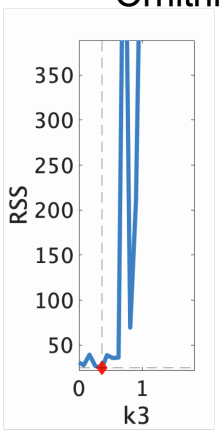
Metabolites	Model # (Population-averaged)	**Subject #1**	**Subject #2**	**Subject #3**
Ornithine	Model #1	Model #3	Model #8	Model #5
Tyrosine	Model #1	Model #8	Model #3	Model #1
3-Hydroxybutyrate	Model #3	Model #3	Model #3	Model #3
Free fatty acid	Model #3	Model #3	Model #3	Model #3
Acetoacetic acid	Model #3	Model #3	Model #3	Model #3
Total ketone body	Model #3	Model #3	Model #3	Model #3
Arginine	Model #4	Model #4	Model #5	Model #1
Proline	Model #4	Model #4	Model #5	Model #4
Isoleucine	Model #5	Model #8	Model #5	Model #5
Leucine	Model #5	Model #8	Model #5	Model #5
Phenylalanine	Model #5	Model #4	Model #5	Model #1
Valine	Model #5	Model #5	Model #5	Model #5
α-Amino-n-butyric acid	Model #5	Model #3	Model #8	Model #3
Asparagine	Model #8	Model #8	Model #5	Model #5
Citrulline	Model #8	Model #1	Model #8	Model #8
Methionine	Model #8	Model #4	Model #3	Model #8
Serine	Model #8	Model #4	Model #8	Model #1
Threonine	Model #8	Model #4	Model #5	Model #8

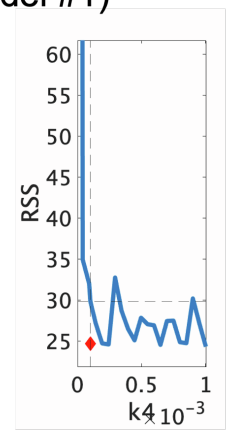
Figure. S12 The selected for each subject. Related to Figure 1.

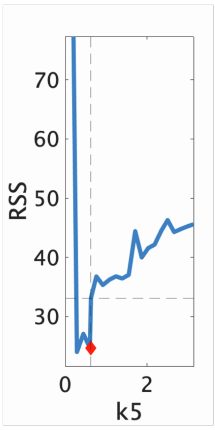
Metabolites, numbers of selected models, model structures selected for each subject.

Ornithine (Model #1)

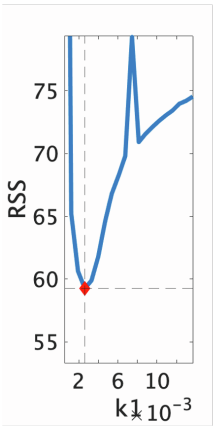


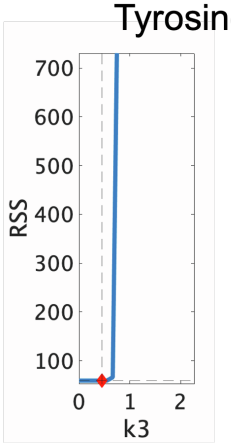


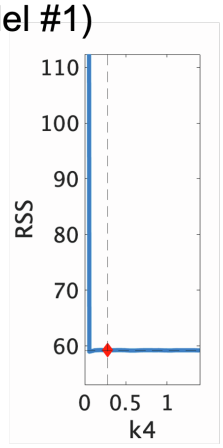


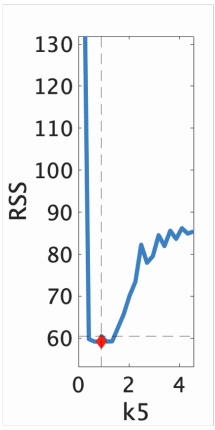


Tyrosine (Model #1)

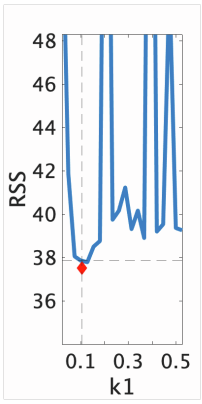


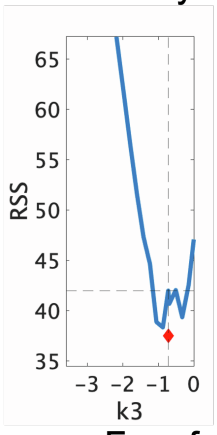


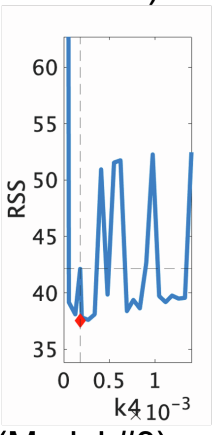


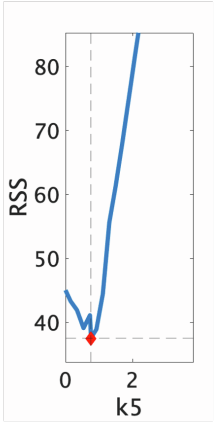


3-Hdroxybutyrate (Model #3)

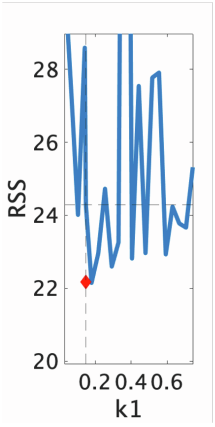


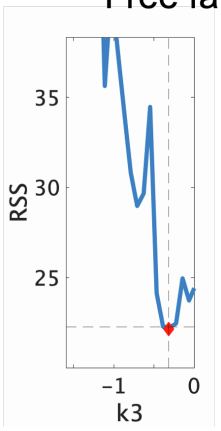


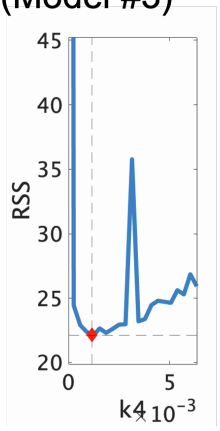


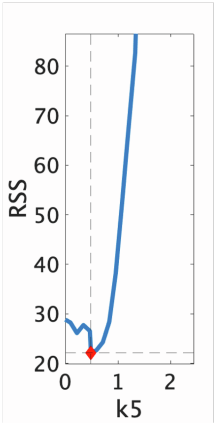


Free fatty acid (Model #3)

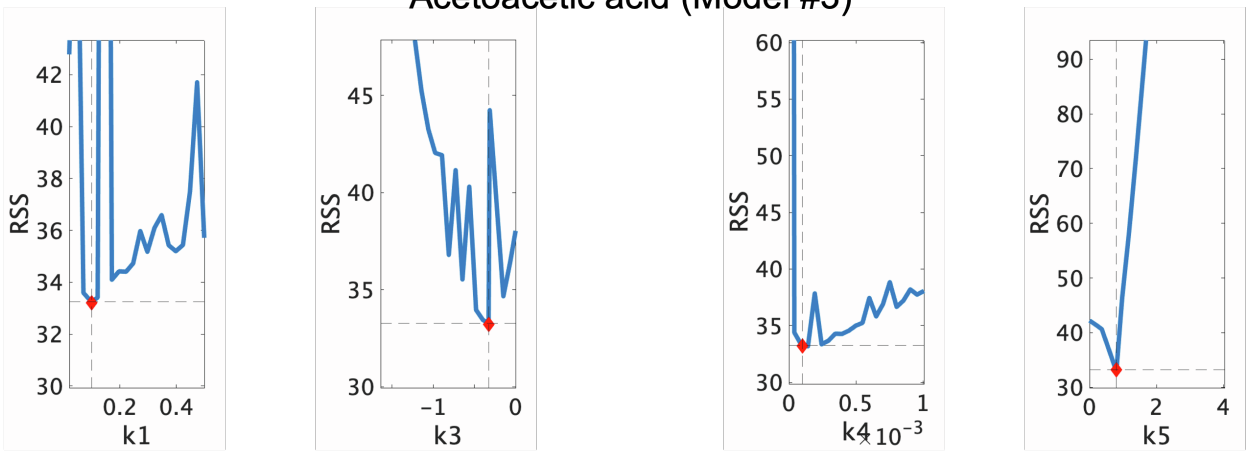




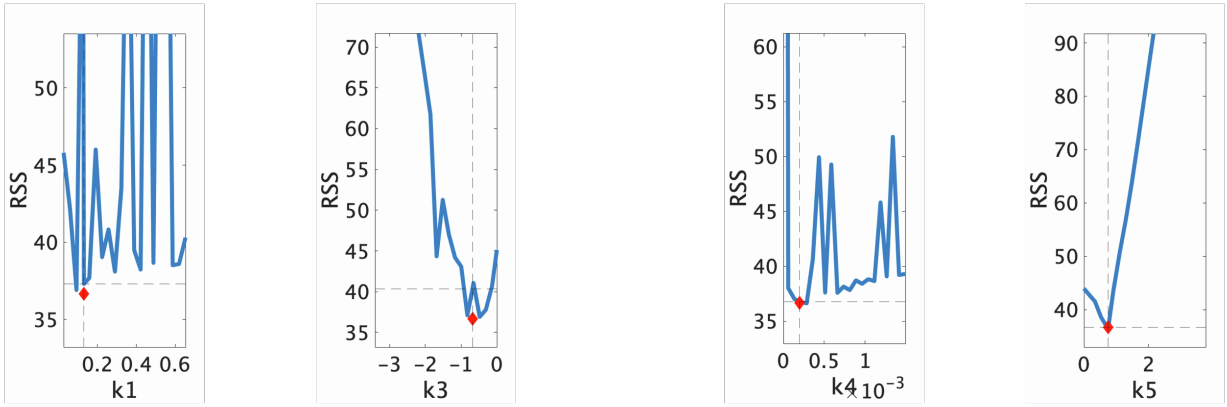




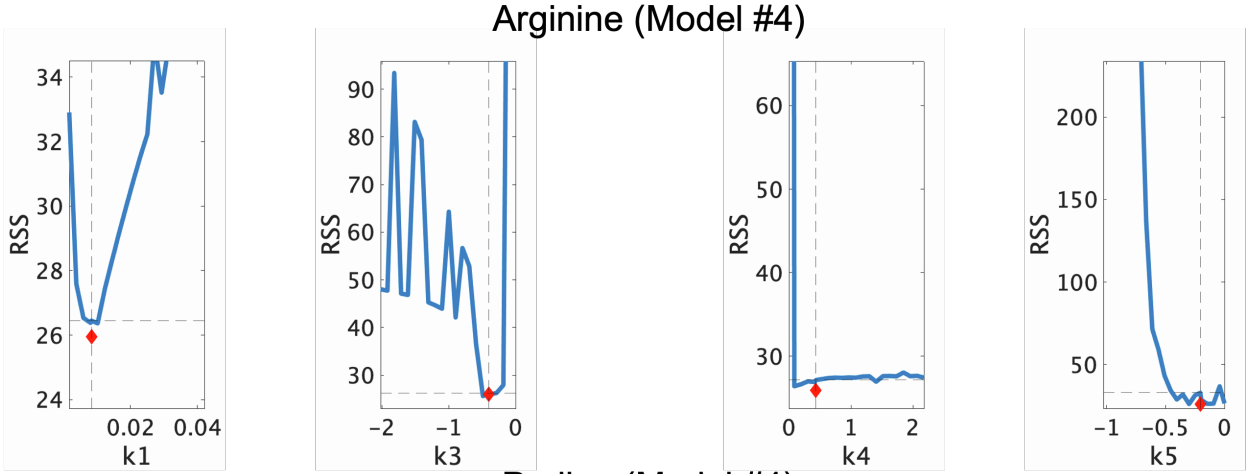
Acetoacetic acid (Model #3)



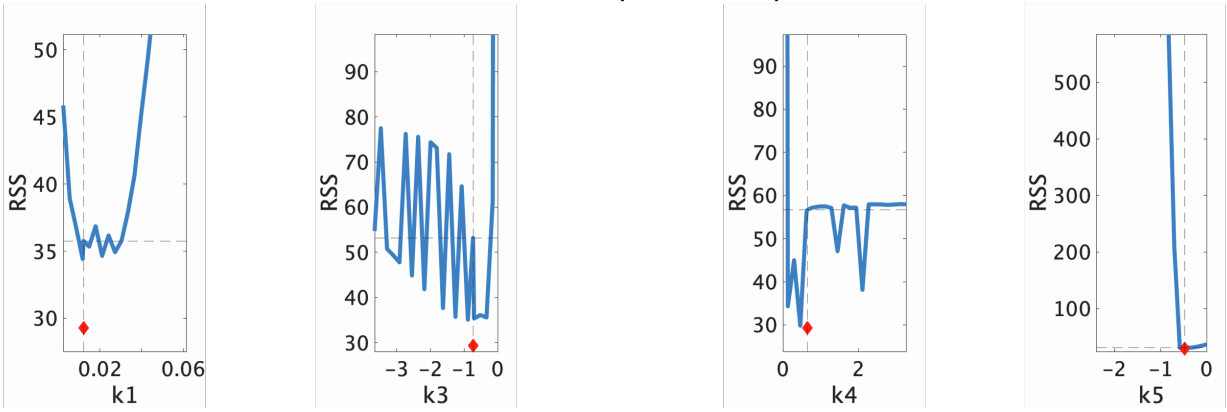
Total ketone body (Model #3)



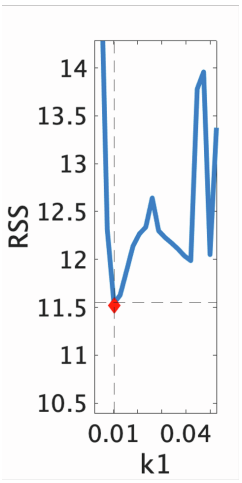
Arginine (Model #4)

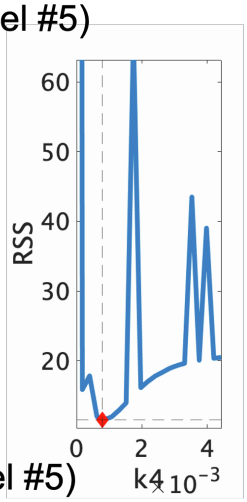


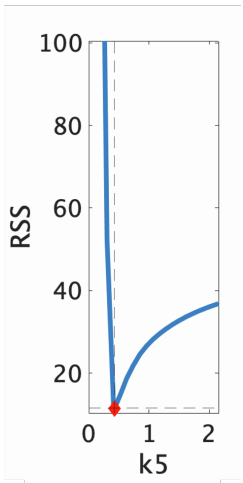
Proline (Model #4)



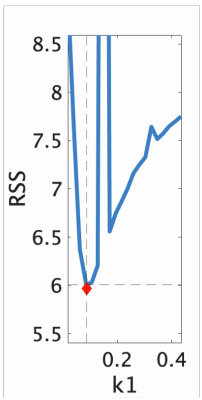
Isoleucine (Model #5)

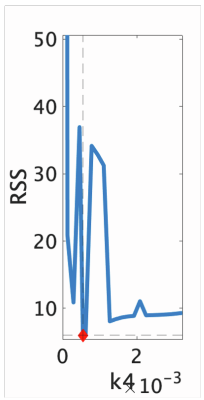


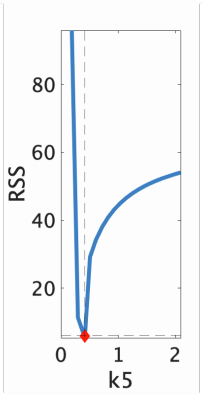




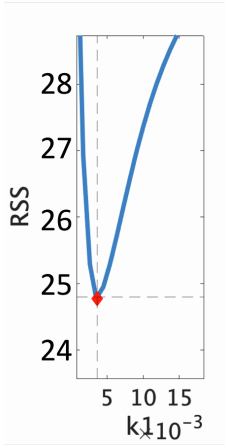
Leucine (Model #5)

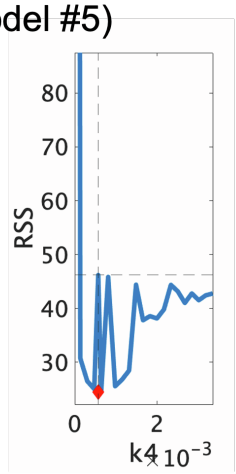


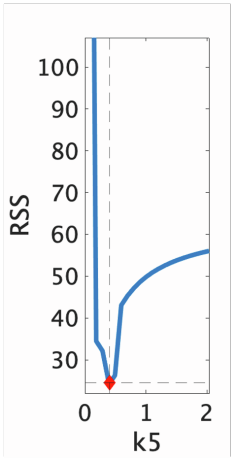




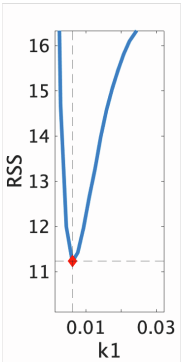
Phenylalanine (Model #5)

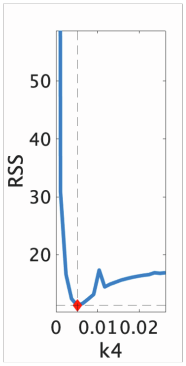


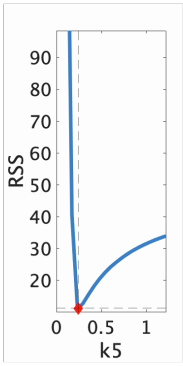




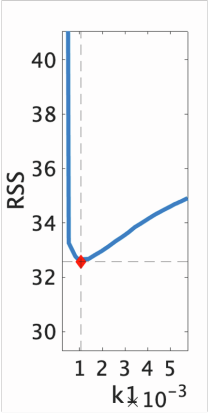
Valine (Model #5)

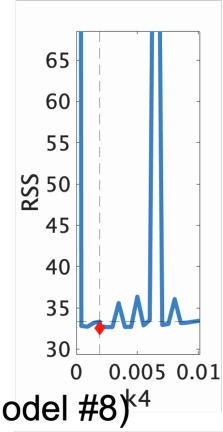


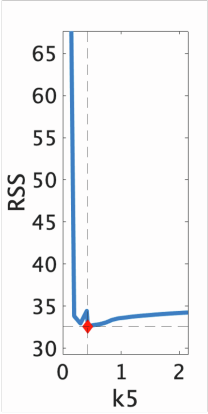




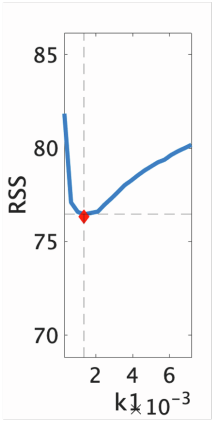
α-Amino-n-butyric acid (Model #5)

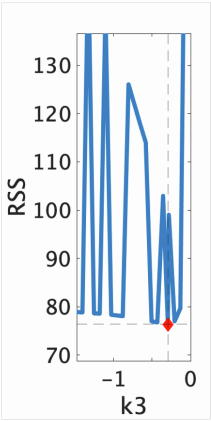


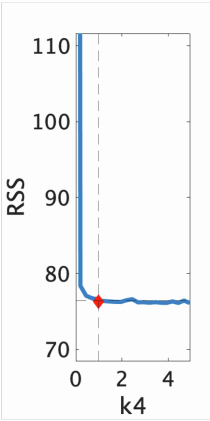




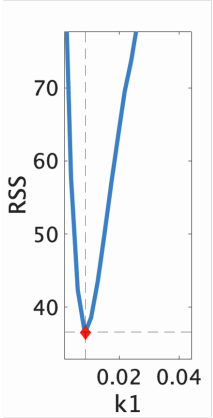
Asparagine (Model #8)

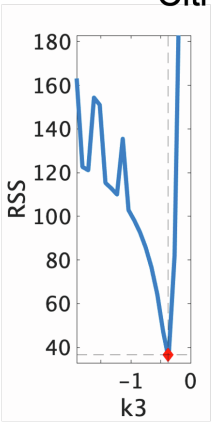


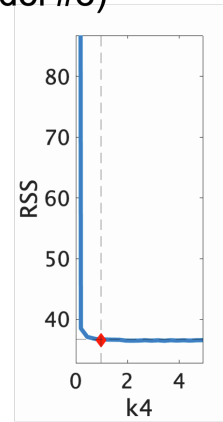




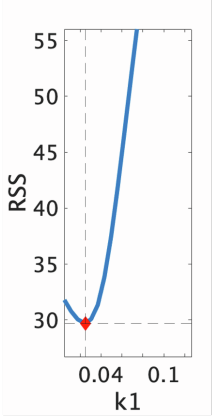
Citrulline (Model #8)

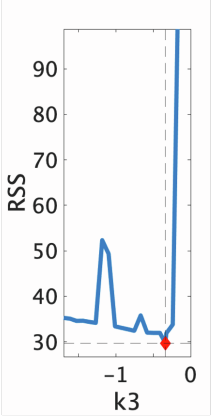


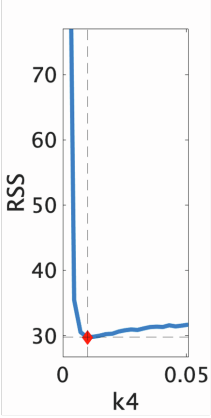




Methionine (Model #8)







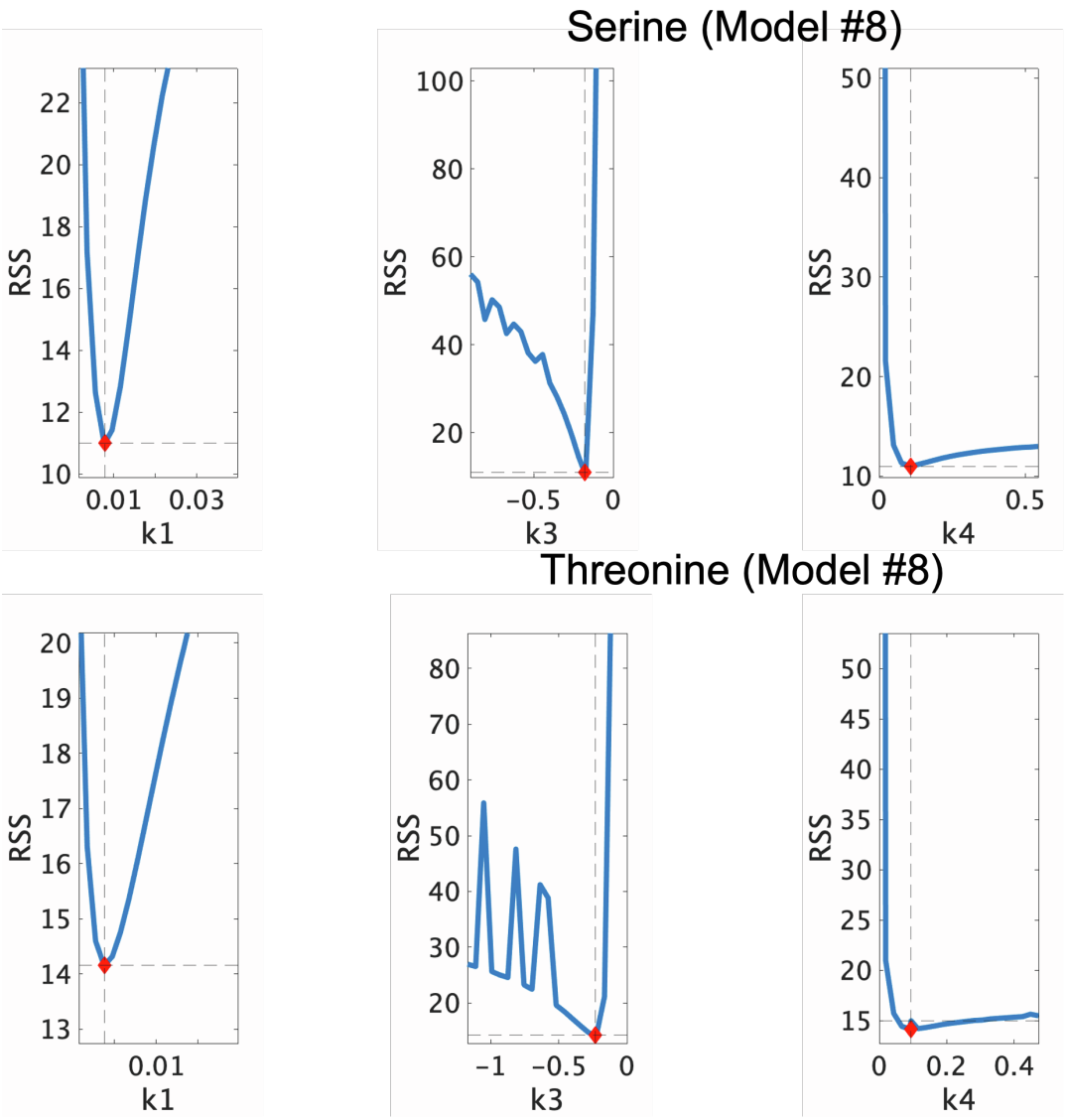
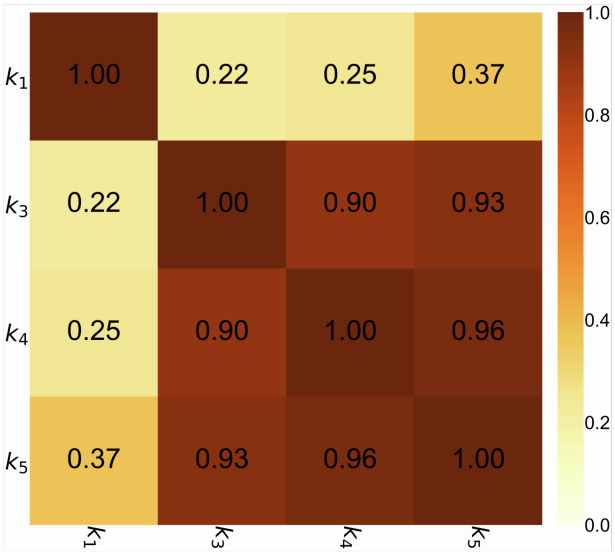


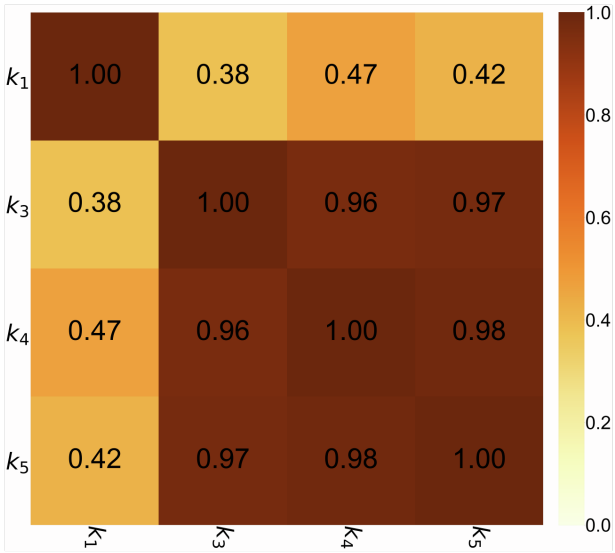
Figure. S13 Identifiability of the parameters in the model structures selected for each metabolite. Related to Figure 1 and Figure 2.

The red star indicates the RSS of the model fitted using the optimal parameter values estimated from the data, and the blue line indicates to the RSS when other parameter values are re-estimated after iterative adjustment of the parameter values. The dashed line represents the RSS when re-estimated using the same values as the optimal parameter values. Parentheses denotes the selected model.

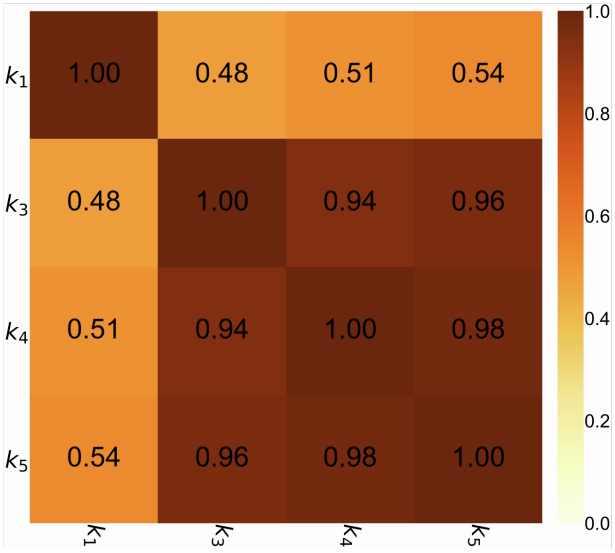
3-Hdroxybutyrate (Model #3)



Acetoacetic acid (Model #3)



Free fatty acid (Model #3)



Total ketone body (Model #3)

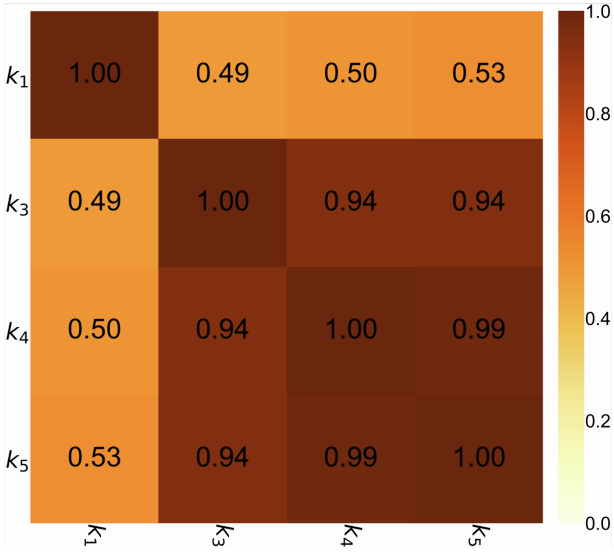
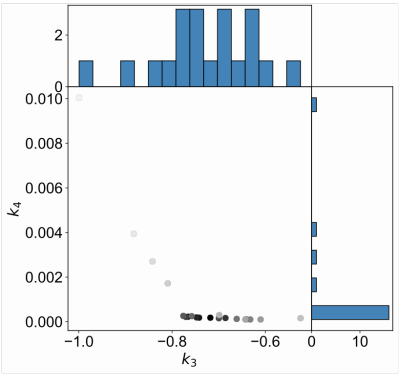
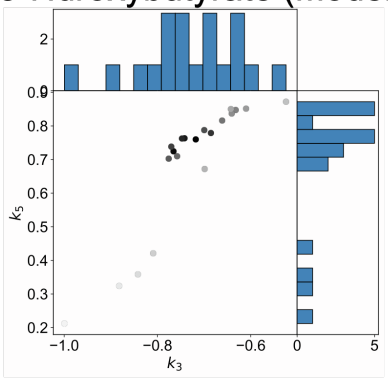


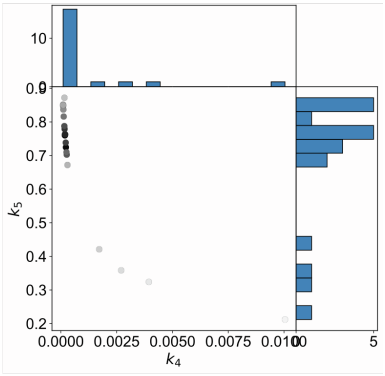
Figure. S14 Correlation among the parameters for lipids. Related to Figure 1 and Figure 2.

Correlation among the estimate parameters for selected model for lipids. The darker the color, the higher the correlation.

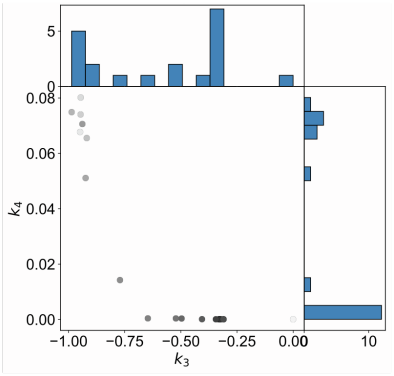
3-Hdroxybutyrate (Model #3)

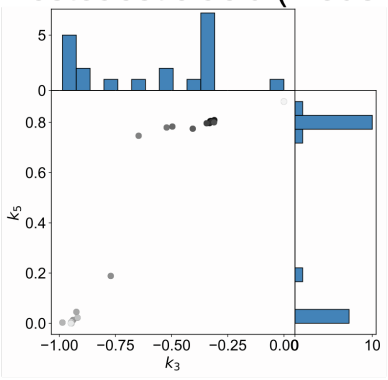


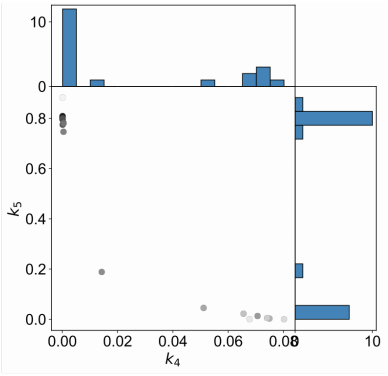




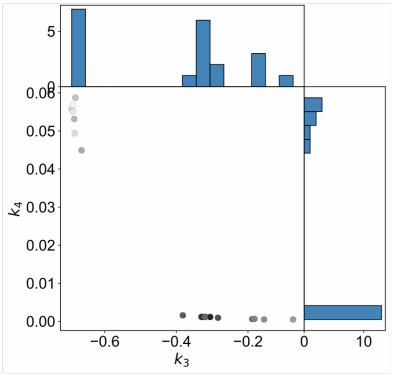
Acetoacetic acid (Model #3)

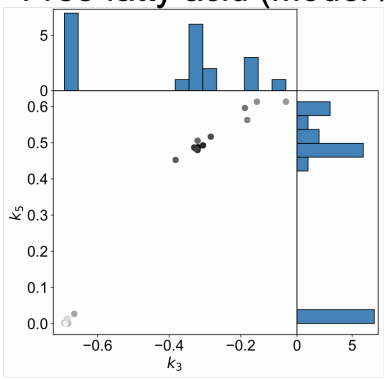


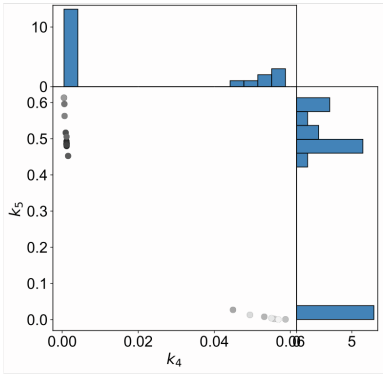




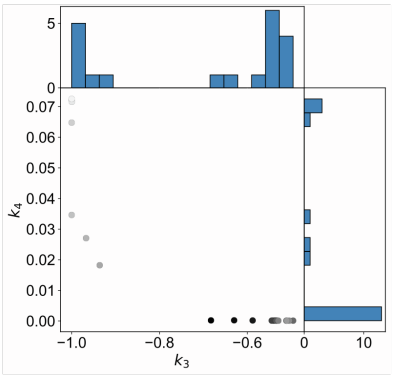
Free fatty acid (Model #3)

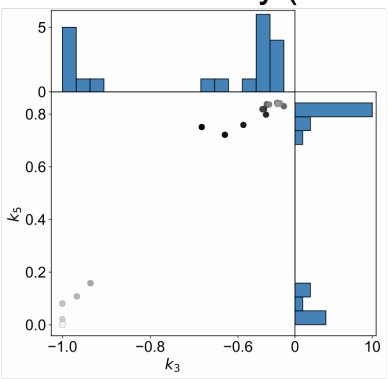






Total ketone body (Model #3)





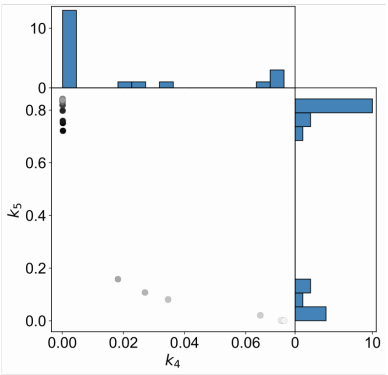


Figure. S15 Distribution of highly correlated parameter pairs for lipids. Related to Figure 1 and Figure 2.

The darker the color of the dots, the lower the RSS.