대사체학을 이용한 당뇨합병증 마커 발굴

Prediction of diabetes complications based on metabolomics

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Contents

- **Metabolomics: an introduction**

- **Plasma Metabolomics in patient with type 2 diabetes mellitus**
    - **diabetic retinopathy**
    - **diabetic macular edema**
- **Hepatic metabolomic and lipidomic analysis of obese Type 2 diabetes in a rat model: Drug mechanism**
- **Oxylipins**

DNA?? Phenotype?? → Metabolomics

DNA? (The music of life)

- Twins

- Ginseng *vs* Wild ginseng

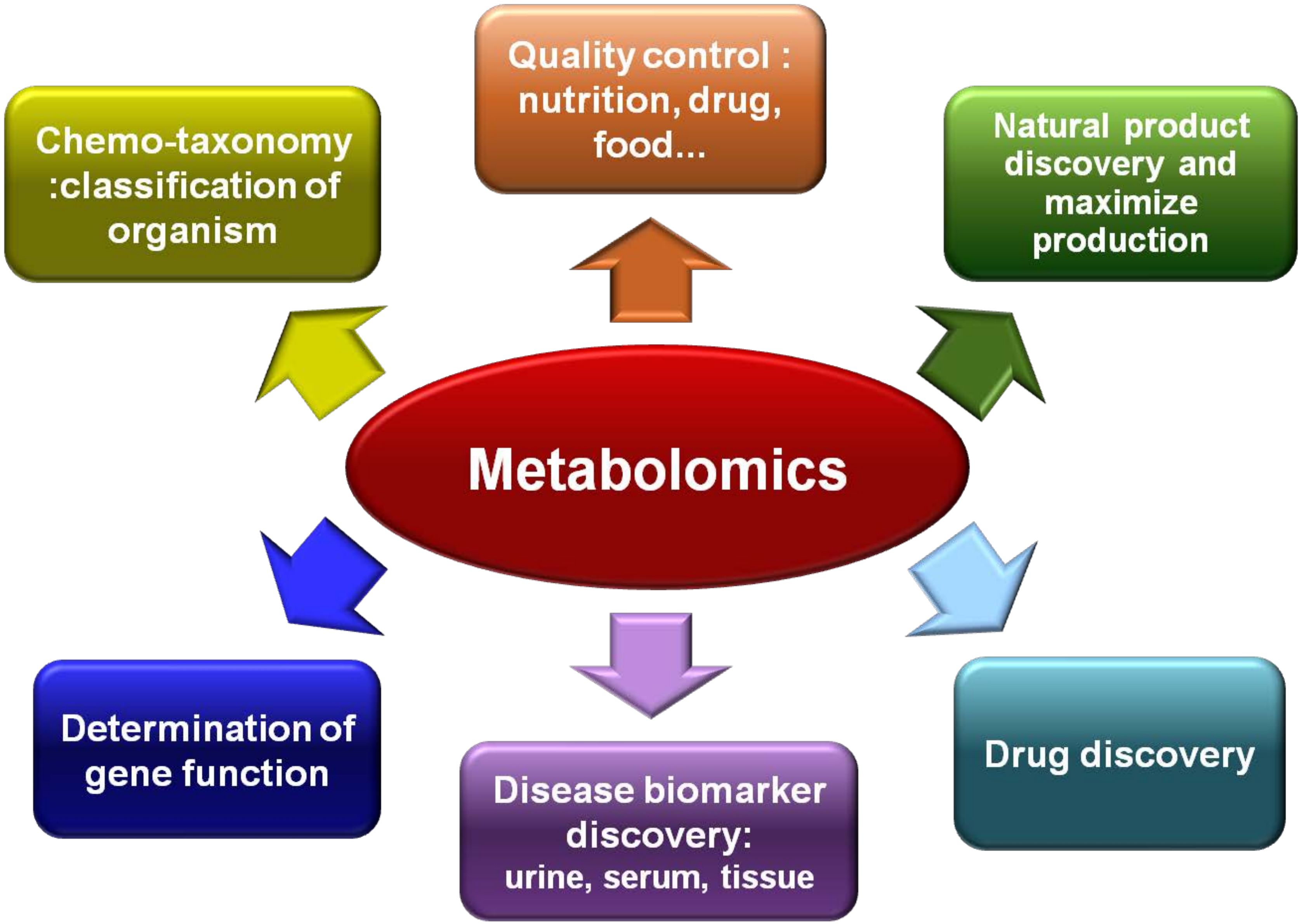
Genome, Transcriptome, Proteome, Metabolome

Genome	Transcriptome	Proteome	Metabolome
gene, chromosome	mRNA	protein	metabolite
Genetic information	Genetic information	Protein function	Phenotype
100,000–120,000	100,000–120,000	5,000–20,000	100– 5,000
mapping, sequencing	sequencing	separation, characterization	separation, characterization, determination, quantitation
DNA sequencing	HT-northern analysis	Two-D-gel	IR, MS, NMR

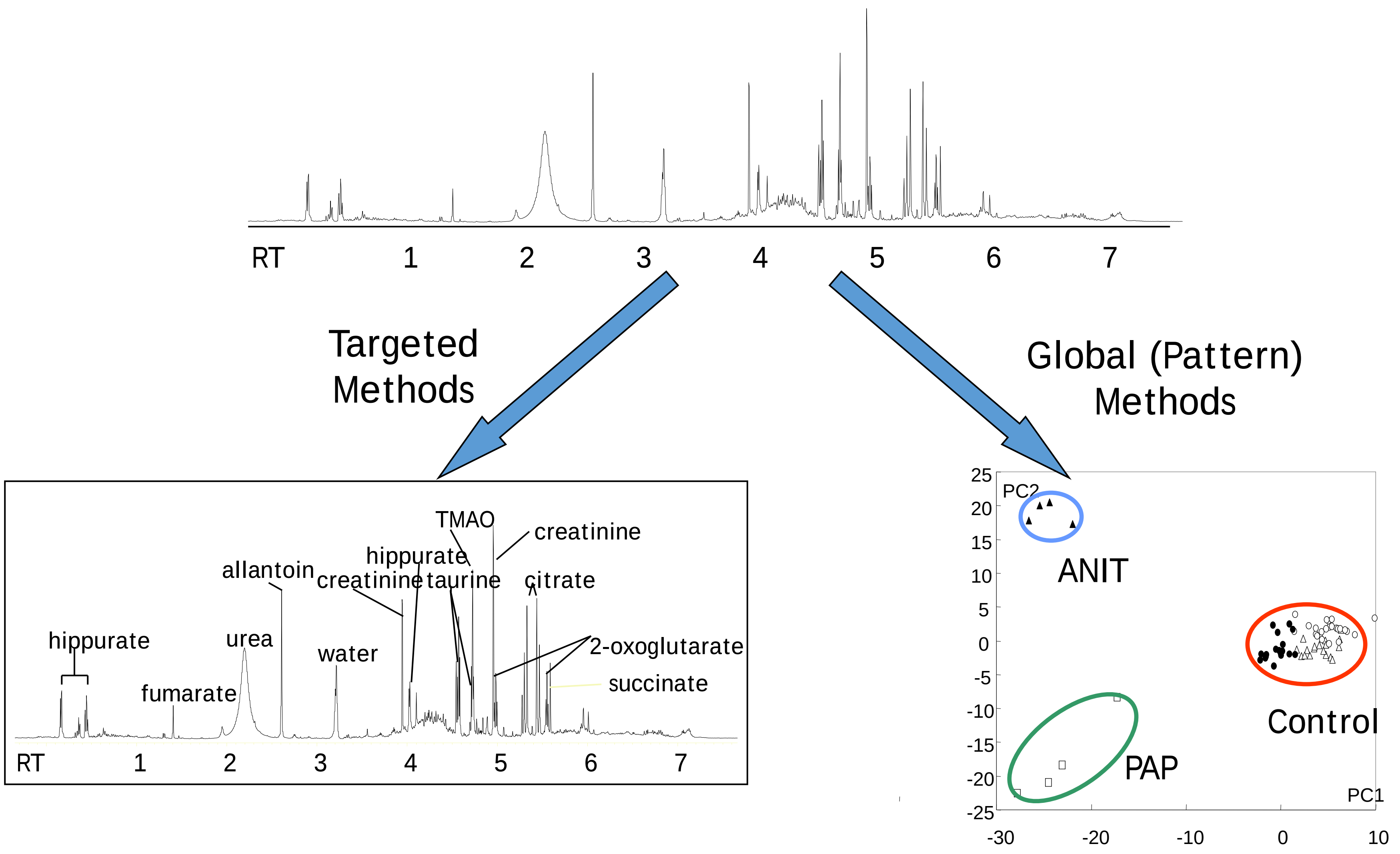
How many Metabolites?

- M/Os: >20,000
- Plants: >200,000
- Mammals: >2,500 - 8,000

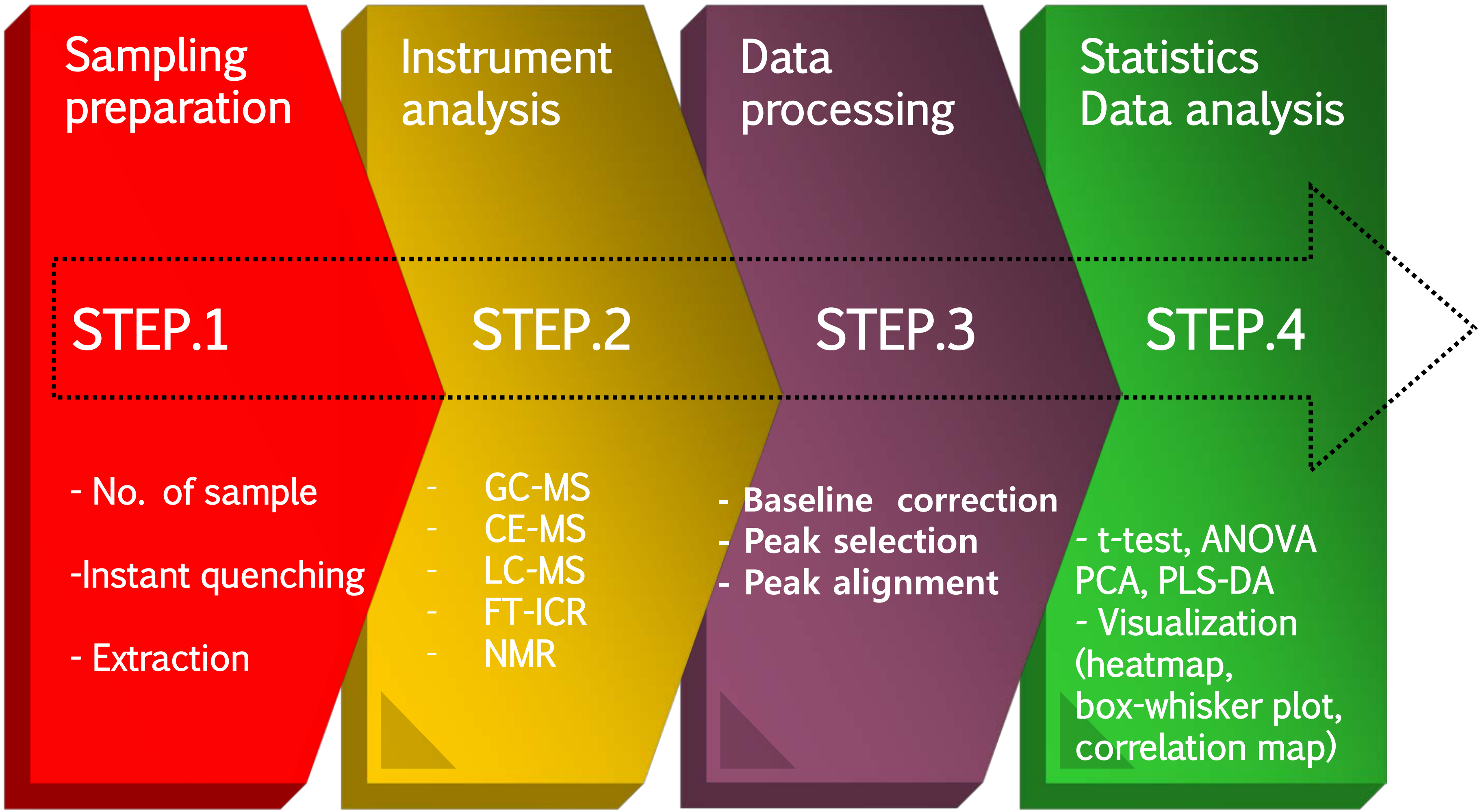
Application of Metabolomics



Two Routes to Metabolomics



Work flow of Metabolomics



What is a Metabolite?

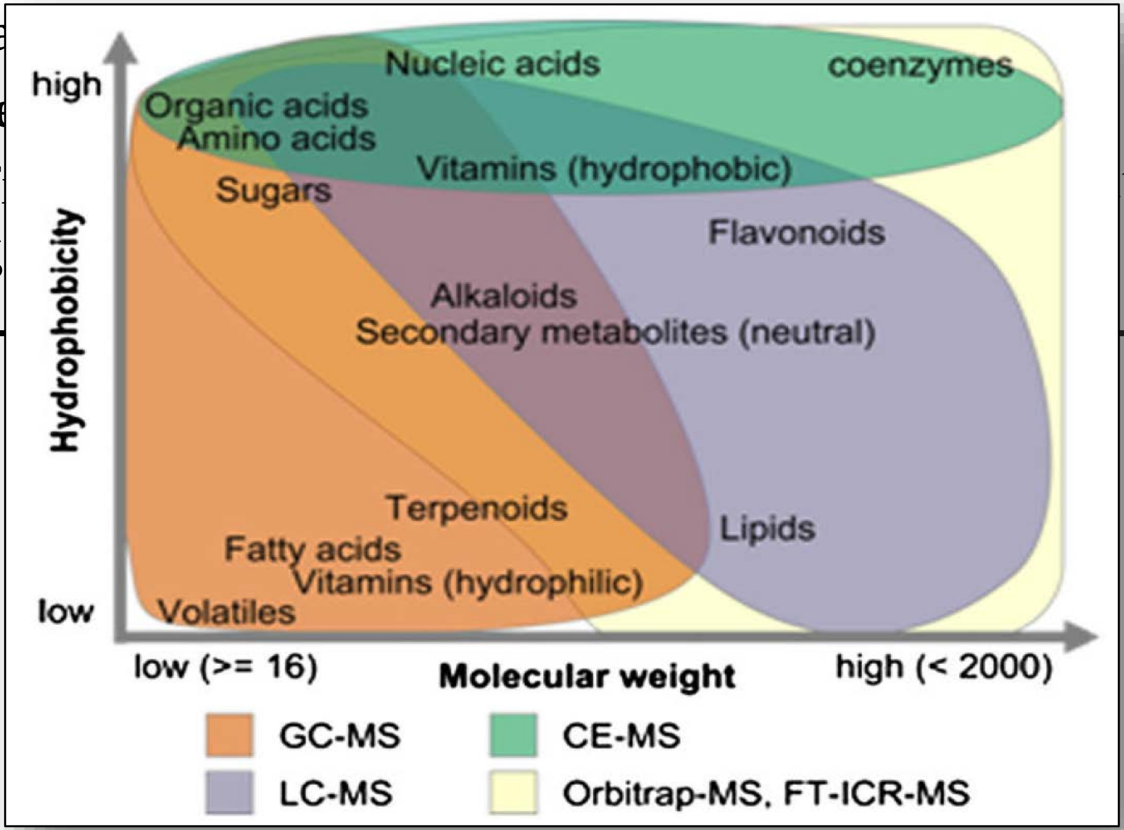
- Any organic molecule detectable in the body with a :
  MW < 1000 Da

- Includes peptides, oligonucleotides, sugars, nucelosides, organic acids, ketones, aldehydes, amines, amino acids, lipids, steroids, alkaloids and drugs (xenobiotics) → Structural diversity

- How many Metabolites?
  - M/Os: >20,000
  - Plants: >200,000
  - Mammals: >2,500 - 8,000 (HMDB)

Major technologies for Metabolomics

MS	NMR
◆ Identify and quantify metabolite **after sepa** ◆ Very **se** ◆ Identifi according	◆ Does not rely on the separation of tivity column)



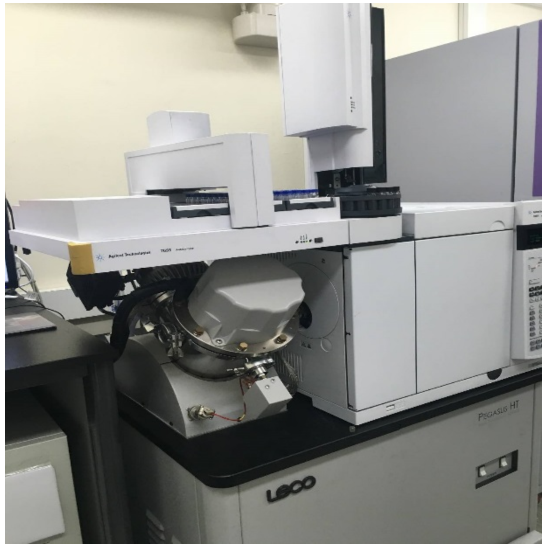
Metabolomics: an introduction

[Equipment]

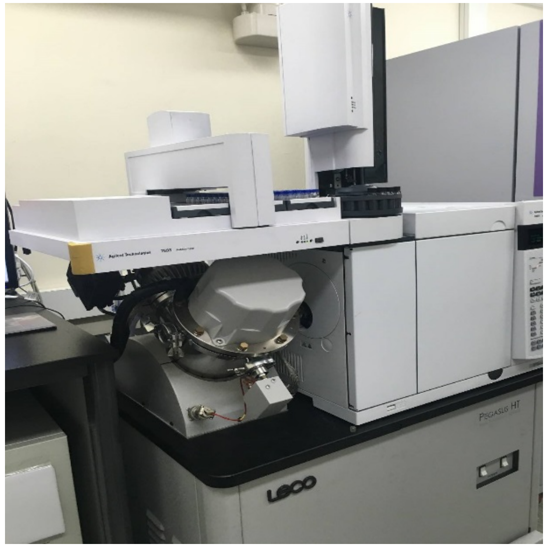
- **UHPLC-LTQ-Orbitrap-MS**

- **Triple-Q MS**

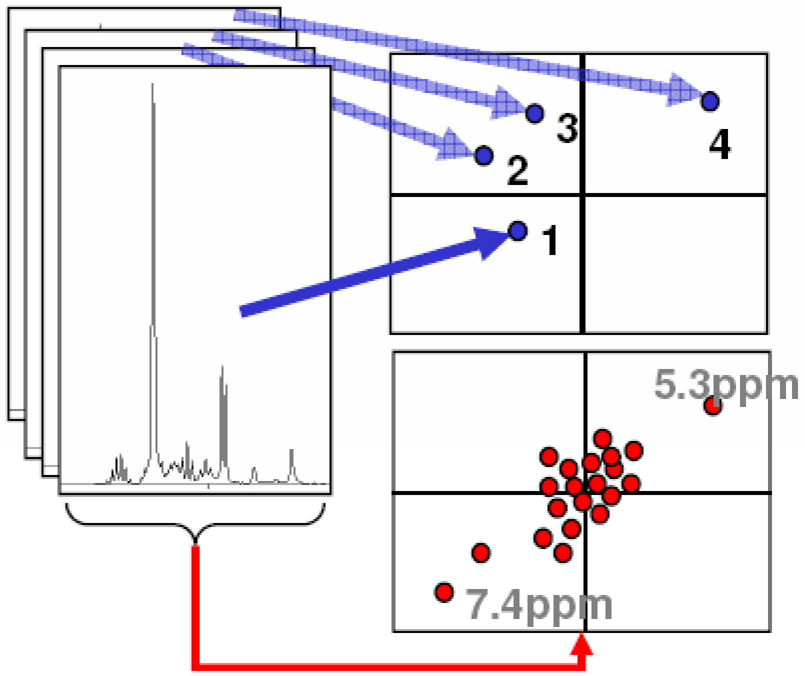
- **GC-TOF-MS (SPME)**



- **GC-TOF-MS II**



Principal Component Analysis (PCA)

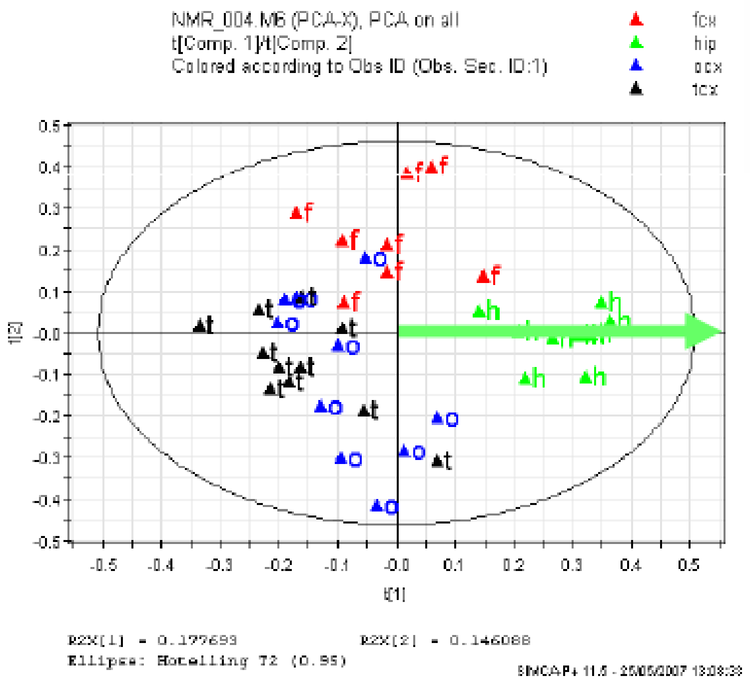


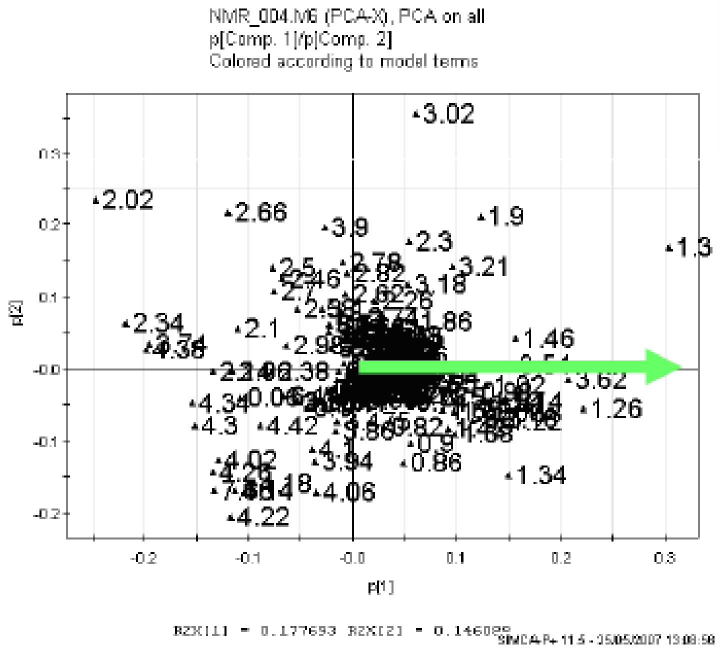
- Spectrum (observation) becomes a point in PCA **Scores plot**
- Variables (ppm or m/z) shown in PCA **Loadings Plot**

- Using plots together allows trends in the sample spectra to be ***interpreted*** in terms of chemical shift

Principal Component Analysis (PCA)

- Scores
  - Observations (spectra)
  - Trends, patterns, groups
- Loadings
  - Variables (ppm)
  - Correlation, influence

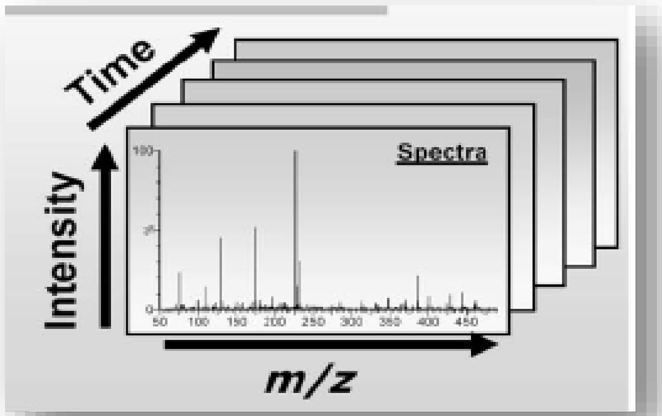




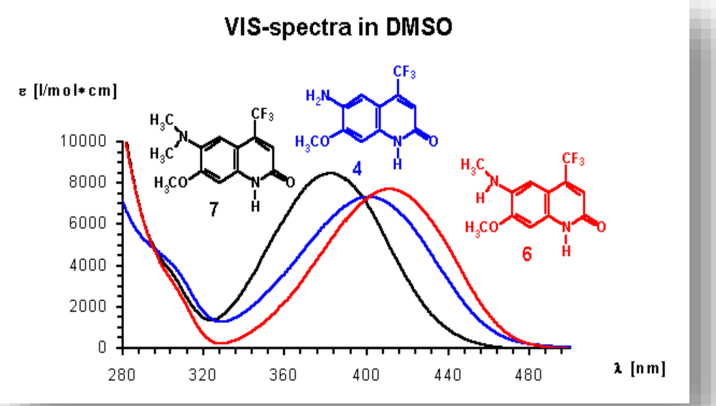
MS/MS Databases

- Large amount of data: MS, MS/MS, HRMS, isotope ratio
- Need for databases that can be easily searched
- In-house MS-DB

Identification of metabolites



Matching



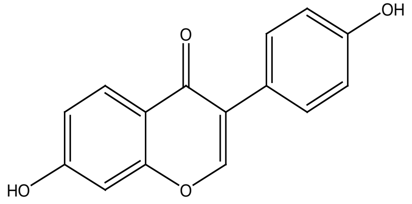
Mass Spectral Library with Search Program

Standard compound

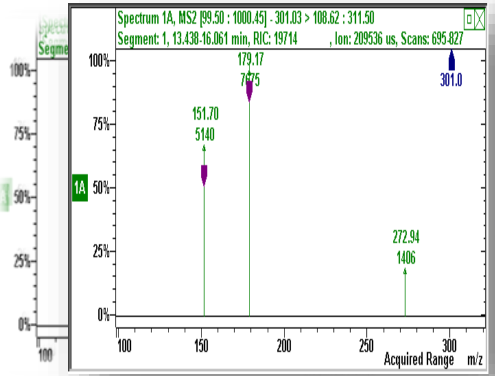
In house library

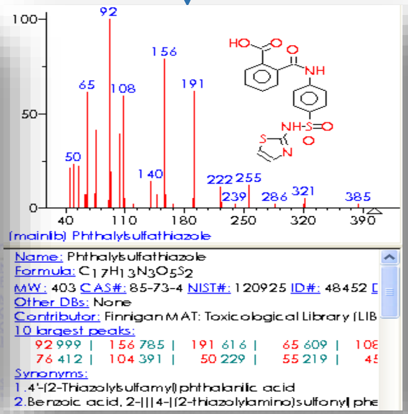
NIST

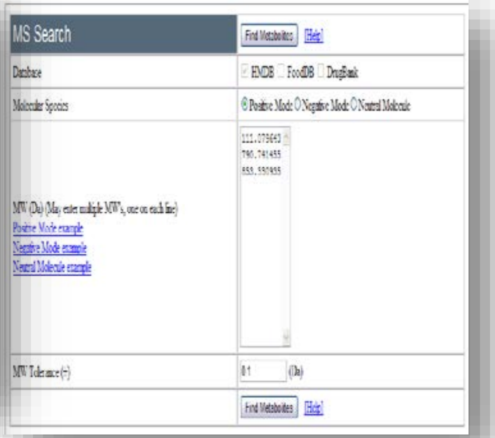
HMDB



Daidzein

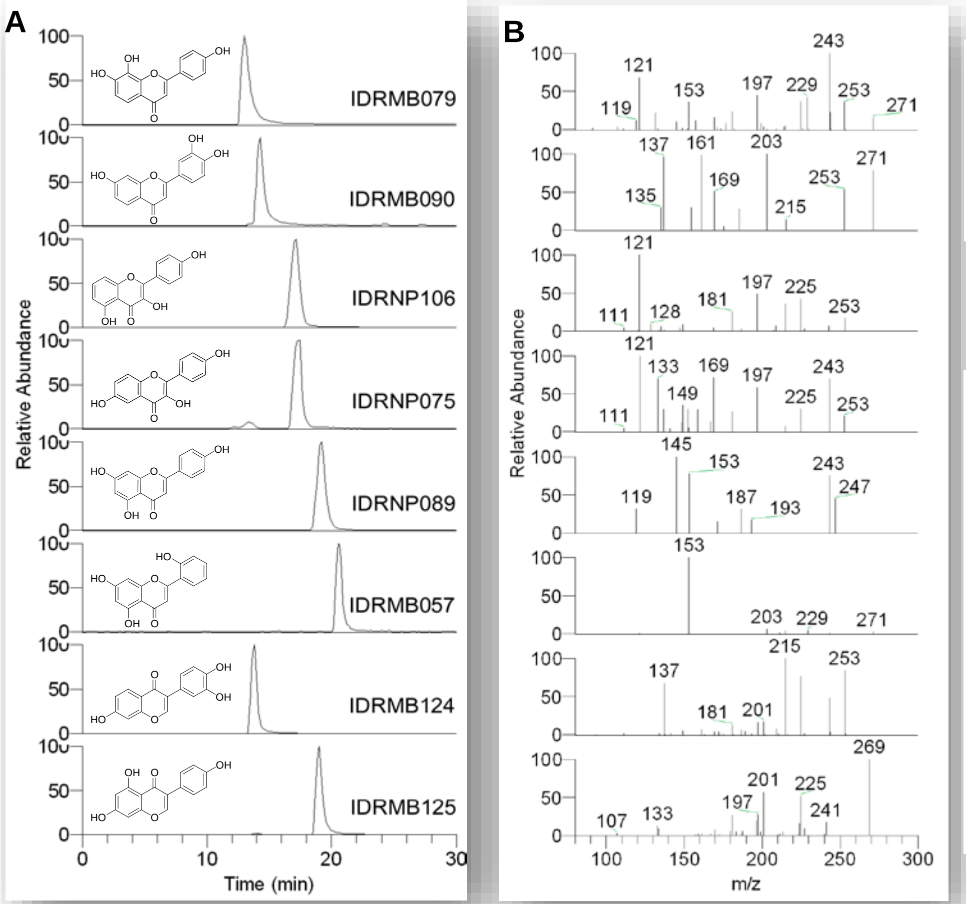






In House LC/MS/MS DB for 2nd metabolites

Contains 6,500 spectra of metabolites



RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2005; 19: 3539–3548
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.2230

RCM

Identification of flavonoids using liquid chromatography with electrospray ionization and ion trap tandem mass spectrometry with an MS/MS library

Table 1. Subclasses of flavonoids

Class	Flavonoids
Flavonols	Quercetin, kaempferol, myricetin, isorhamnetin
Flavones	Luteolin, apigenin
Flavanones	Hesperetin, naringenin, eriodictyol, pentahydroxylflavanone
Flavans	Catechin, gallocatechin, epicatechin, epigallocatechin, dihydrokaempferol, dihydroquercetin, dihydromyricetin
Isoflavones	Daidzein, genistein, glycitein
Anthocyanidins	Cyanidin, delphinidin, malvidin, pelargonidin
Chalcones	Chalcone, tetrahydroxychalcone

Contents

- **Metabolomics: an introduction**

- **Plasma Metabolomics in patient with type 2 diabetes mellitus**
  - Glutamine, glutamic acid, and its ratio can be new biomarkers to predict the prognosis of **diabetic retinopathy**
  - Plasma amino acids and oxylipins as potential multi-biomarkers for predicting **diabetic macular edema**
- **Drug mechanism: Hepatic metabolomic and lipidomic analysis of obese Type 2 diabetes in a rat model**

- **Oxylipins**

- **Metabolome-Microbiome analysis reveals green tea alleviates UVB-damaged mouse skin**

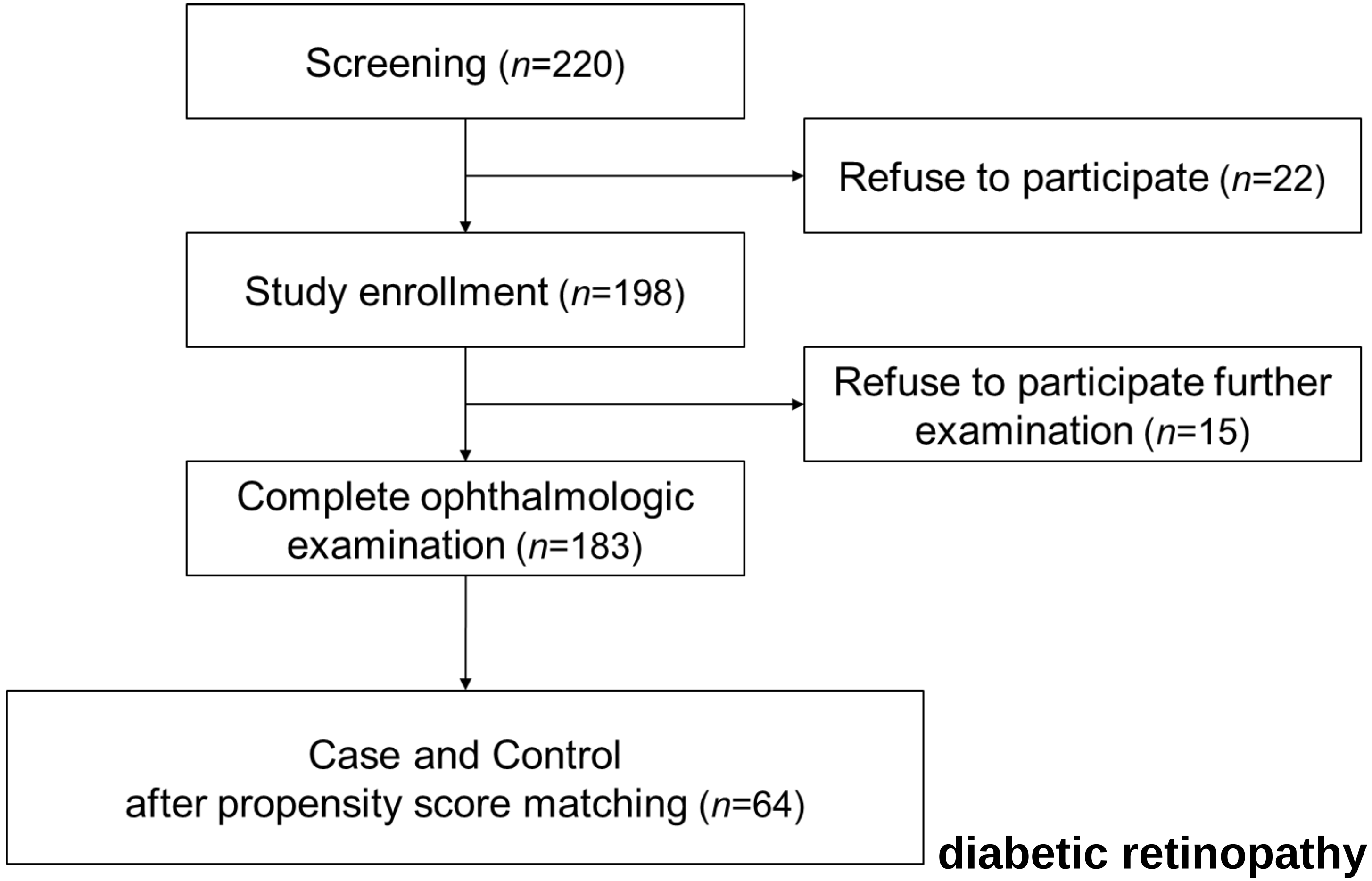
Metabolomics (2018) 14:89
https://doi.org/10.1007/s11306-018-1383-3

ORIGINAL ARTICLE

Plasma glutamine and glutamic acid are potential biomarkers for predicting diabetic retinopathy

Sang Youl Rhee[1] · Eun Sung Jung[2] · Hye Min Park[2] · Su Jin Jeong[3] · Kiyoung Kim[4] · Suk Chon[1] · Seung-Young Yu[4] · Jeong-Taek Woo[1] · Choong Hwan Lee[2]

Study Progression

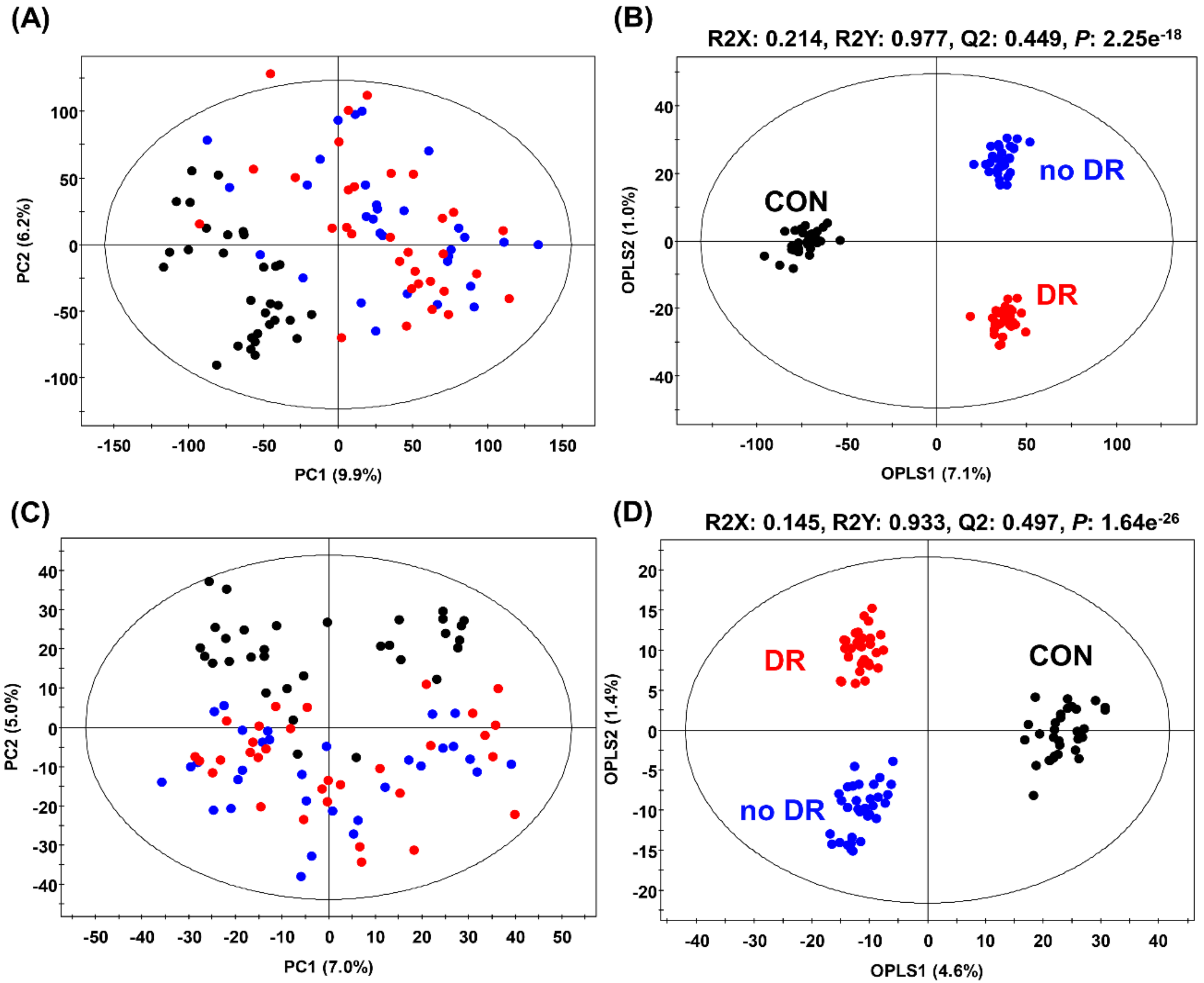


diabetic retinopathy

Principal component analysis (PCA) (A,C) and orthogonal partial least squares discriminant analysis (OPLS-DA) (B,D) score plots for plasma of non-diabetic control, no DR, and DR subjects analyzed by GC-TOF-MS (A,B), and UPLC-Q-TOF-MS (C,D).

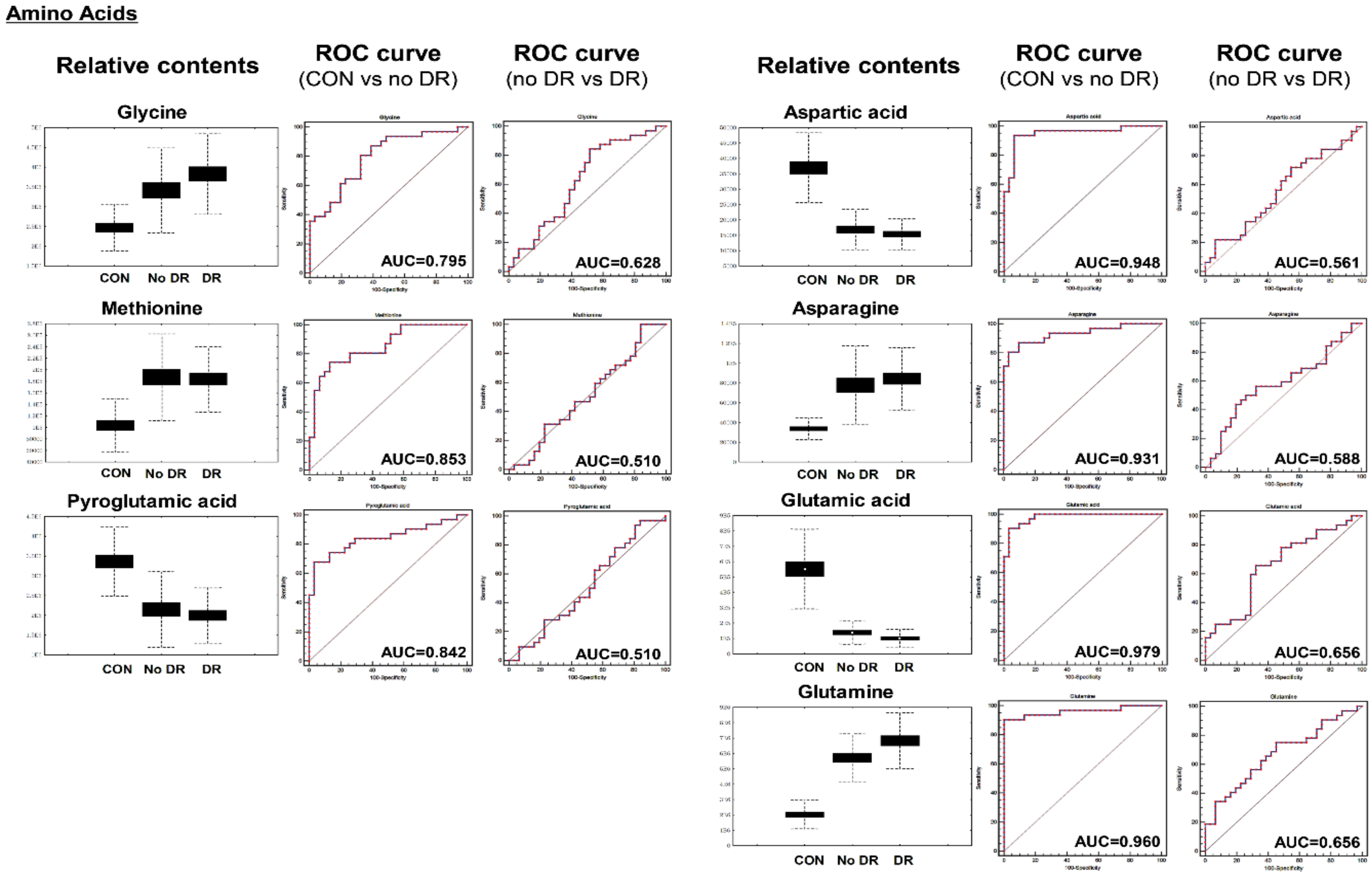
GC-TOF-MS (A,B)

UPLC-Q-TOF-MS (C,D)



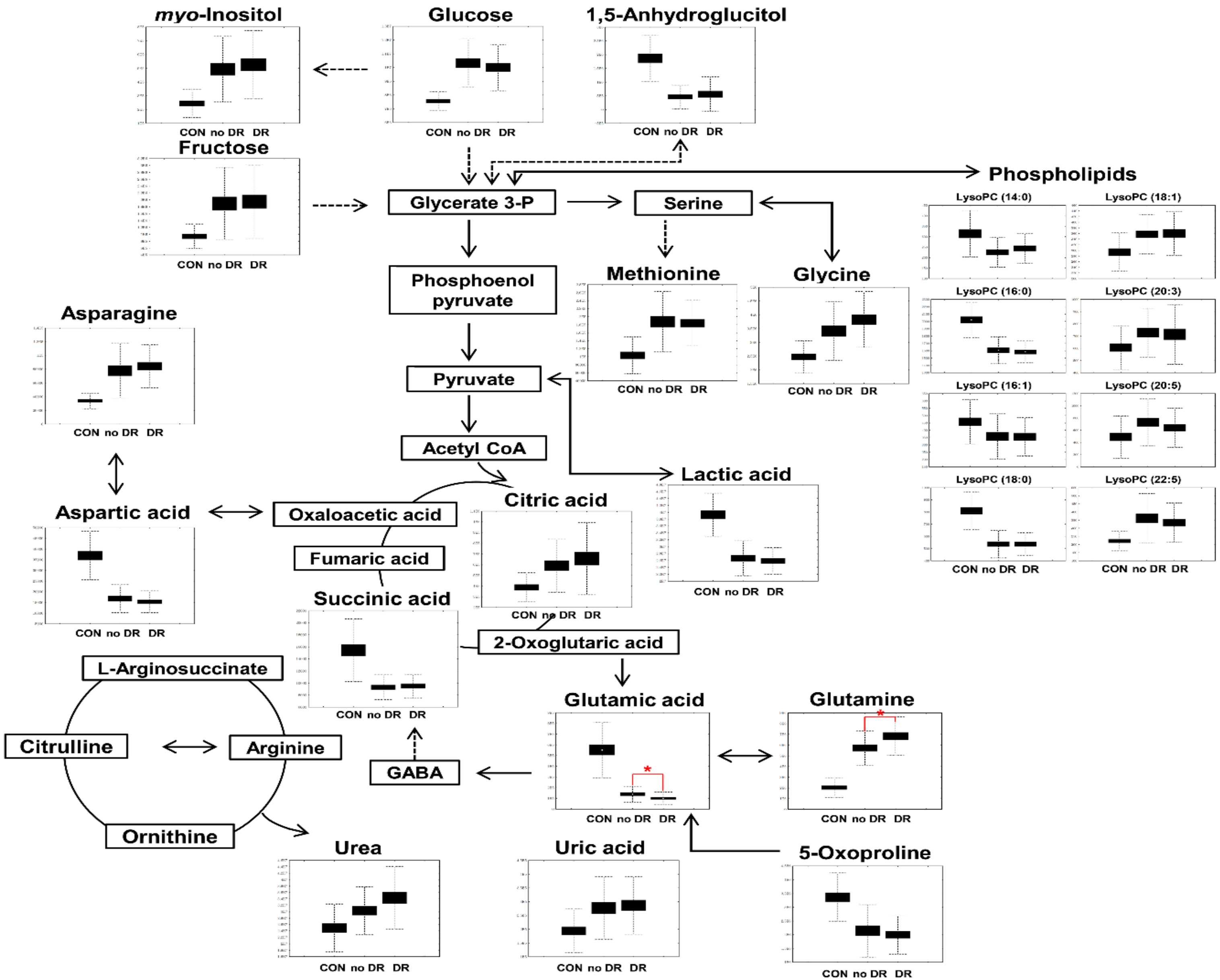
diabetic retinopathy

Box and whisker plots and ROC curves of amino acids in plasma which significantly distinguish non-diabetic control, no DR, and DR subjects. The AUC values of each metabolites are shown in inside of ROC curve.



diabetic retinopathy

A schematic diagram of a proposed metabolic pathway using metabolites shows significantly different levels among experimental groups including non-diabetic control, no DR, and DR subjects.



diabetic retinopathy

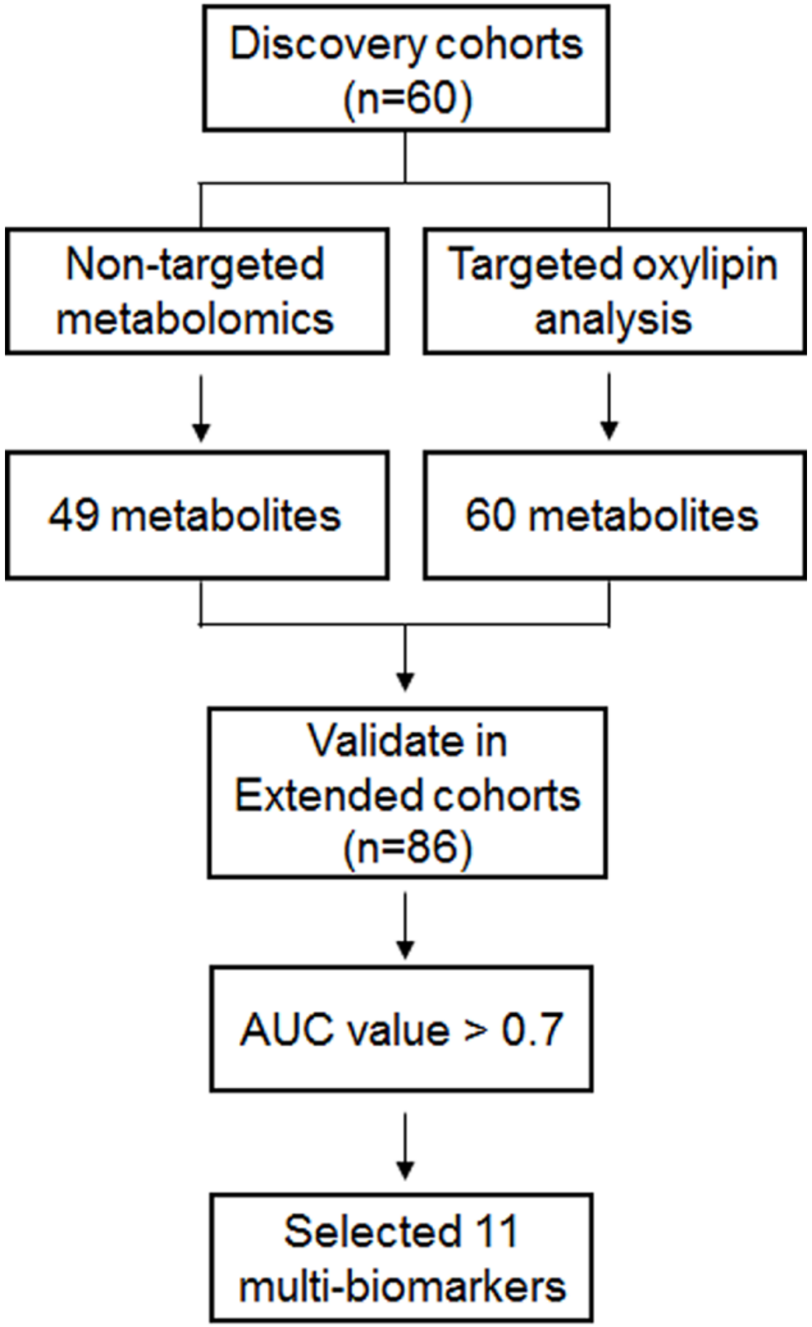
Summary of diabetic retinopathy

- Glutamine and glutamic acid were identified as the most accurate marker for the presence of DR in subjects. ROC analysis showed high diagnostic value of glutamine (AUC=0.671), glutamic acid (AUC=0.656) and its ratio (AUC=0.742) for DR.

- Our study suggests that **glutamine, glutamic acid, and its ratio** can be useful as new biomarkers to predict the prognosis of DR in elderly T2DM patients.

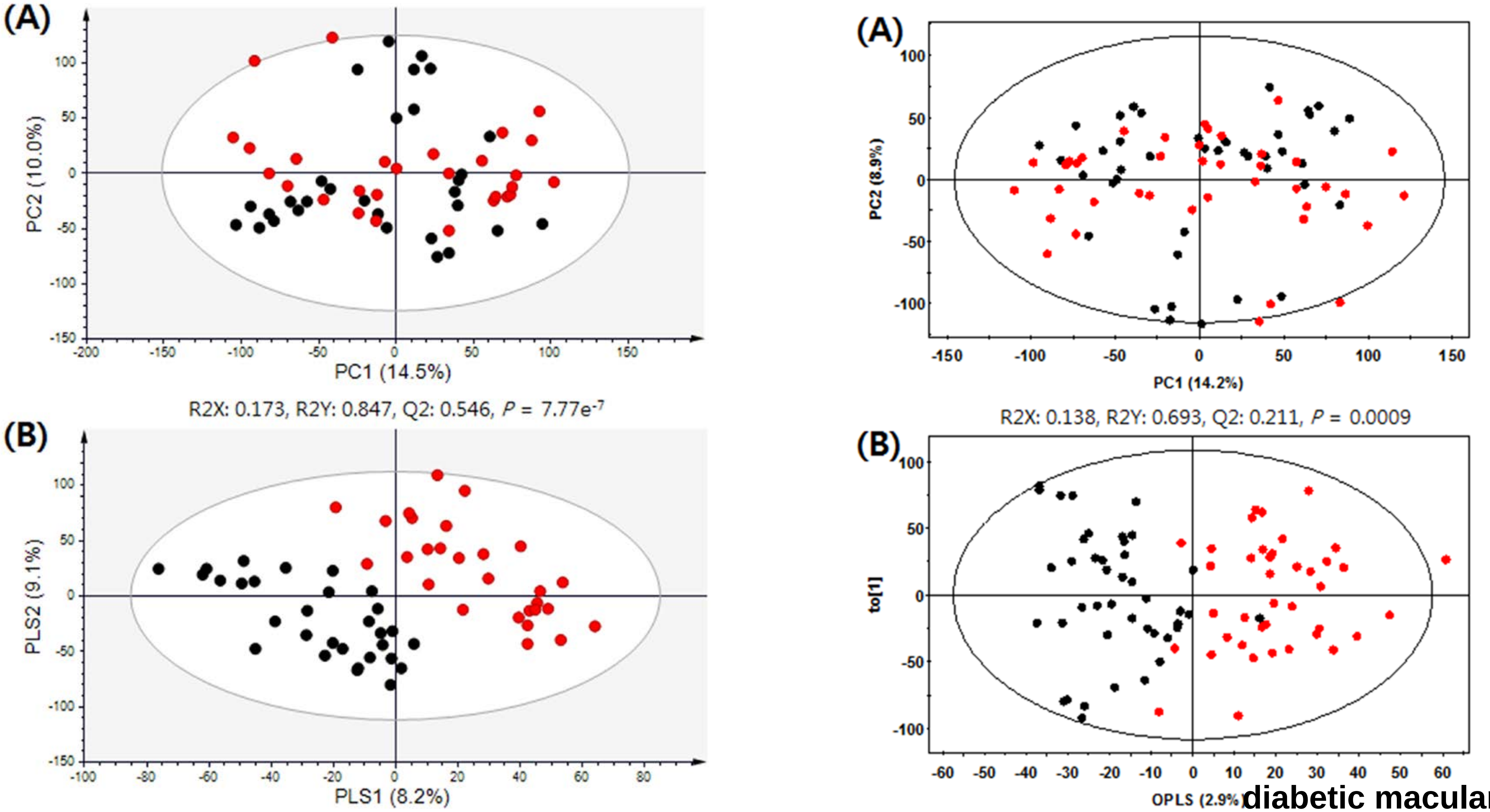
Plasma amino acids and oxylipins as potential multi-biomarkers for predicting diabetic macular edema

Sang Youl Rhee[1*], Eun Sung Jung[2*], Su Jin Jeong[3], Kiyoung Kim[4], Suk Chon[1], Seung-Young Yu[4], Jeong-Taek Woo[1**], Choong Hwan Lee[2,5,6**]

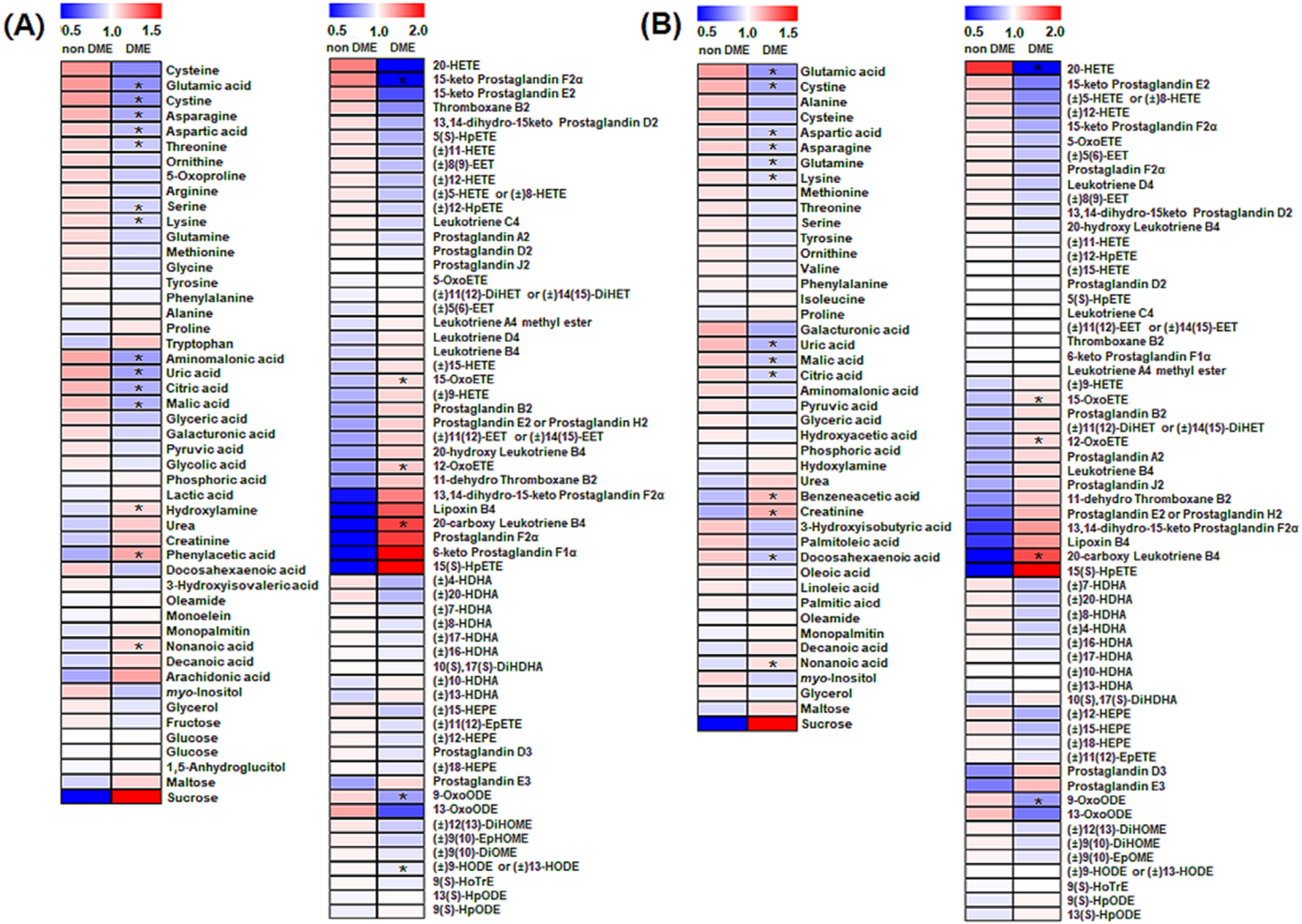


Scientific Report *in press*

Principal component analysis (PCA) (A) and partial least squares discriminant analysis (PLS-DA) (B) score plots for candidate plasma markers in diabetic macular edema (DME) and non-DME subjects analyzed by GC-TOF-MS. ●—non-DME group, ●—DME group



diabetic macular edema



diabetic macular edema

Receiver operating characteristic (ROC) curve of potential metabolite biomarkers distinguishing diabetic macular edema (DME) versus non-DME subjects, and combined ROC curves of those multi-biomarkers.

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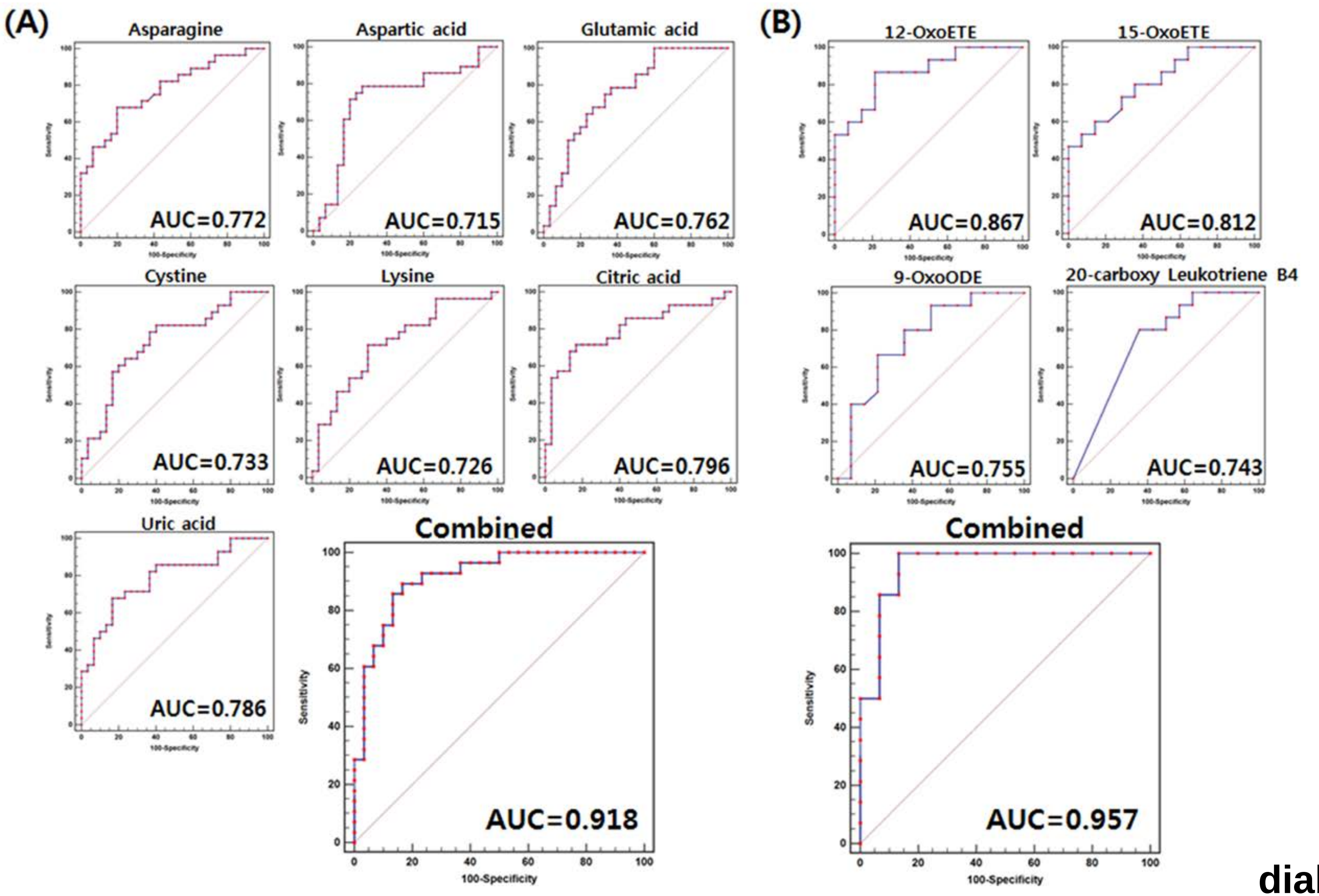
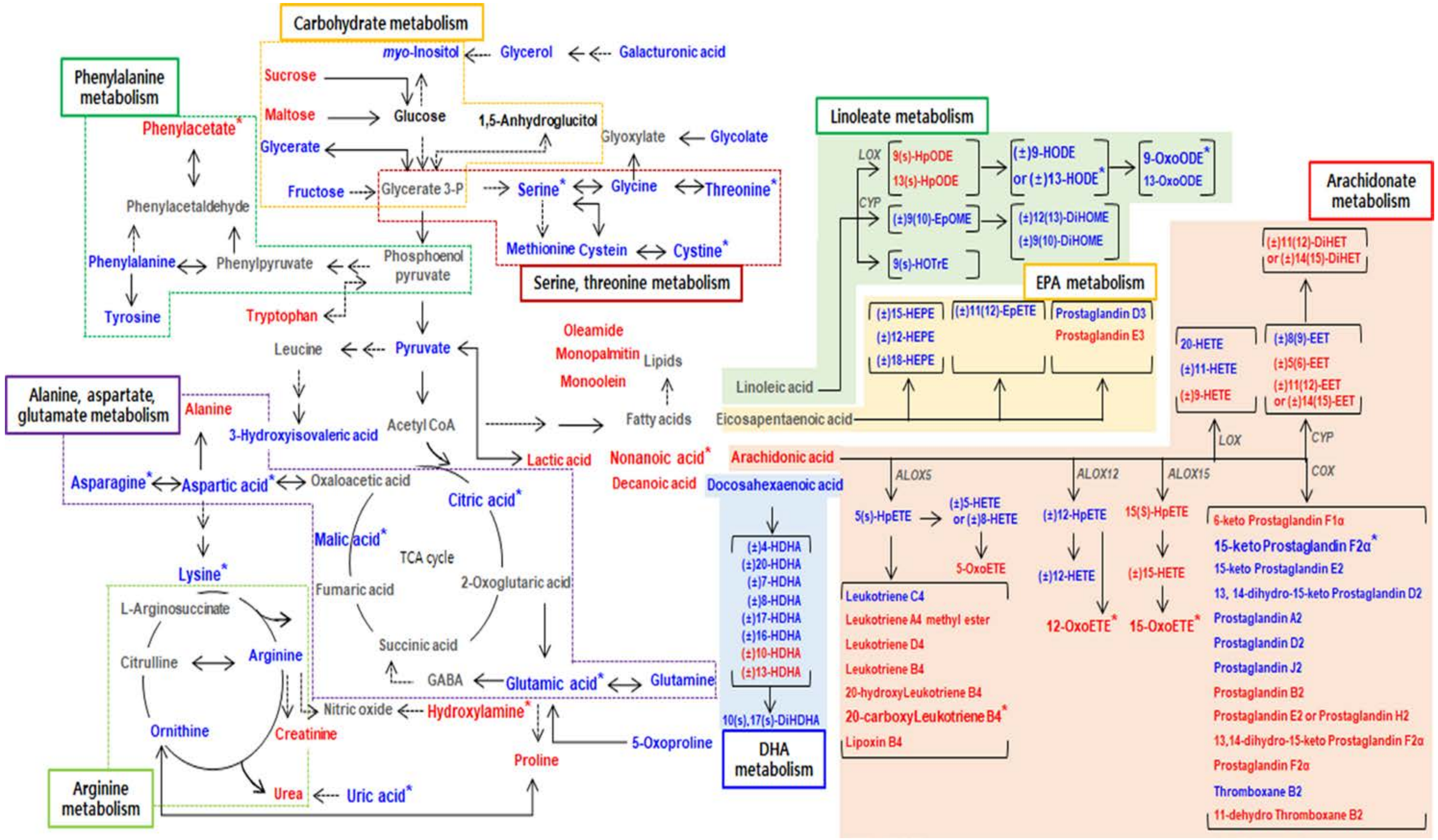


diabetic macular edema



Schematic diagram of a proposed metabolic pathway using plasma metabolites derived from metabolite and lipid profiling of experimental groups including diabetic macular edema (DME) and non-DME subjects. Metabolites labelled with *blue characters* indicate that relative metabolite levels were lower in DME cases than in non-DME subjects. Metabolites labelled with *red characters* indicate that relative metabolite levels were higher in DME cases than in non-DME patients. Asterisks indicate statistically significant differences in levels of metabolites distinguishing DME and non-DME individuals ($p < 0.05$). The metabolic pathway was modified from the reported Kyoto Encyclopedia of Genes and Genomes pathway (KEGG, http://www.genome.jp/kegg/).

diabetic macular edema

Summary of diabetic macular edema

- From metabolomic studies of plasma, 5 amino acids (asparagine, aspartic acid, glutamic acid, cysteine, and lysine), 2 organic compounds (citric acid and uric acid) and **4 oxylipins (12-oxoETE, 15-oxoETE, 9-oxoODE, 20-carboxy leukotriene B4)** were identified as candidate multi-biomarkers which can guide DME diagnosis among non-DME subjects.

- Our study suggests that multi-biomarkers may be useful for predicting DME in elderly T2DM patients

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- **Metabolomics: an introduction**
- **Diabetes complications: Plasma Metabolomics in patient with type 2 diabetes mellitus**
- **Hepatic metabolomic and lipidomic analysis of obese Type 2 diabetes in a rat model: Drug mechanism**

  - The effect of pioglitazone on hepatic steatosis

  - **Oxylipins**

- **Metabolome-Microbiome analysis reveals green tea alleviates UVB-damaged mouse skin**

British Journal of Pharmacology

British Journal of Pharmacology (2018) •• ••–•• 1

RESEARCH PAPER

Metabolomic and lipidomic analysis of the effect of pioglitazone on hepatic steatosis in a rat model of obese Type 2 diabetes

Correspondence Dr Choong Hwan Lee, Department of Bioscience and Biotechnology, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, South Korea, and Dr Cheol-Young Park, Division of Endocrinology and Metabolism, Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, 29 Saemunan-ro, Jongno-gu, Seoul 03181, South Korea. E-mail: chlee123@konkuk.ac.kr; cydoctor@chol.com

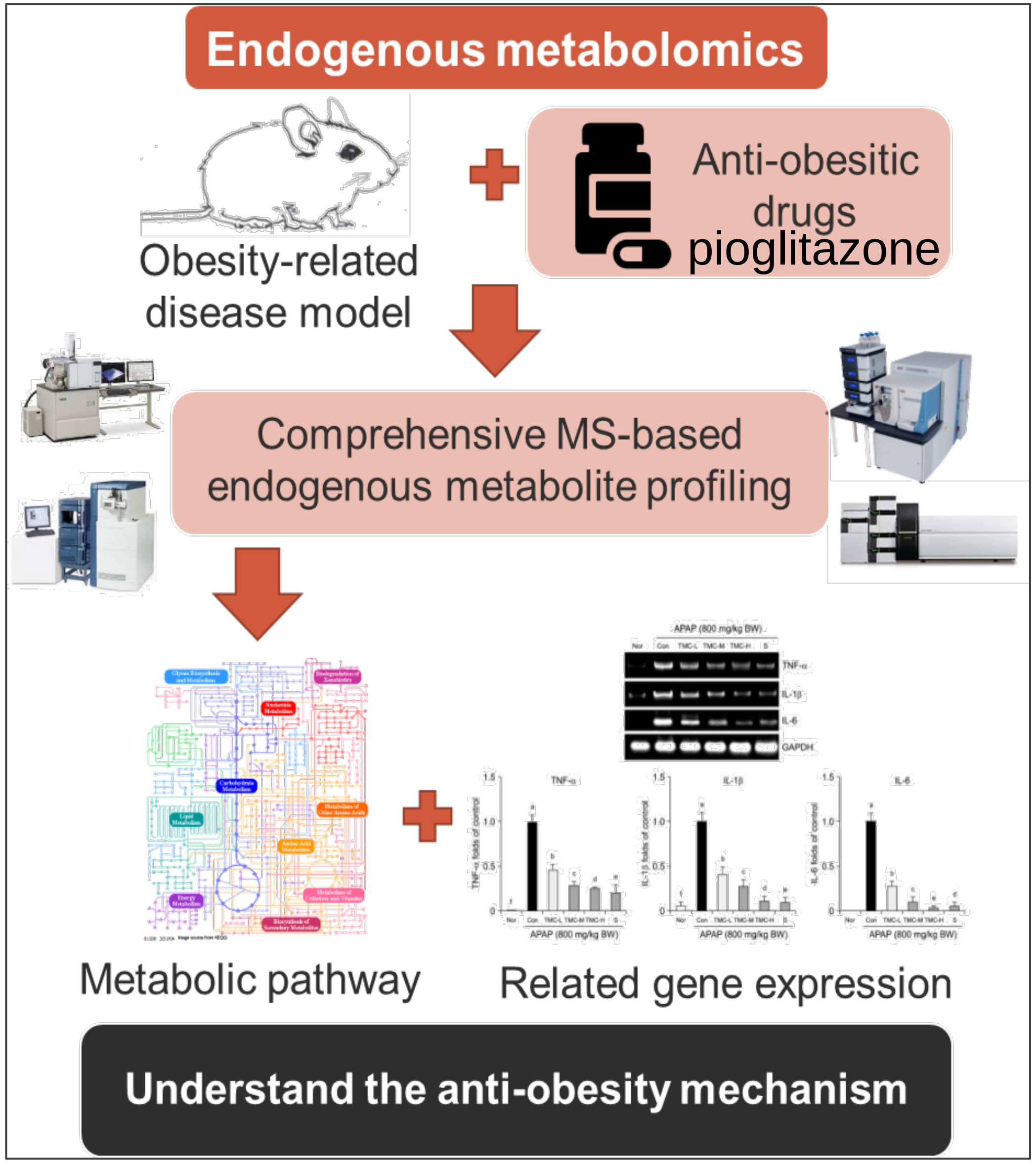
Received 28 May 2018; **Accepted** 8 June 2018

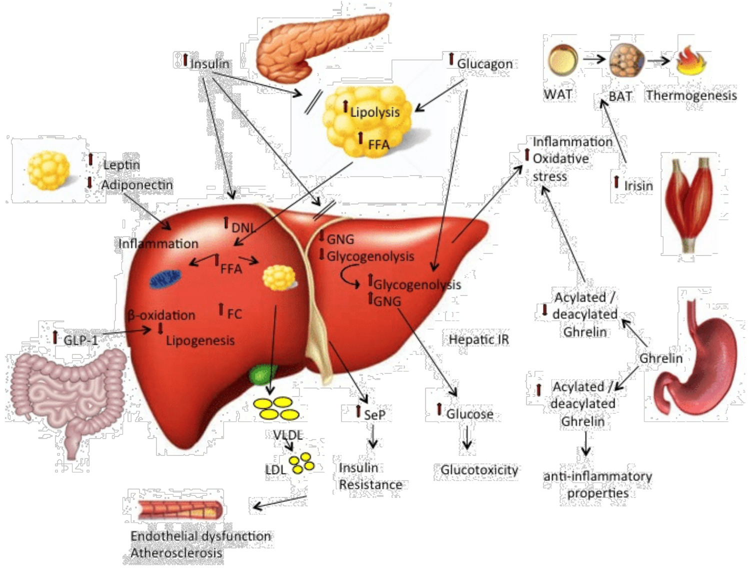
Hyekyung Yang[1,*], Dong Ho Suh[3,*], Dae Hee Kim[1], Eun Sung Jung[3], Kwang-Hyeon Liu[4], Choong Hwan Lee[3] and Cheol-Young Park[1,2]

Research design

- Male OLETF rats were orally administered pioglitazone (30-mg·kg1) and fed a high-fat diet (60% kcal fat) for 12 weeks.
- Hepatic metabolites were analysed *via* **metabolomic and lipidomic analyses**.
- **Gene expression** and PLA2 activity were analysed in livers from pioglitazone-treated and control rats.

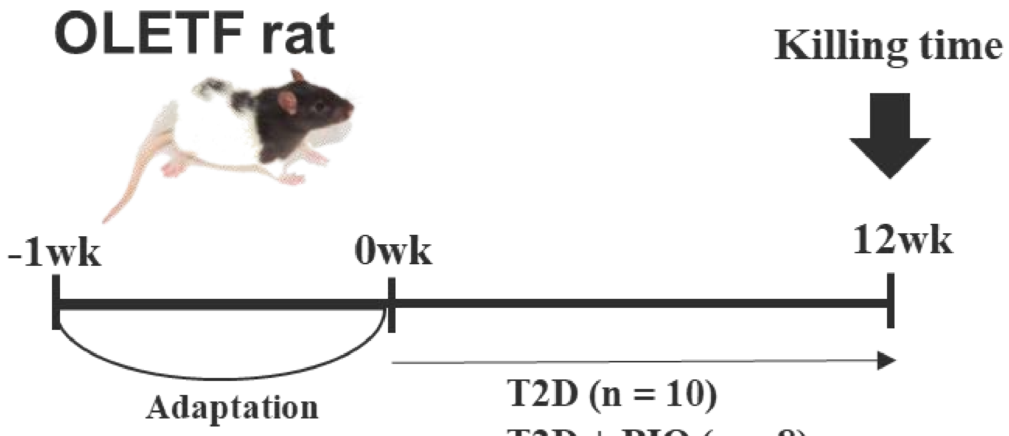
Endogenous metabolomics in animal model

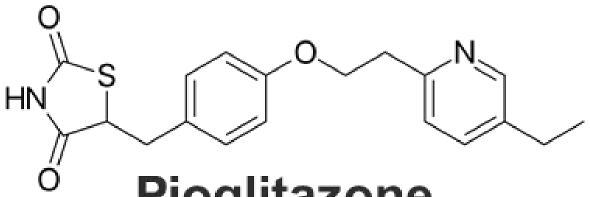




pioglitazone on hepatic steatosis

Anti-obesity effects of single drug (Pioglitazone)

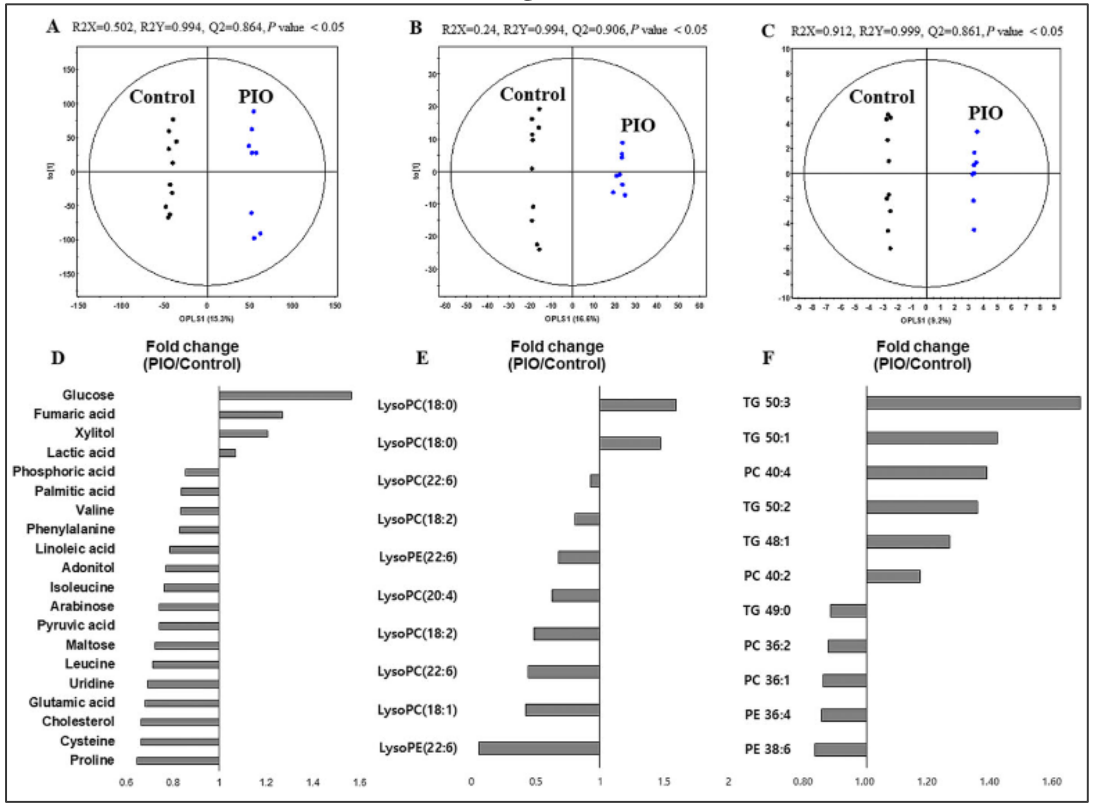




Pioglitazone
(Single drug)

Comprehensive metabolite profiling

GC-TOF-MS UPLC-Q-TOF-MS Nanomate-LTQ

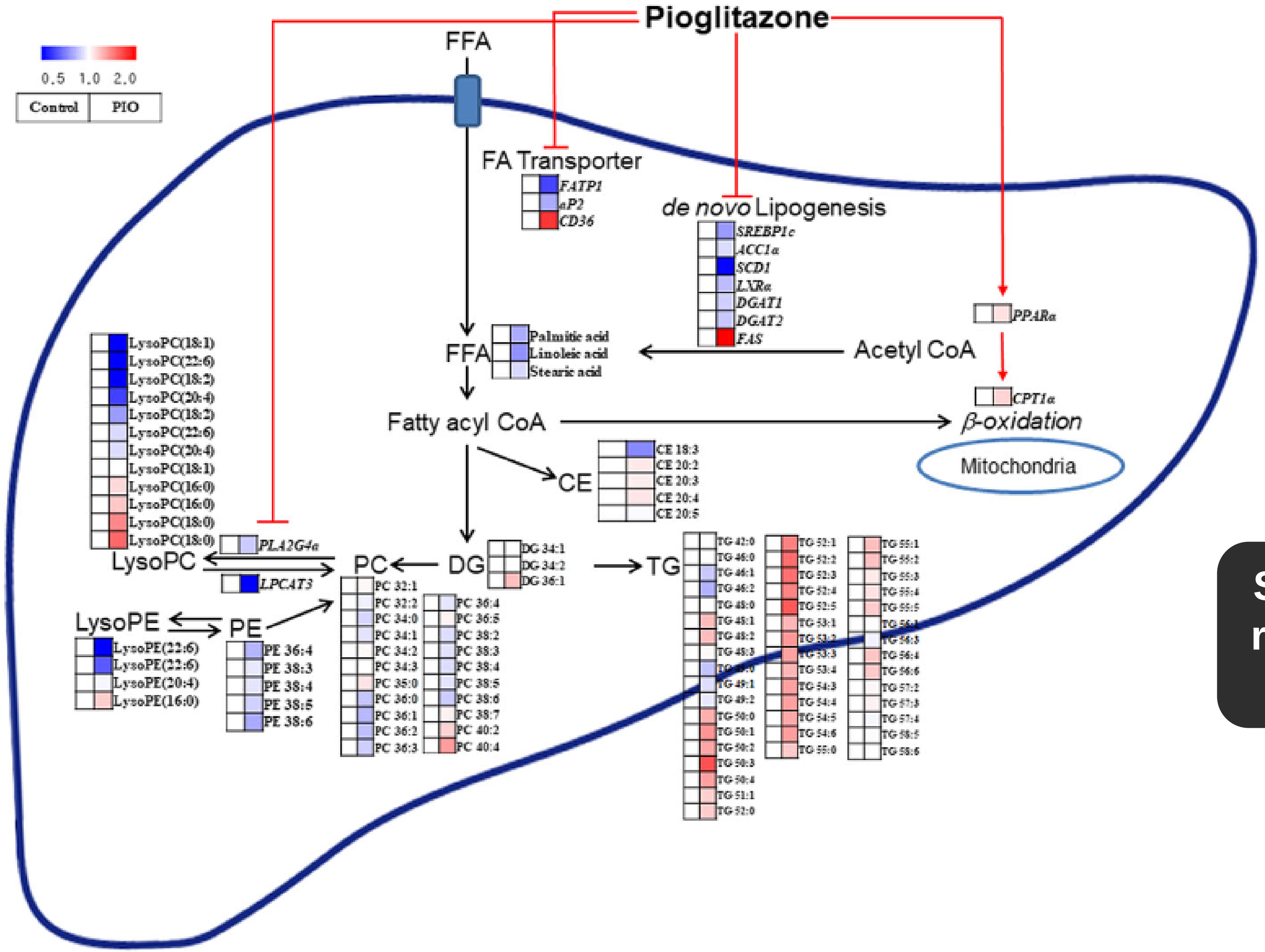


British Journal of Pharmacology (2018)

pioglitazone on hepatic steatosis

Anti-obesity effects of single drug (Pioglitazone)

Metabolic pathway (hepatic metabolites + gene expression)



Scheme of the mechanism by which PIO regulates hepatic metabolites, lipids and related gene-expression patterns

British Journal of Pharmacology (2018)

Summary of pioglitazone on hepatic steatosis

- Pioglitazone treatment significantly altered levels of hepatic metabolites, including free fatty acids, lysophosphatidylcholines and phosphatidylcholines, in the liver.

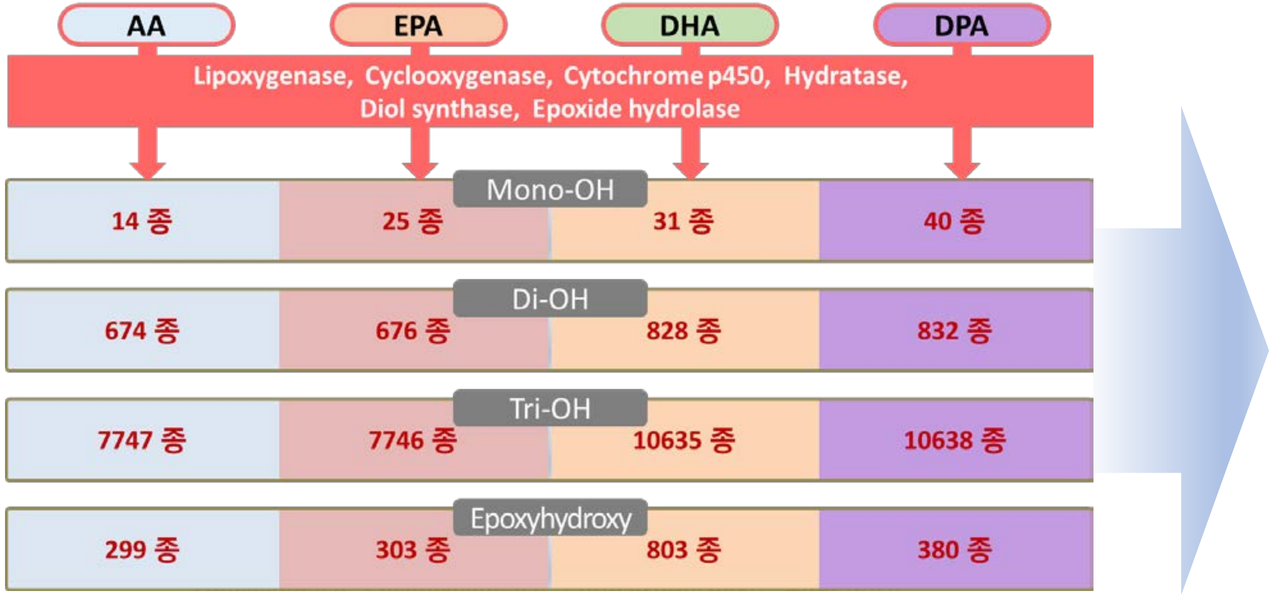
- reduced the expression of genes involved in hepatic *de novo* lipogenesis and fatty acid uptake and transport, whereas genes related to fatty acid oxidation were upregulated.

- Gene expression and enzyme activity of PLA2, which hydrolyzes phosphatidylcholines to release lysophosphatidylcholines and free fatty acids, were significantly decreased.

Suh and Lee, British Journal of Pharmacology (2018)

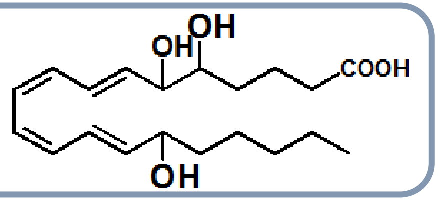
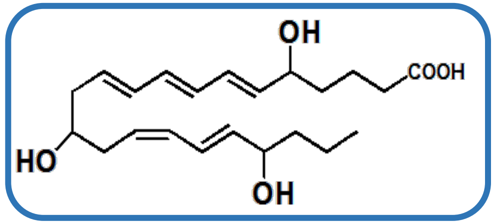
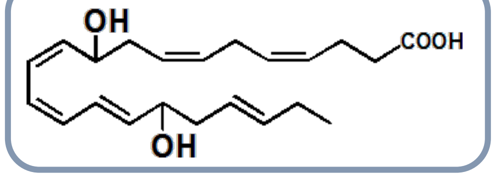
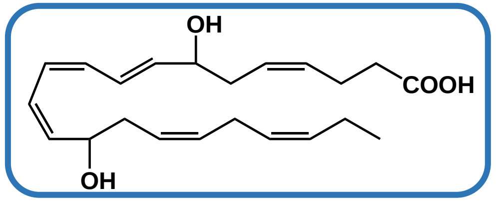
Oxylipins: 인체 내의 옥시리핀(산화지방산)이란?

- 인체 내 옥시리핀 (mono-, di-, tri-hydroxy fatty acids, epoxyhydroxy fatty acids): 약 110여종; 염증해소, 통증완화, 조직 재생, 신호전달, 항상성 유지 등에 관여
- 인체 내 옥시리핀은 Arachidonic acid (AA), EPA, DHA, DPA로부터 지방산화 효소 (위치특이적 5*S*,8*S*,8*R*,11*S*,12*S*,15*S*-lipoxygenases, cyclooxyganse, cytochrome p450, hydratase, diol synthase 등)의 조합반응으로 생성



산화위치에 따라 약 41,000 여 종 신규 옥시리핀 발견 가능

Oxylipins: 염증해소 특화지방산(SPM)의 정의 및 효능

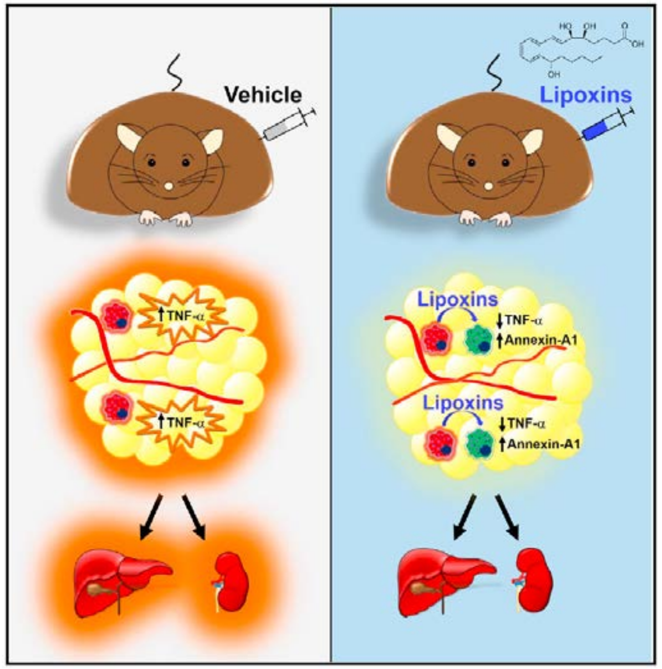
SPM	화학 구조	물질 특징 및 주요 효능
Lipoxin (e.g. Lipoxin A_4)		4종; 당뇨/비만치료, 급성염증 치료, 알러지에 대한 항염증, 식균작용 촉진 Cell 140, 717 (2010)
Resolvin (e.g. Resolvin D_3)		13종 ; 염증성 장 질환 치료, 염증질환 해결, 상처치료, 항생제 사용량 감소 Science 339, 166 (2013)
Protectin (e.g. Protectin D_x)		7종; 항염증, 망막손상 보호, 세포퇴행 예방, 바이러스 제거 촉진 Cell 153, 112 (2013)
Maresin (e.g. Maresin 1)		8종; 염증질환 치료, 알러지 치료, 상처 치료, 사멸세포 제거 촉진 Nature 510, 92 (2014)

Nature **447**, 869-874, 2007; Nature **484**, 524-528, 2012; Nature **490**, 107-111, 2012; Science **339**, 166-172, 2013; Cell **153**, 112-125, 2013, Nature, **510**, 92-101, 2014

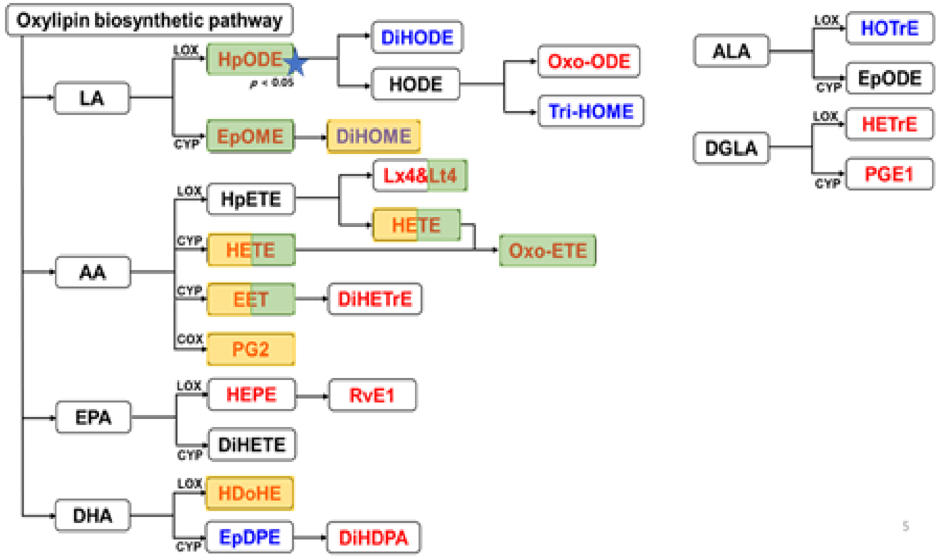
oxylipins

Oxylipins: 옥시리핀의 비만과의 연관성

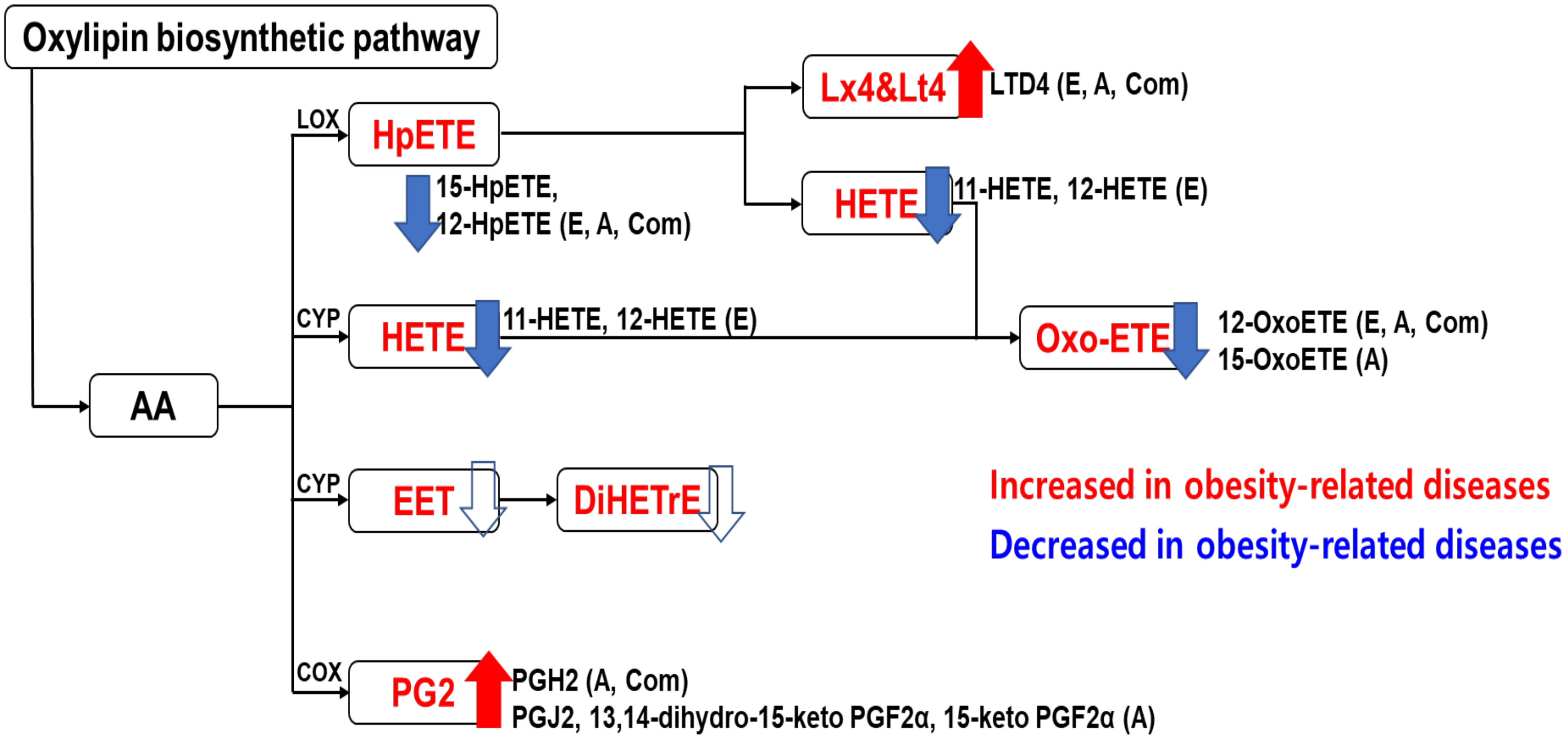
- 옥시리핀은 지방조직 염증/비만 유도 질병 치료에 관여함.
- 인체 내 염증/비만 치료제를 투여하면 치료 과정 중 다양한 옥시리핀이 발생됨.
- 인체 내 염증/비만 치료에 관여하는 옥시리핀의 동정이 필요함.



옥시리핀의 비만질환 치료
Cell Metabolism, **22**, 125 (2015)



항 당뇨/비만 약물 투입 후 관련 옥시리핀 생성
빨강색: 비만관련 질병 증가, 파란색: 비만관련 질병 감소

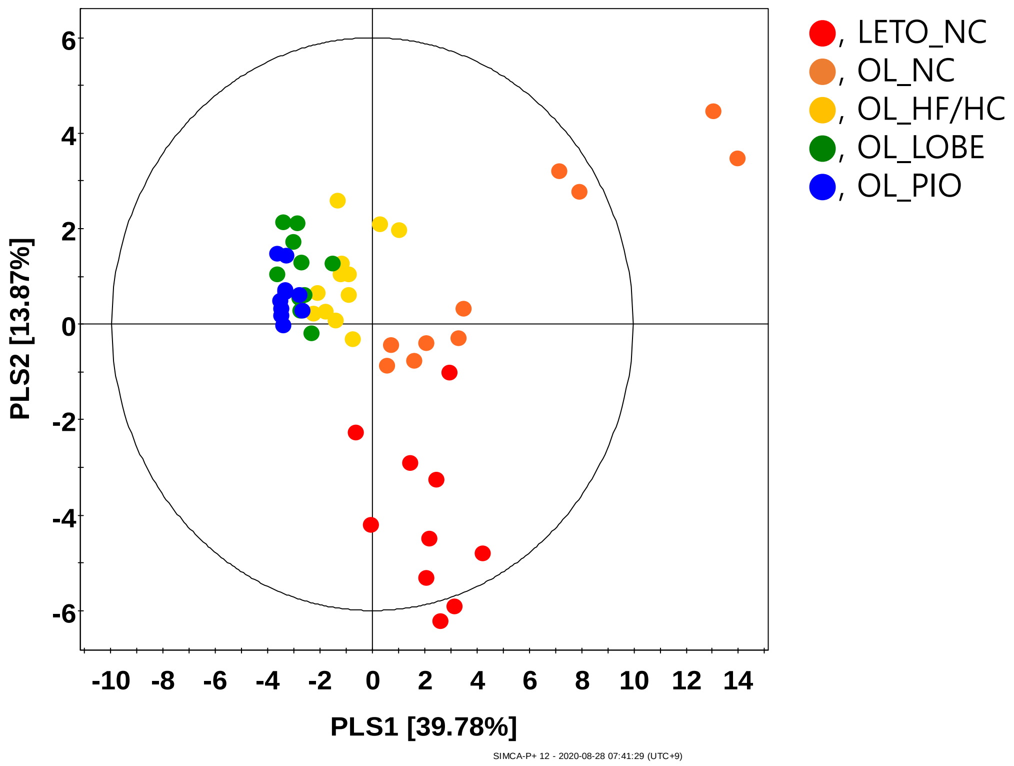

Oxylipin biosynthetic pathway
LOX
HpETE
15-HpETE,
12-HpETE (E, A, Com)
Lx4&Lt4
LTD4 (E, A, Com)
HETE
11-HETE, 12-HETE (E)
CYP
HETE
11-HETE, 12-HETE (E)
Oxo-ETE
12-OxoETE (E, A, Com)
15-OxoETE (A)
AA
CYP
EET
DiHETrE
COX
PG2
PGH2 (A, Com)
PGJ2, 13,14-dihydro-15-keto PGF2α, 15-keto PGF2α (A)
Increased in obesity-related diseases
Decreased in obesity-related diseases

oxylipins

Oxylipins: Targeted metabolomic analysis in Liver

PLS-DA

R^2X: 0.604 R^2Y: 0.590 Q^2: 0.409 p-value < 0.05



LE_NC	OL_NC	OL_HF/HC	OL_LOBE	OL_PIO	Oxylipins & Fatty acids	
1.00	0.36	0.28	0.18	0.22		Arachidonic acid 유래
1.00	0.49	0.48	0.39	0.37		
1.00	0.50	0.49	0.27	0.29		
1.00	0.54	0.33	0.22	0.29		
1.00	0.94	0.38	0.36	0.48		
1.00	0.42	0.44	0.43	0.77		
1.00	0.77	0.55	0.44	0.47		
1.00	0.59	0.29	0.32	0.44		
1.00	0.61	0.56	0.47	0.57		
1.00	0.57	0.29	0.20	0.28		
1.00	0.52	0.27	0.25	0.31		
1.00	0.39	0.19	0.14	0.19		
1.00	0.37	0.18	0.13	0.17		
1.00	0.38	0.51	0.15	0.21		
1.00	0.68	0.39	0.22	0.37		
1.00	0.49	0.51	0.27	0.30		
1.00	0.49	0.36	0.23	0.37		
1.00	0.42	0.29	0.18	0.27		
1.00	1.05	0.00	0.00	0.00		
1.00	1.34	0.35	0.31	0.31		
1.00	1.09	0.42	0.28	0.47		
1.00	1.08	1.35	1.68	3.96		
1.00	1.42	0.65	1.83	2.22		
1.00	1.94	0.89	2.27	3.20		
1.00	0.23	0.37	0.44	0.51		DHA 유래
1.00	0.63	0.67	0.89	1.15		
1.00	0.47	0.57	0.71	1.01		
1.00	0.53	0.56	0.54	0.75		
1.00	0.55	0.56	1.46	1.29		EPA 유래
1.00	0.89	0.38	1.08	1.51		
1.00	0.12	0.08	0.04	0.08		
1.00	0.54	0.81	1.31	1.50		
1.00	1.02	0.62	1.56	2.16		
1.00	0.97	1.12	1.16	0.85		Linolenic acid 유래
1.00	0.96	1.04	1.20	1.10		
1.00	0.75	0.35	0.38	0.38		
1.00	0.66	0.38	0.34	0.33		
1.00	0.71	0.74	0.78	0.67		
1.00	1.59	1.32	0.96	1.14		
1.00	0.66	0.58	0.52	0.40		
1.00	0.49	0.17	0.13	0.14		
1.00	1.15	0.12	0.09	0.16		
1.00	0.88	0.10	0.11	0.16		

oxylipins

Conclusion and application

- **Diabetes complications: Plasma Metabolomics in patient with type 2 diabetes mellitus → Biomarker for prescription**

  - Glutamine, glutamic acid, and its ratio can be new biomarkers to predict the prognosis of **diabetic retinopathy**

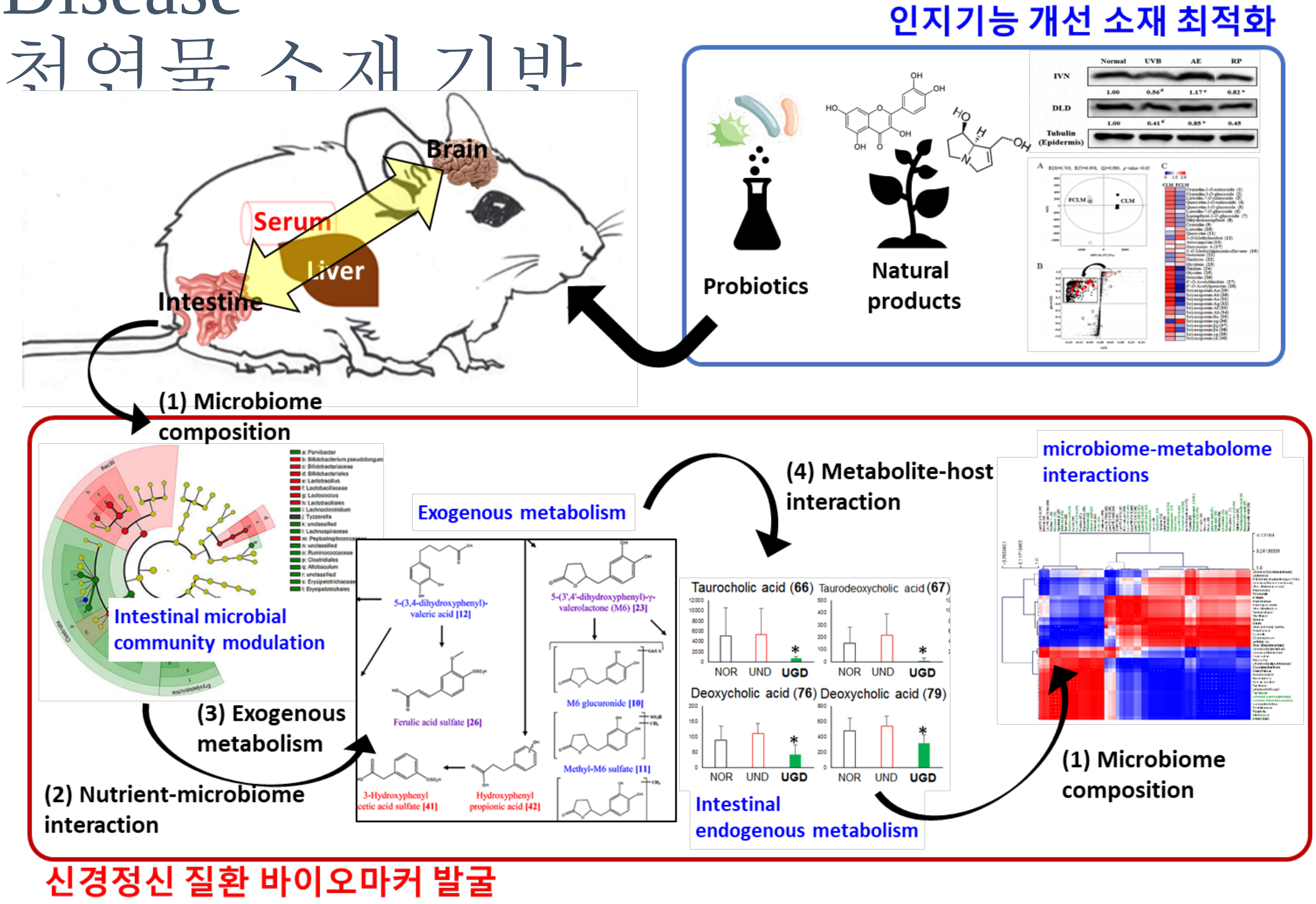
  - Plasma amino acids and oxylipins as potential multi-biomarkers for predicting **diabetic macular edema**

- **Drug mechanism: Hepatic metabolomic and lipidomic analysis of obese Type 2 diabetes in a rat model → Biomarker for drug mechanism**

  - The effect of pioglitazone on hepatic steatosis

- **Oxylipins → Biomarker and Drug**

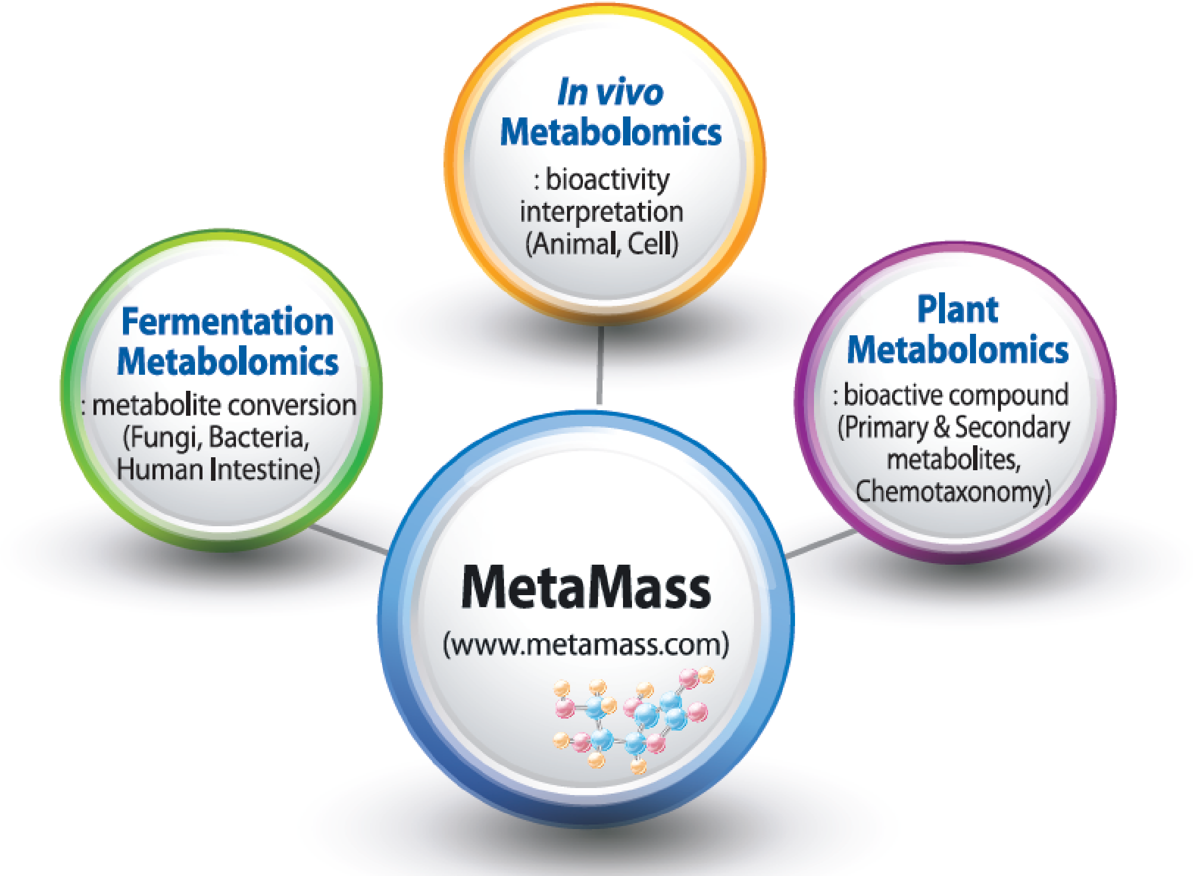
The Human Microbiome: Associated with Disease
천연물 소재 기반



주) 메타매스

"MetaMass"는

'metabolomics'와 'mass spectrometry'의 합성어로서 21세기 과학산업의 핵심인 생명공학 산업에 있어서 기능성 식품, 천연물, 미생물, 생체 등을 바탕으로 표준화 기술을 확립함으로써 다양한 산업분야에서의 고부가 가치를 창출하고자 하는 대사체 해석 전문 회사입니다.



Pathway analysis / Systems biotechnology

Meta Mass

Members of Functional Metabolomics Lab.

http://www.funcmetabol.com

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Functional Metabolomics Lab
생명공학과 기능대사체 연구실