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Metabolic changes in obese diabetic rats treated with '*Andrographis Paniculata*' extracts determined by ^1H NMR-based metabolomics approach



Muhammad Tayyab Akhtar

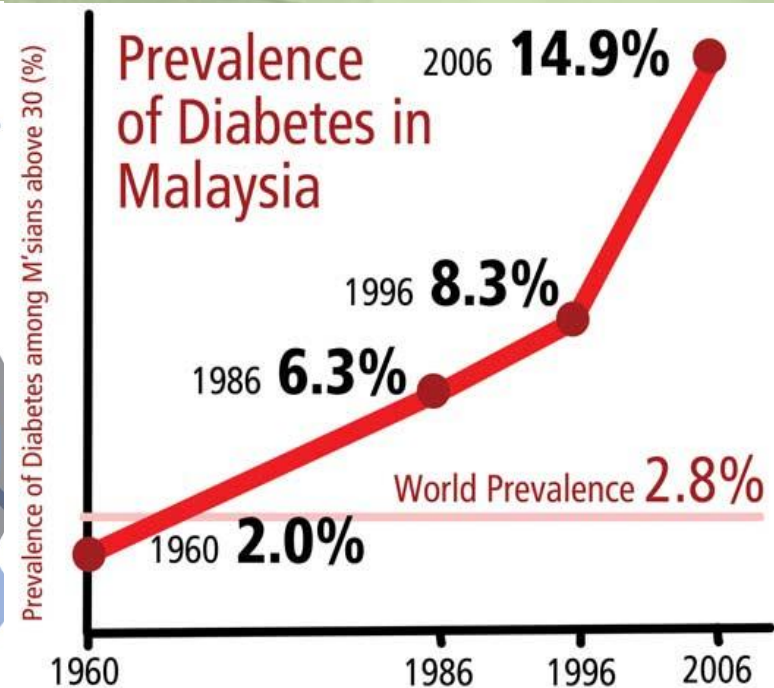
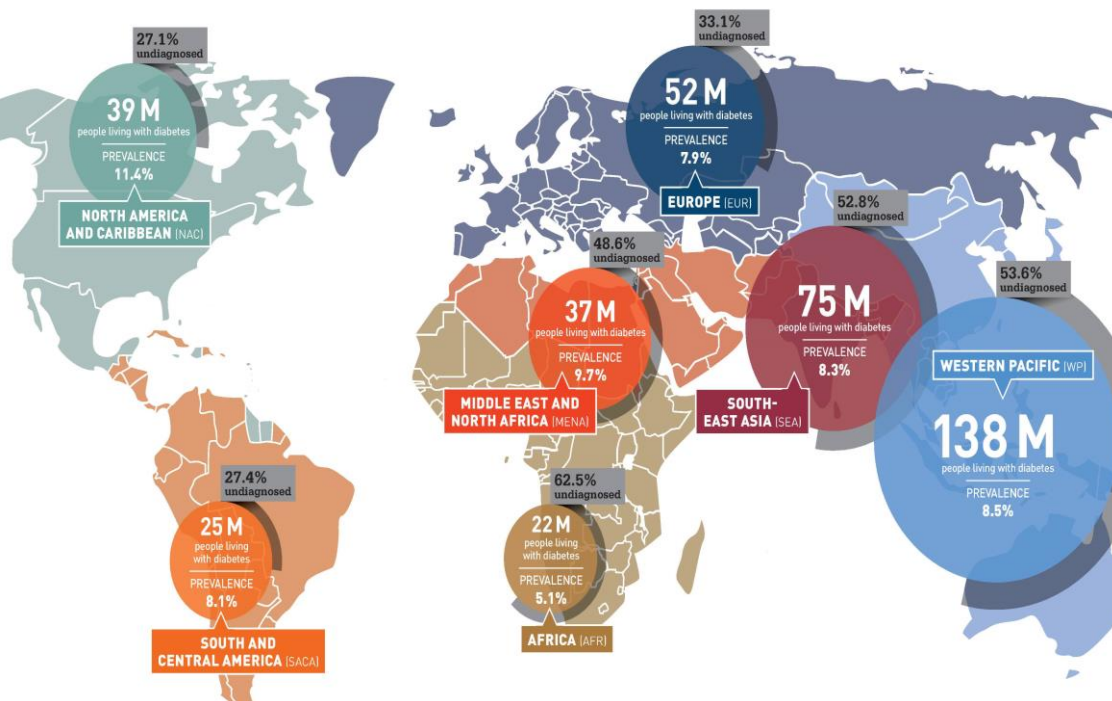
Laboratory of Natural Products
Institute of Bioscience, Universiti Putra Malaysia

Herbals Summit 26 – 27 Oct 2015
Chicago, USA

- ❑ Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose concentration, caused by insulin deficiency and often combined with resistance
- ❑ Causes of diabetes are associated with genetics, environmental factors, nutritional effects, or combination of these
- ❑ Obesity has been identified as one of major syndrome correlate with diabetes especially in non insulin dependent diabetes (Type 2 DM).



- ❑ Diabetes mellitus affects 387 million people worldwide
- ❑ Expected to increase to 592 million by 2035





Andrographis paniculata:

Widely cultivated in southern and southeastern asia, and has been used to treat different Infections and some diseases.

Traditional uses:

- Health tonic
- Detoxifier
- Gastric problems
- Appetizer
- Antimicrobial agent
- Remedy for leprosy, scabies, boils, skin eruptions, chronic and seasonal fevers , and **diabetes mellitus**



Scientific reports:

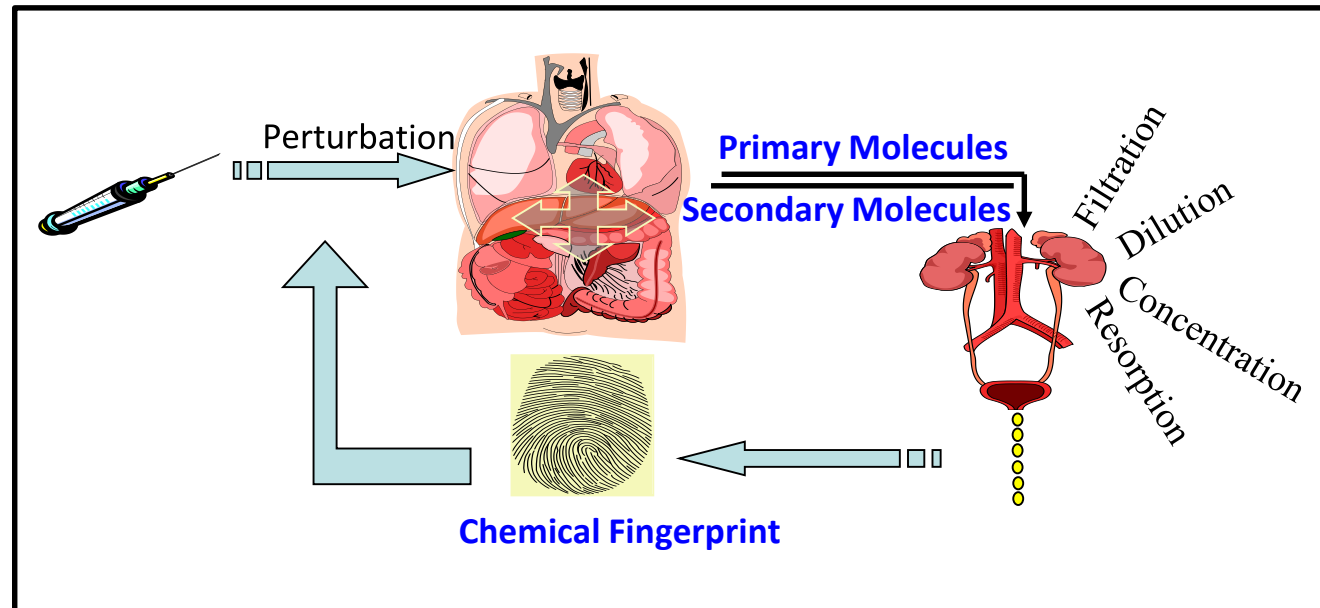
- Anti-inflammatory
- Anticancer
- Anti-HIV
- Antispasmodic
- Anti-pyretic
- Hepatoprotective
- Anti-diabetic
- Immunomodulator

METABOLOMICS

- ❑ Non-biased identification and quantification of all the metabolites present in a system at a particular time
- ❑ Metabolomic provides a unique metabolic fingerprints that a specific cellular processes or biochemical disturbance leave behind

Applications

- ✓ genetic classification
- ✓ quality control and assessments
- ✓ environmental diagnostics
- ✓ plant-host interactions
- ✓ plant bioactivity studies
- ✓ food chemistry
- ✓ biomarker discovery
- ✓ clinical toxicology



1.

Project
Objectives :

1. To discriminate *A. paniculata* varieties cultivated under controlled condition and those collected from different sources
2. To assess the potential use of metabolomics in assessing the effects of *A. paniculata* water extract on the urine of diabetes-induced rat model
3. To determine the biomarkers related to the *in vivo* anti-diabetic effects of *A. paniculata* water extract

Plant cultivation and harvesting

- ❑ *A. paniculata* (H11265) was planted in experimental plot, Institute of Bioscience, UPM.
- ❑ Plants receive watering and NPK
- ❑ Plants harvested at 10 WAT (before flowering) Immediately after harvest the leaves (45-50 kg wet weight) were separated from stems and oven-dried at @ 40°C (FRIM)
- ❑ Dried leaves (11 kg) were ground into fine powder using Wiley mill (0.70 mm mesh size)



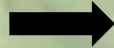
METHODOLOGY

BAHAGIAN PEMBANGUNAN HERBA (HDD)

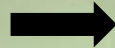
Plant drying and extraction



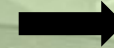
Oven drying
(40°C)



Grinding



Leaf powder



Sonication
30mins,



Dried leaf water extract



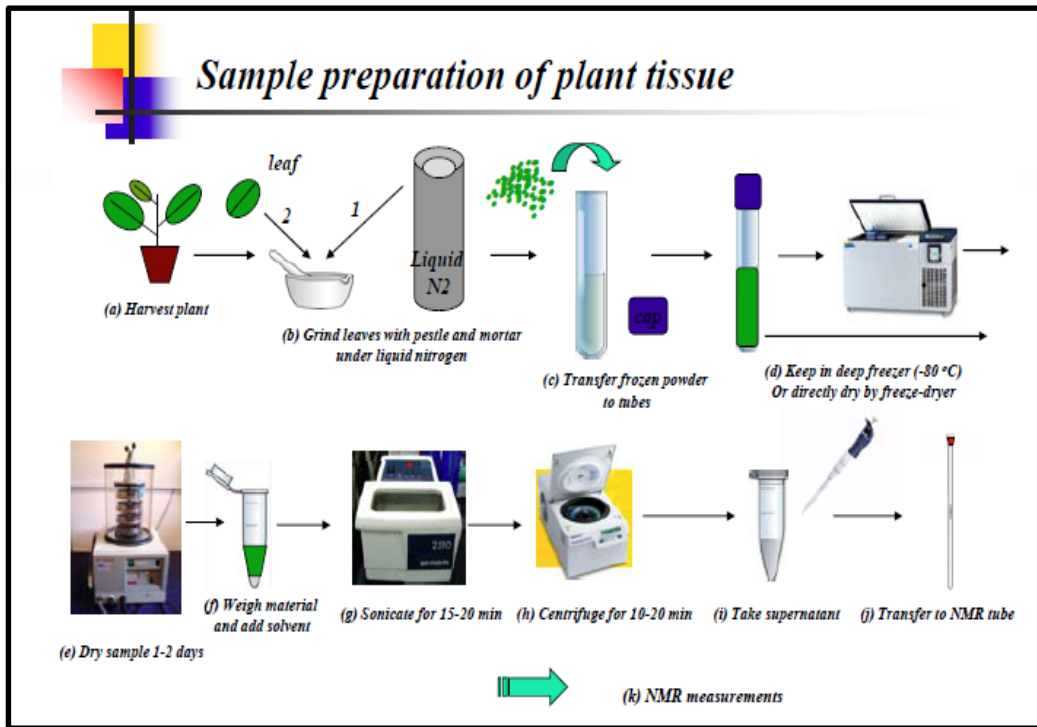
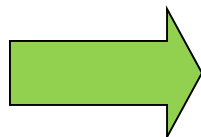
Freeze drying



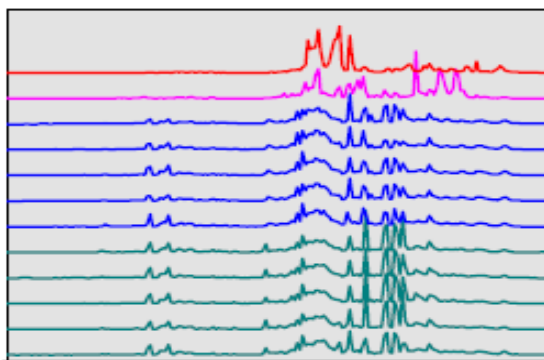
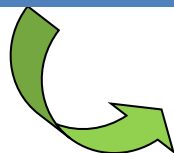
Vacuum evaporation



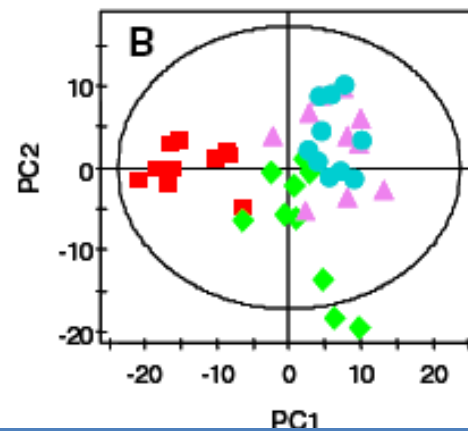
Plant varieties grown under controlled conditions



^1H NMR measurements (Varian 500MHz)

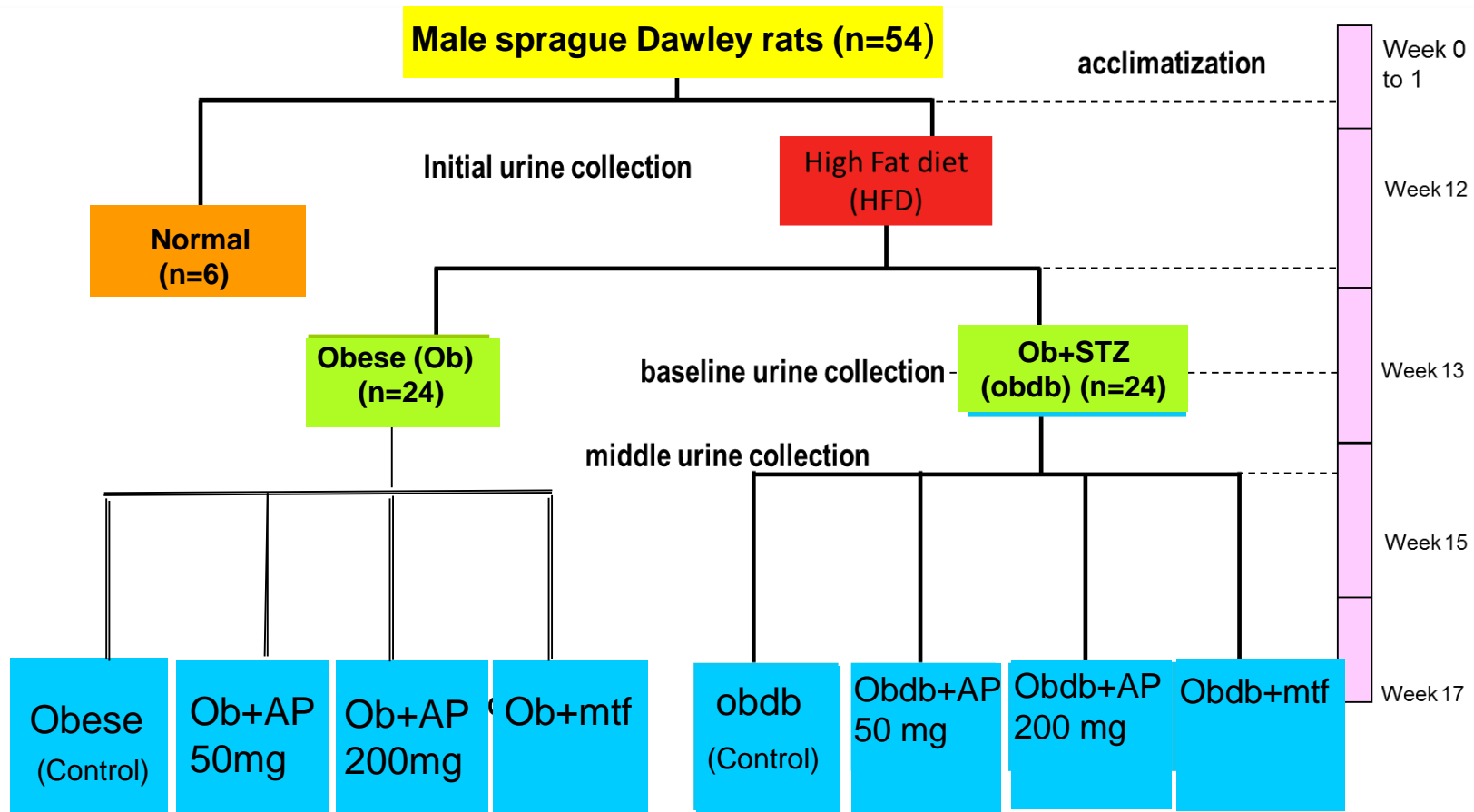


Spectra preprocessing →
Chenomx



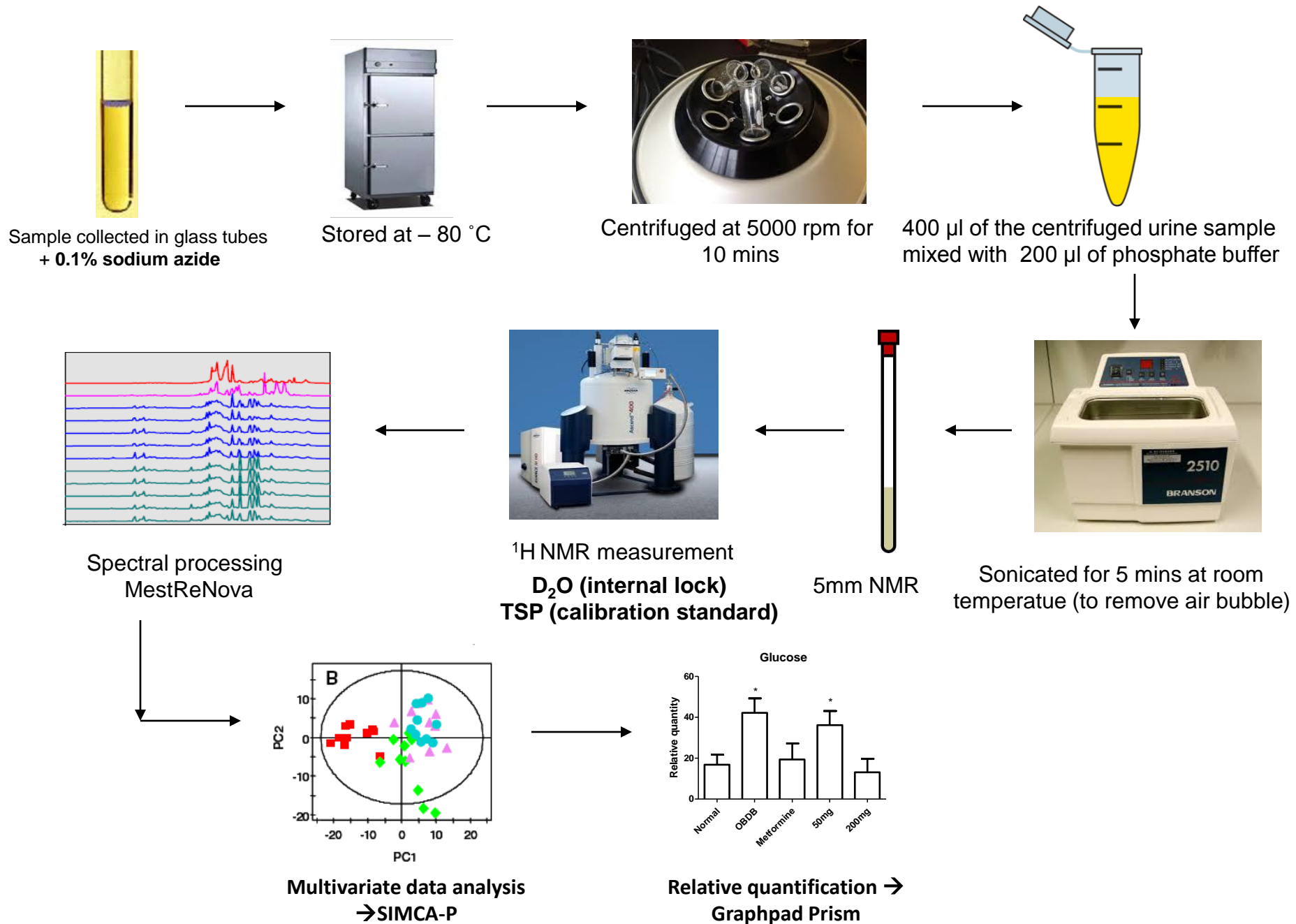
Multivariate data analysis
→SIMCA-P

Experimental design for Type II diabetes animal model study:



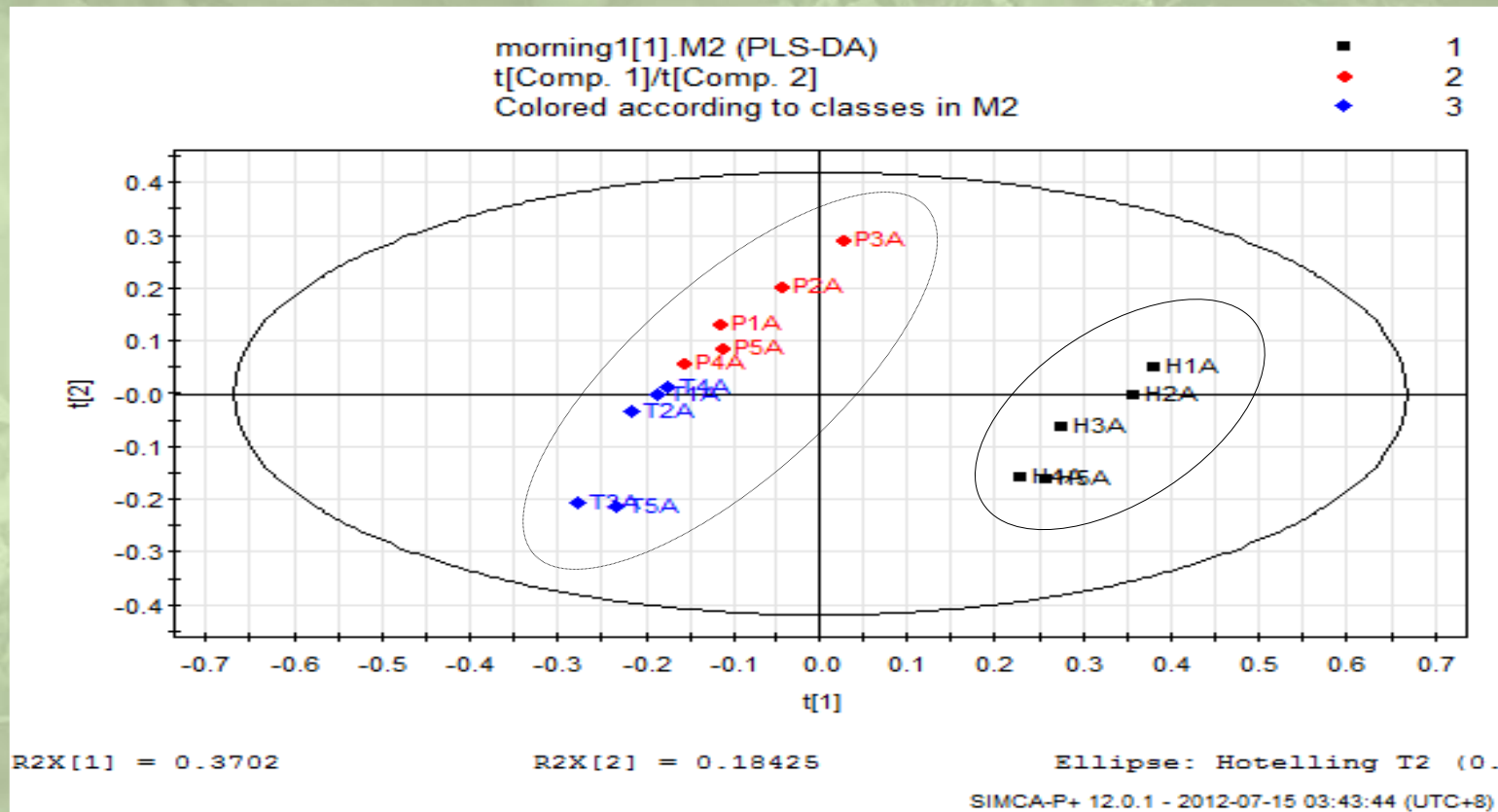
- ❑ After 3 months of HFD, obese rats were given (via intravenous injection) a low dose of STZ (25mg/kgBW)
- ❑ Rats were considered to be diabetic when basal plasma glucose concentrations exceeds 13.9 mmol/L

Preparation of urine samples and acquisition of ^1H -NMR spectra

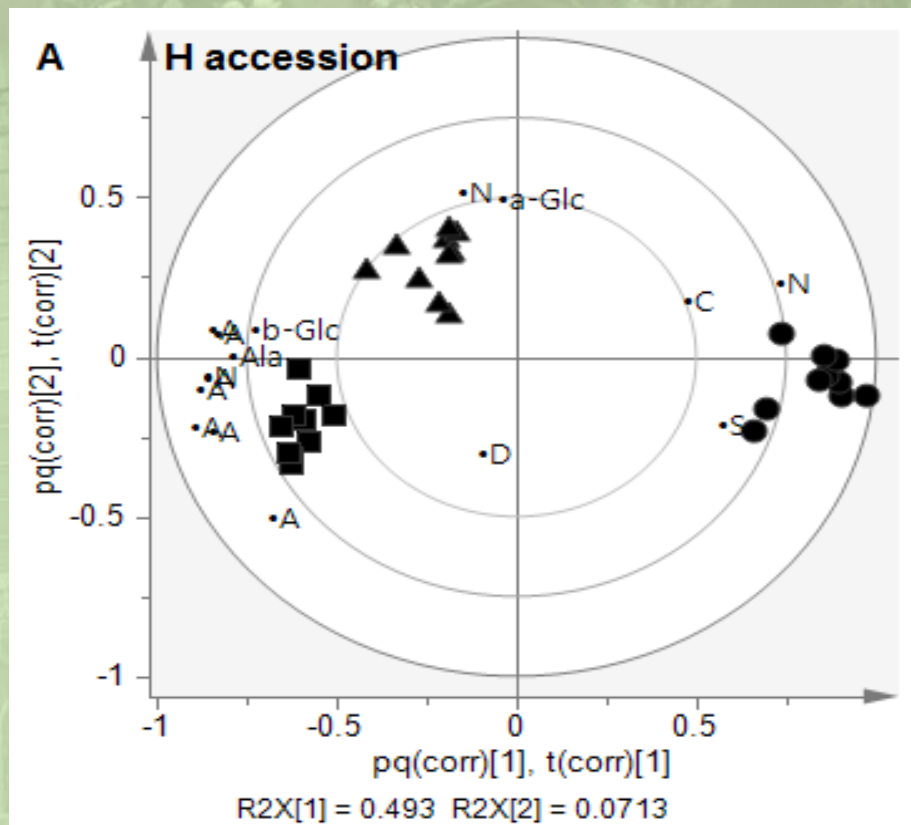
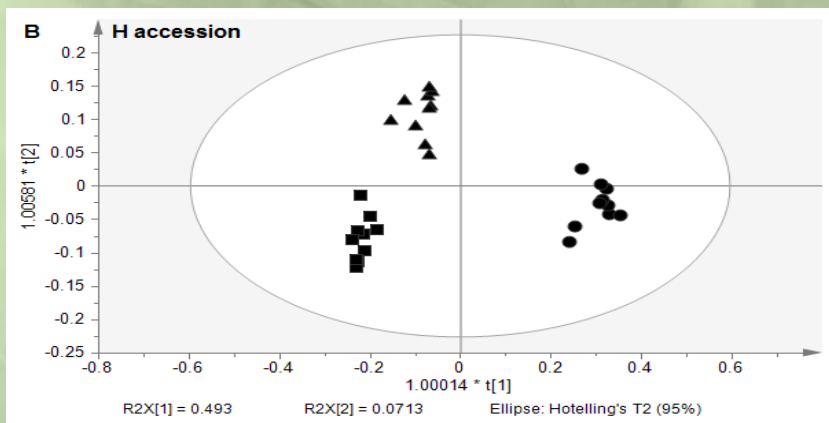


**Study 1:
Discriminative analysis of *Andrographis
paniculata* varieties and determination of
best harvesting age**

OPLS-DA score plot showed clear differentiations among the *A. paniculata* accessions

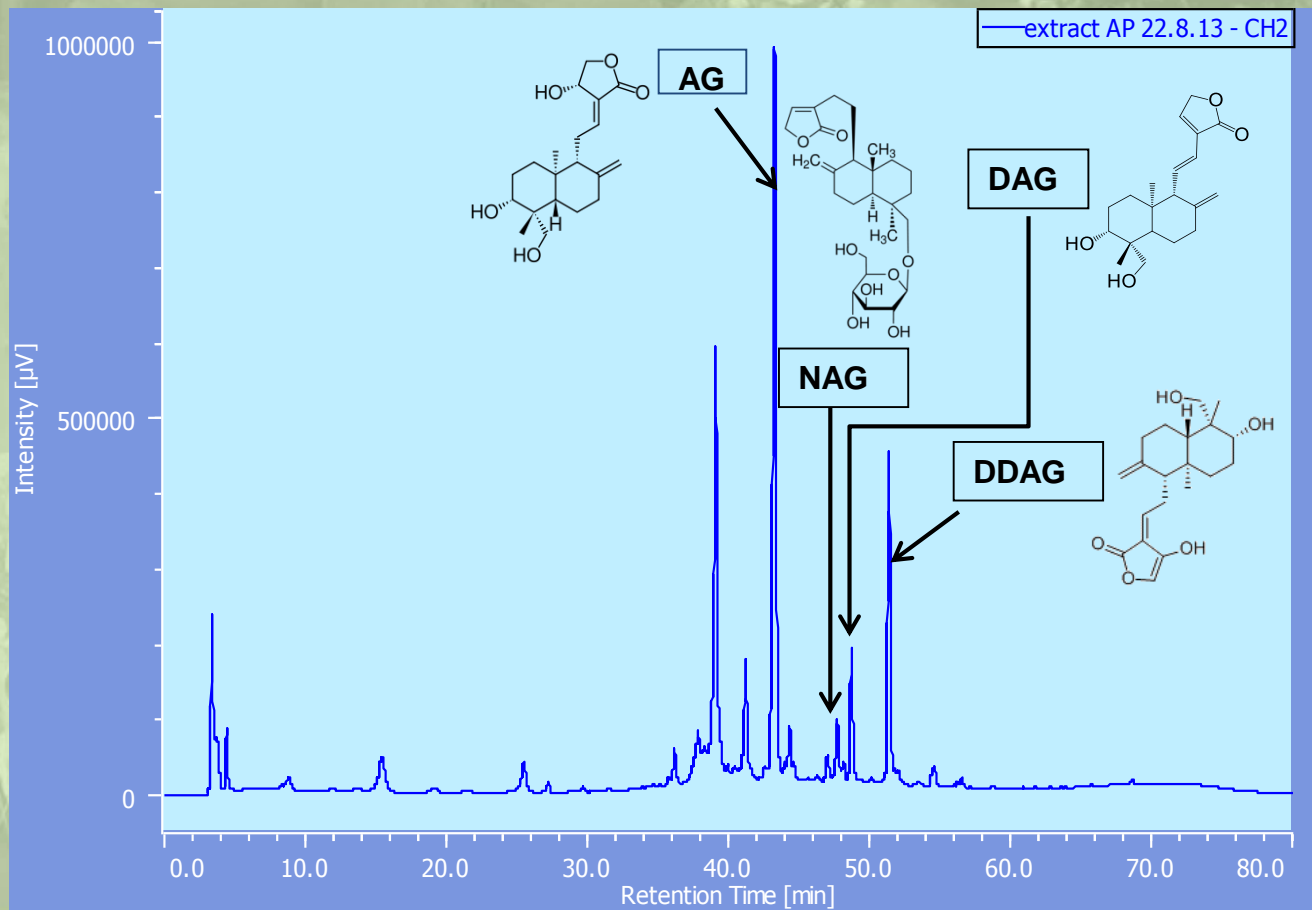


- ❑ *A. paniculata* metabolites were strongly influenced by the age.
- ❑ 120 DAT contained highest levels of andrographolide – **BEST HARVEST TIME**



OPLS-DA score plot and loadings bi-plot of marker compounds in *Ap* and their correlation to the different harvesting ages (DAT).
 Andrographolide (A) Deoxyandrographolide (D)
 Neoandrographolide (N)
 Alanine (Ala), Choline (C), Valine (V), α -glucose (a-Glc), β -glucose (b-Glc)
 ● 180 DAT ▲ 150 DAT ■ 120 DAT

HPLC of the aqueous ethanolic extract of fresh leaves of *A. paniculata*

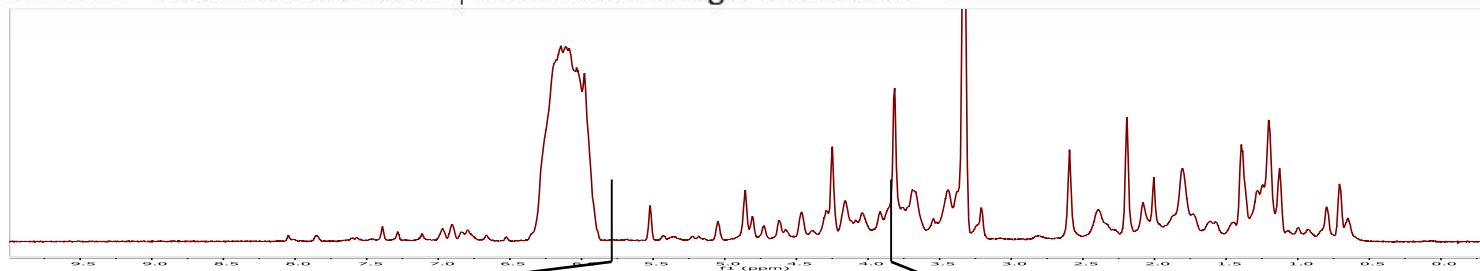




^1H QNMR analysis of *Andrographis paniculata* leaf water extract

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Compound	mg/ 25 mg of extract
Andrographolide	2.4
Dehydroandrographolide	2.1
Deoxyandrographolide	2.65
Neoandrographolide	1.88

14
13
12
11
10
9
8
7
6
5
4
3
2
1

Reference Standard

Andrographolide

Dehydroandrographolide

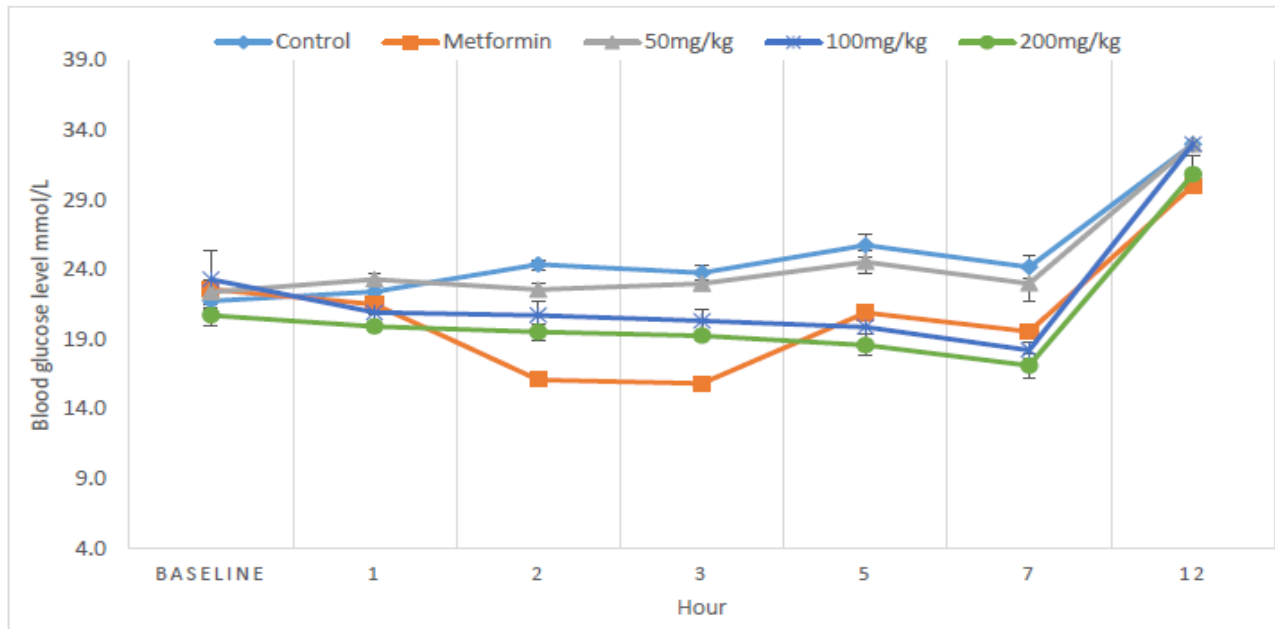
Deoxyandrographolide

Neoandrographolide

5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9
f1 (ppm)

**Study 2:
Understanding the effects of *Andrographis
paniculata* water extract on the urine of
diabetes-induced rats *via*
¹H NMR-based metabolomics**

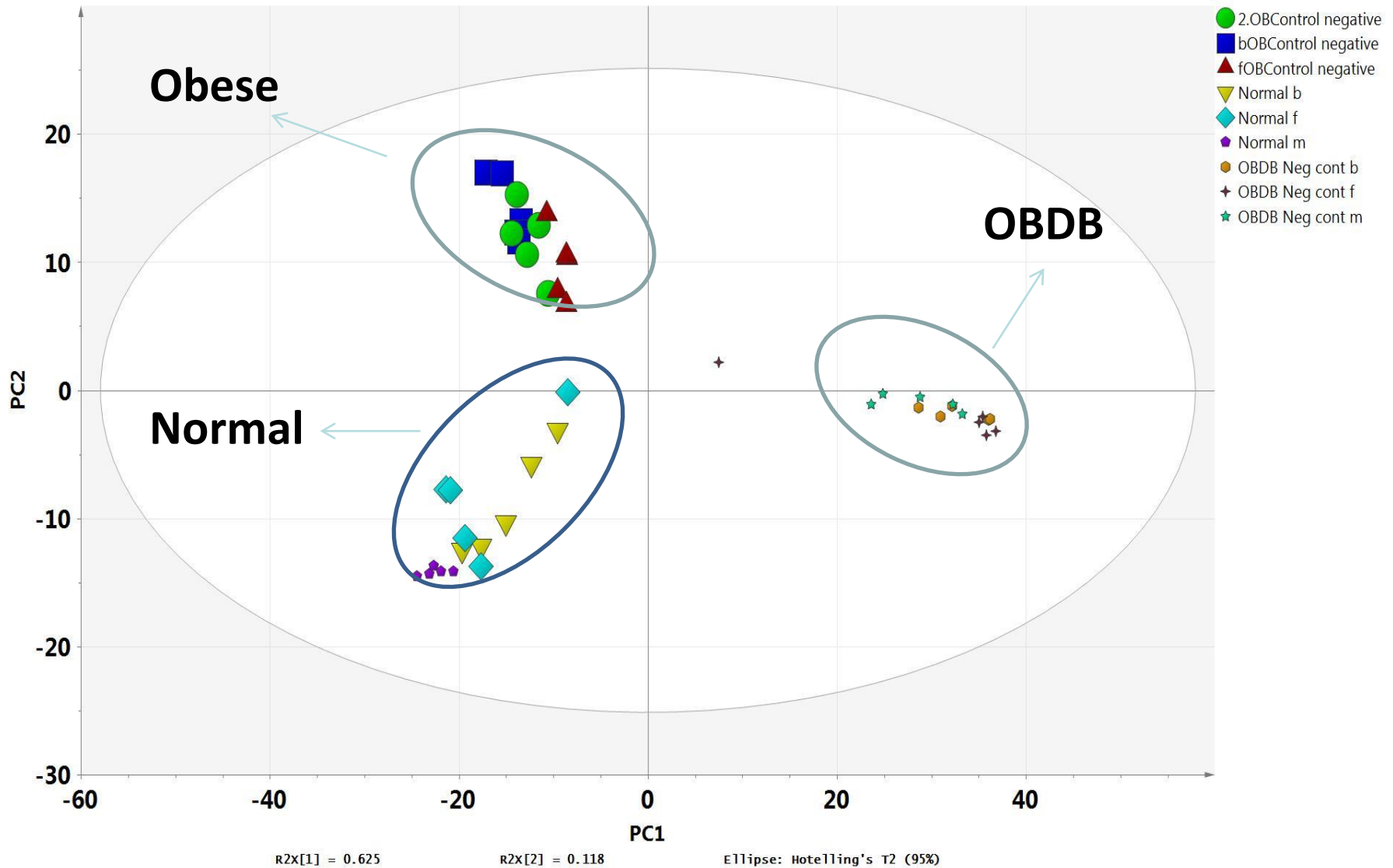
Oral glucose tolerance test for obese-diabetic group treated and non-treated with *A. paniculata*



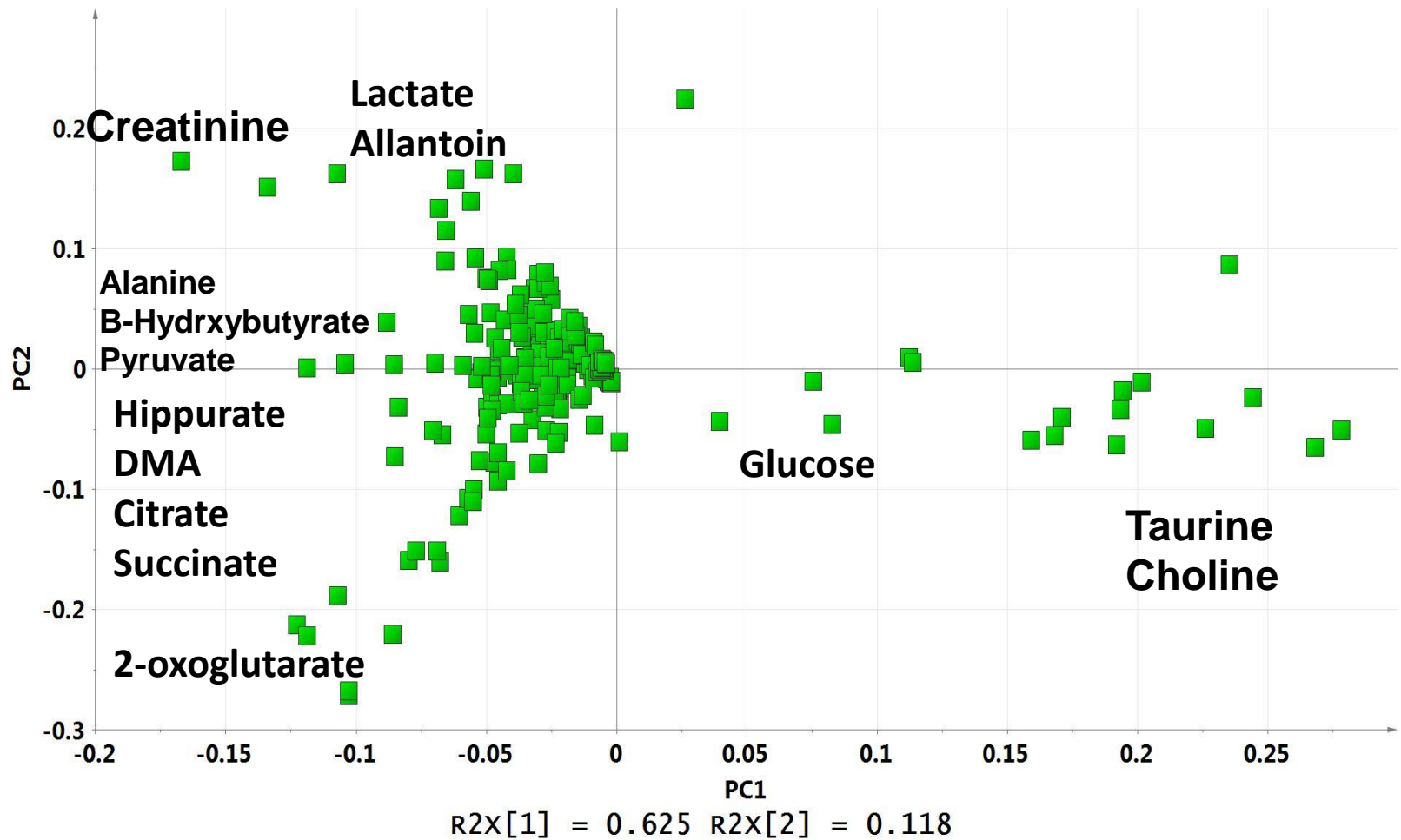
Groups	Blood glucose						
	Baseline	1hour	2hours	3hours	5hours	7hours	12hour (non-fasting)
Control DB	21.8 ±0.65	22.4 ±0.51	24.4 ±0.34**	23.8 ±0.54	25.8 ±0.83	24.2 ±0.77	33.0 ±0.00
Metformin	22.6 ±0.62	21.5 ±0.74	16.1 ±0.45**	15.9 ±0.38**	20.9 ±0.50**	19.6 ±0.49**	30.0 ±0.46
50 mg/Kg	22.4 ±0.46	23.3 ±0.46	22.6 ±0.49**	23.0 ±0.25	24.6 ±0.87	23.0 ±1.25	33.0 ±0.00
100 mg/Kg	23.3 ±2.04	20.9 ±0.94	20.7 ±1.00**	20.3 ±0.84**	19.9 ±0.50**	18.2 ±0.60**	33.0 ±0.00
200 mg/Kg	20.7 ±0.80	19.9 ±0.41**	19.5 ±0.62**	19.3 ±0.39**	18.6 ±0.74**	17.1 ±0.86**	30.8 ±1.37

*: No significant different; ±SEM

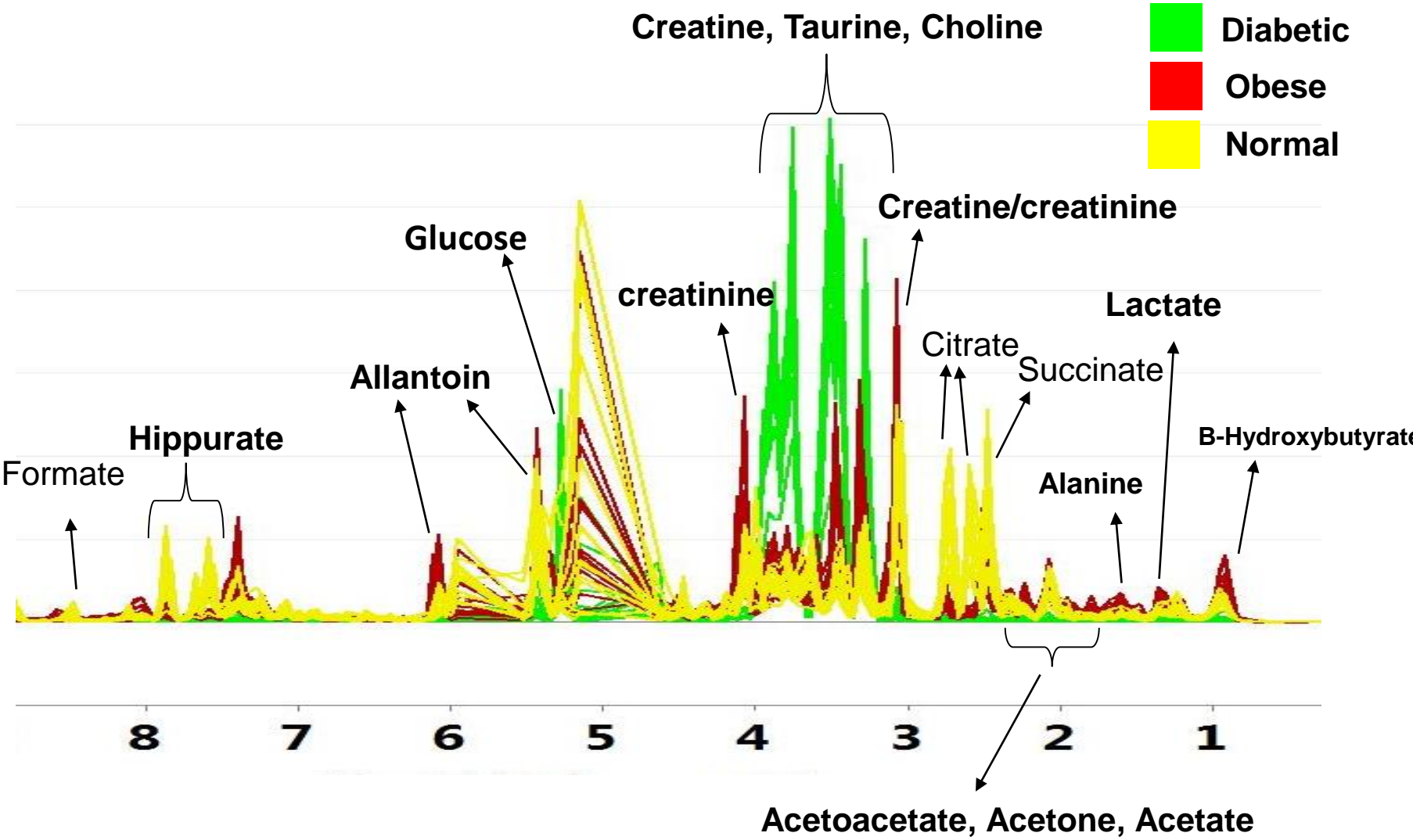
PCA Score plot of Normal, Obese and Obese diabetic rats



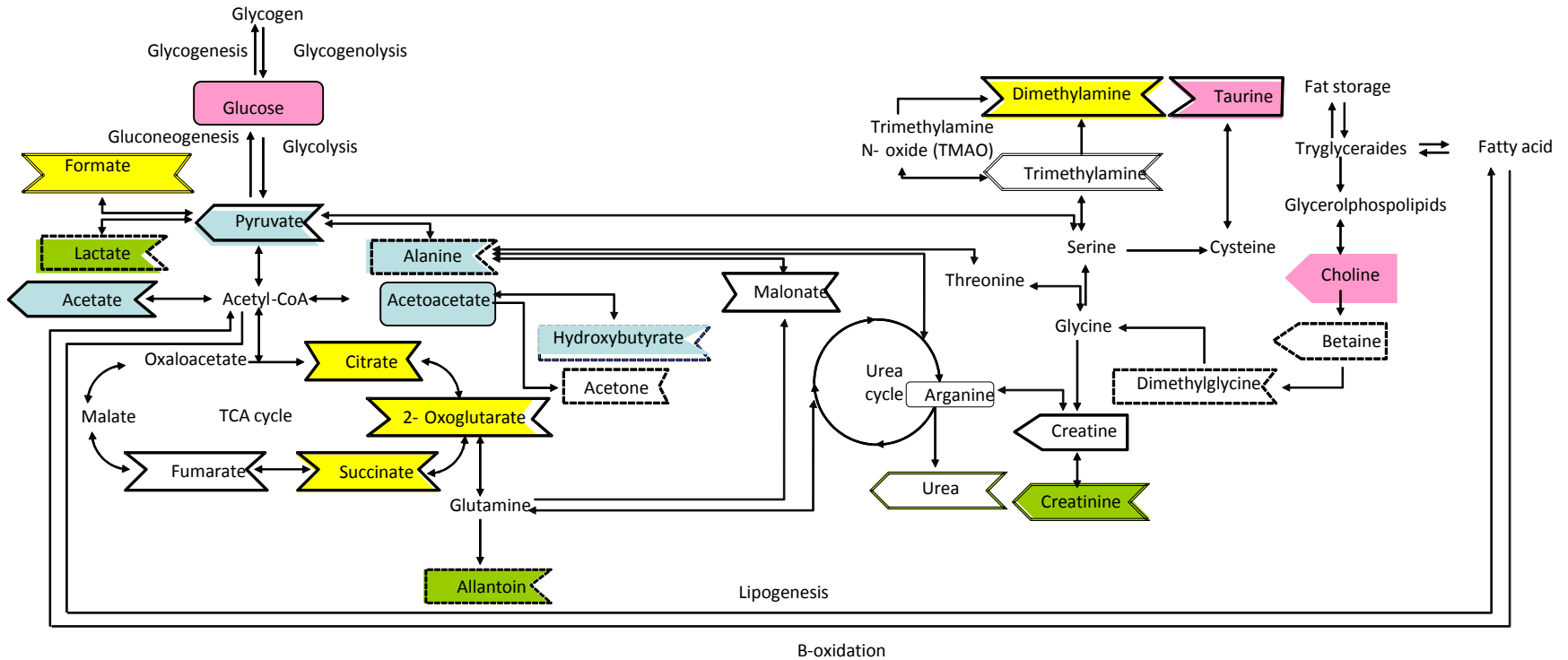
Loading plot of Normal, Obese and Obese diabetic rats



Binned 1H-NMR spectra overlay (ppm) of nd, ob and obdb rats.

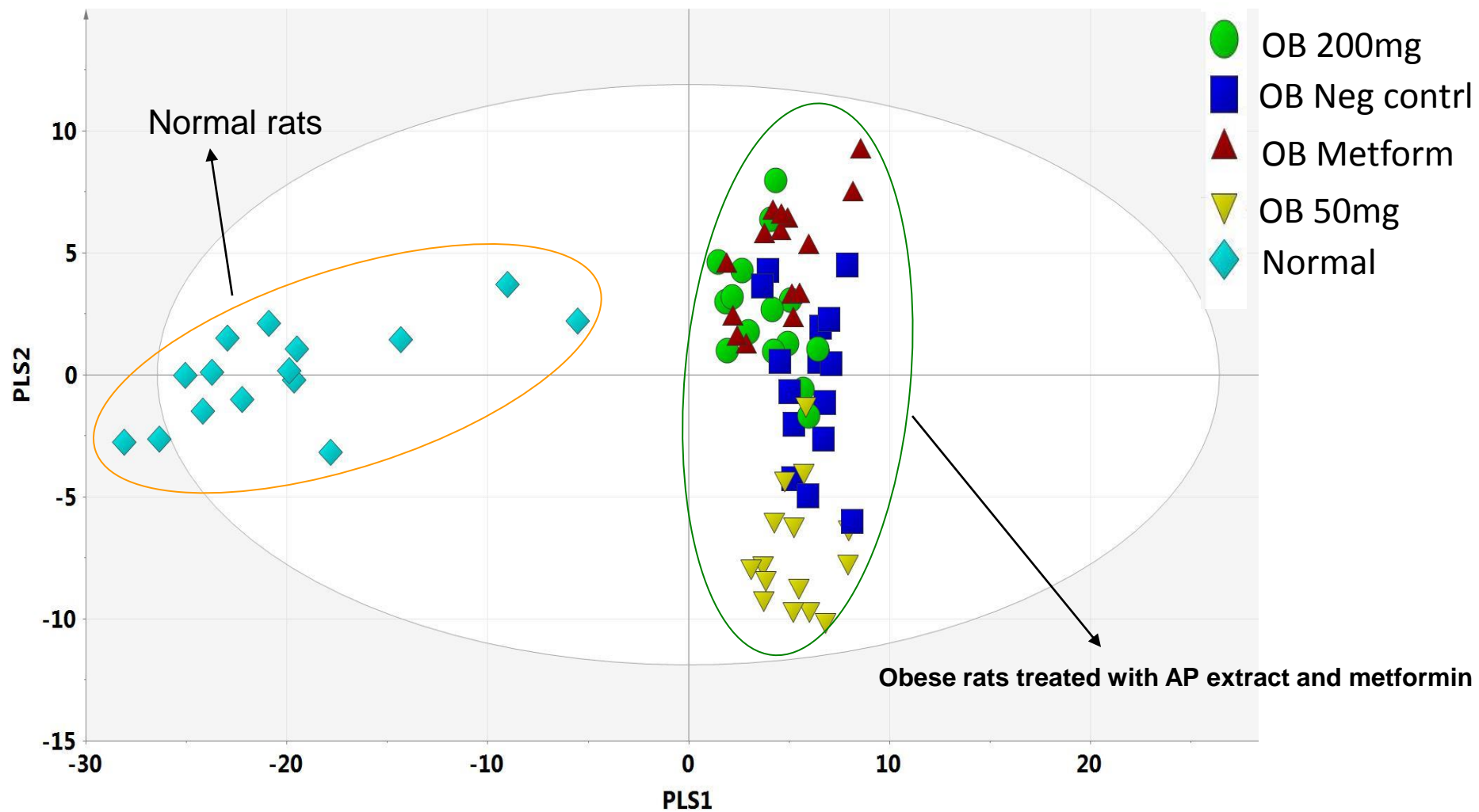


Metabolic pathway network in obese and obese diabetic rats

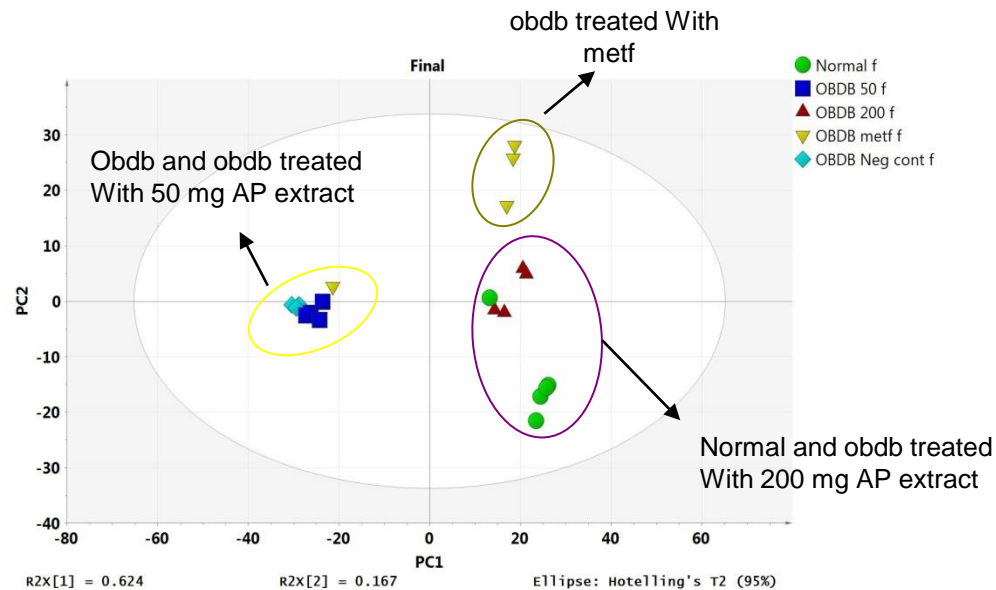
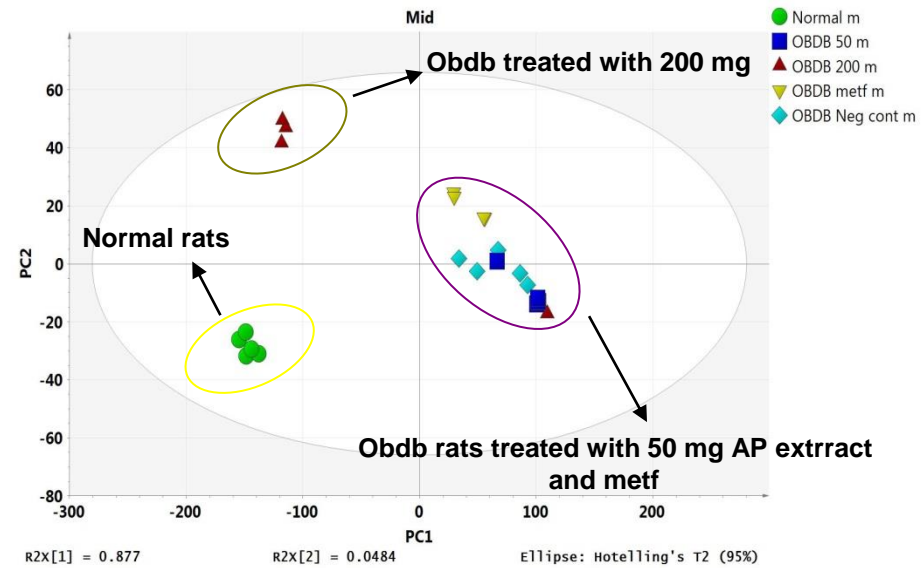
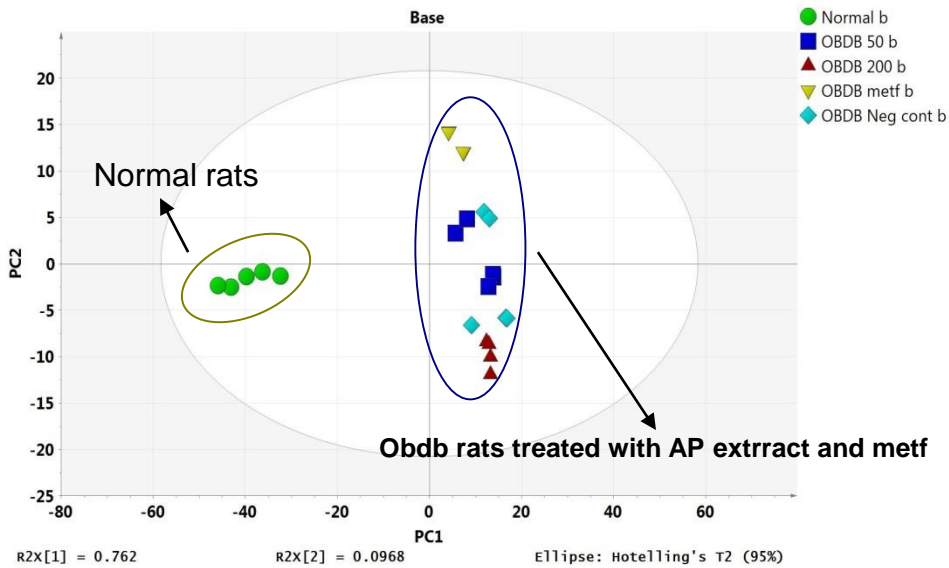


- Increased in obdb rats
- Increased in ob rats
- Decreased in OB and OBDB rats
- Similar in OB and Normal, decreased in obdb rats

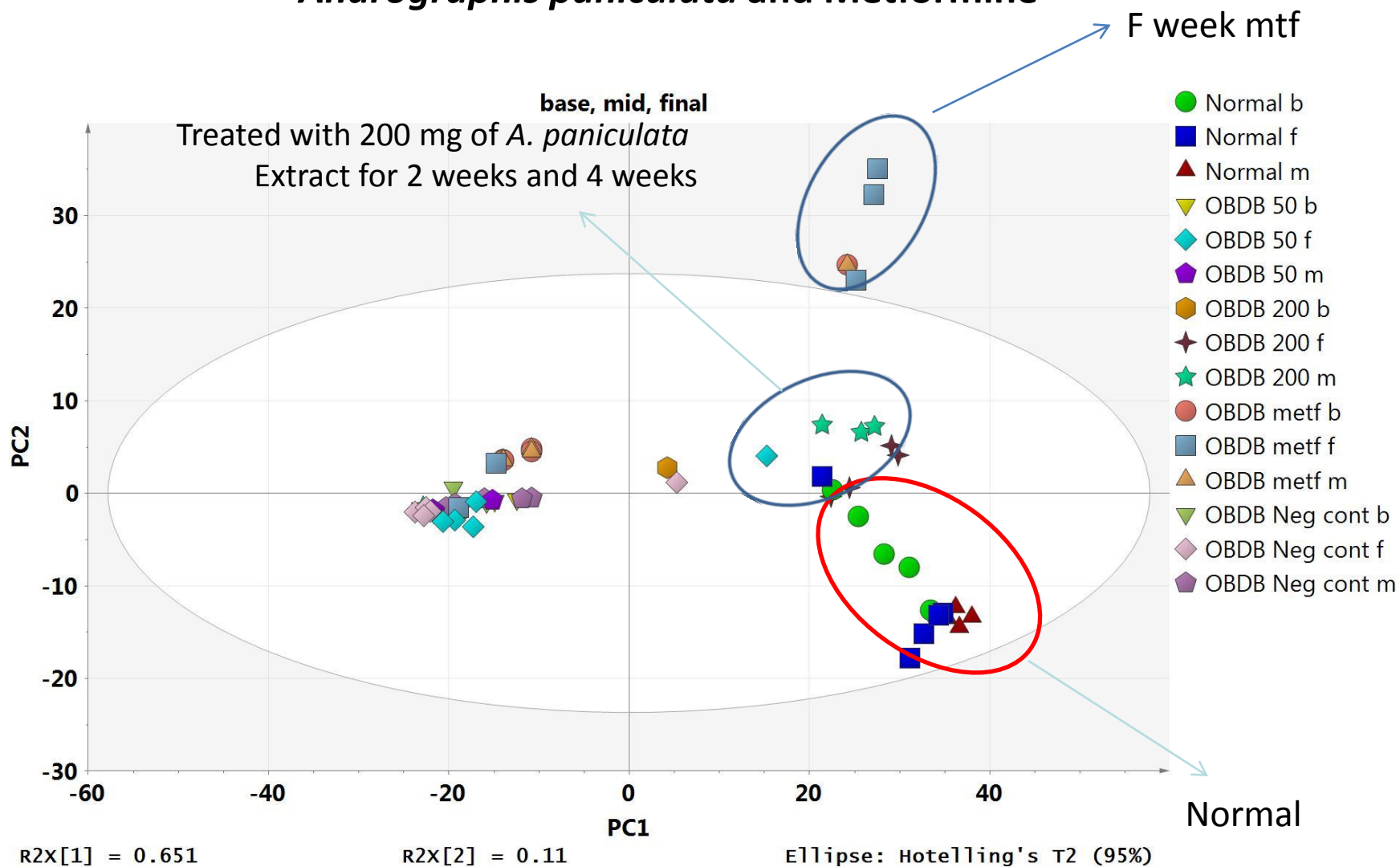
OPLS-DA Score plot of obese rats treated with *Andrographis paniculata* and Metformine



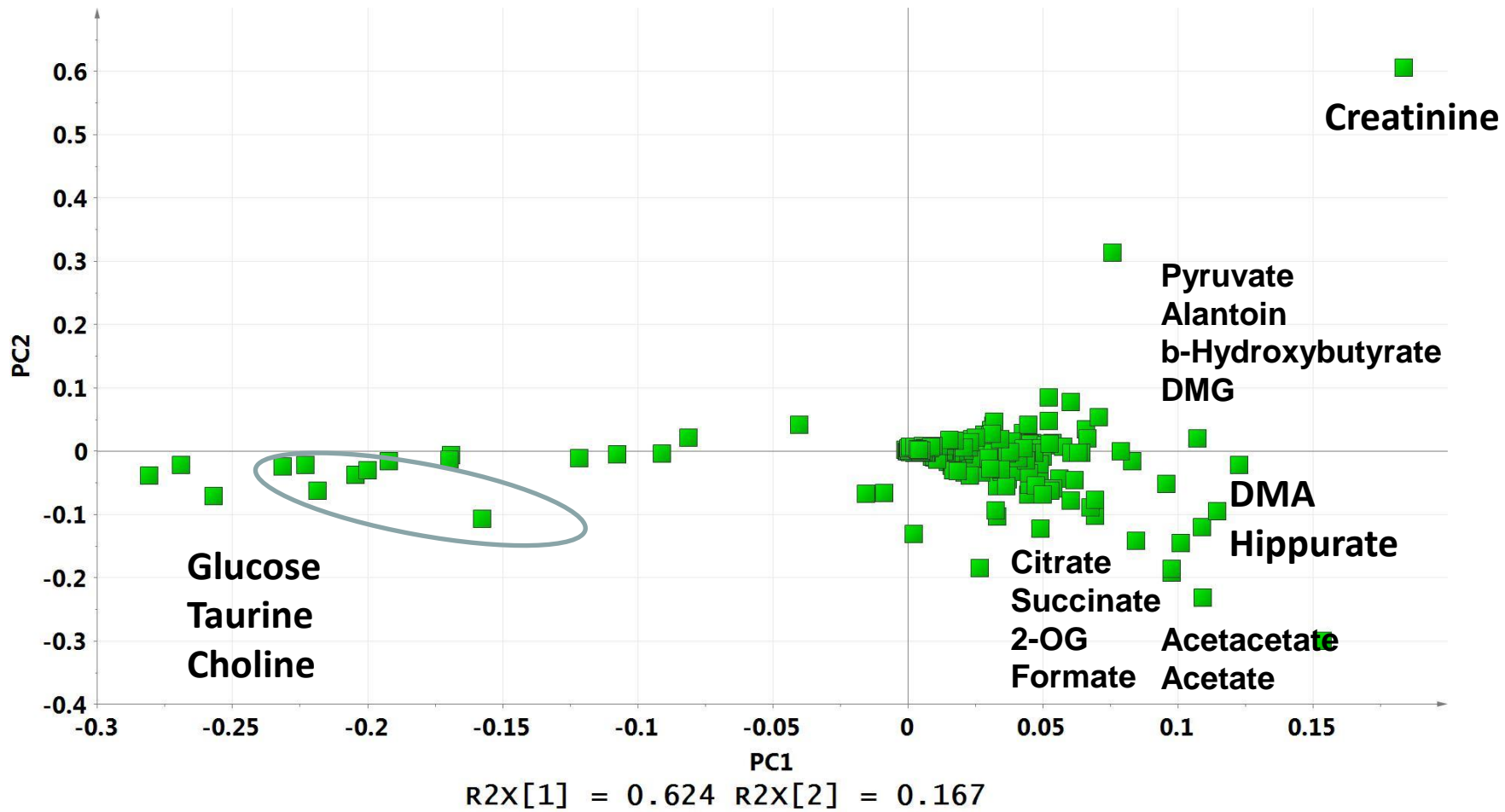
PCA score plots for obese diabetic rats treated with *Andrographis paniculata* and Metformine



Metabolite changes in obese diabetic rats treated with *Andrographis paniculata* and Metformine



Loading plot of Final week



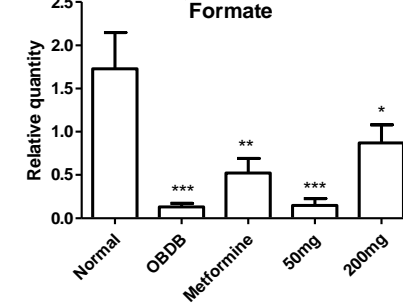
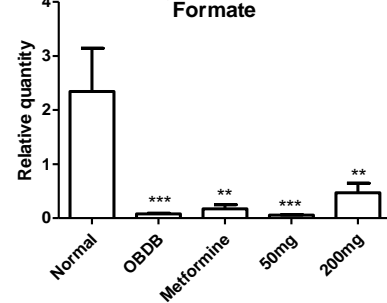
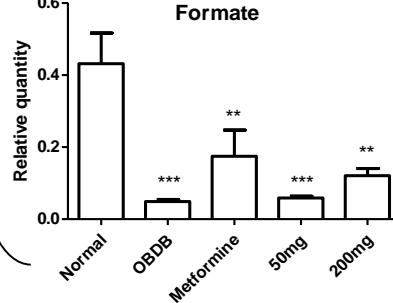
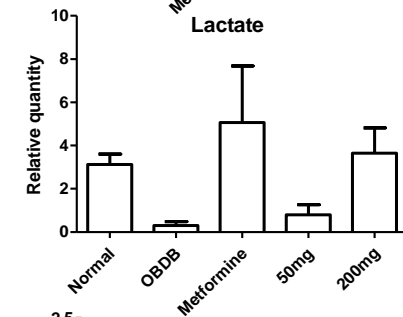
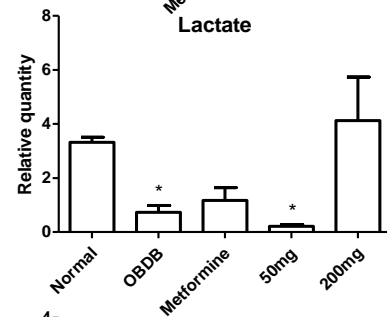
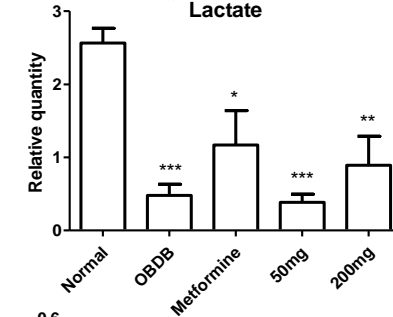
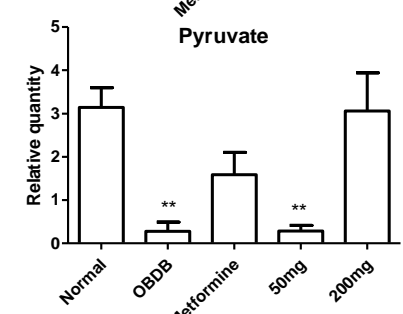
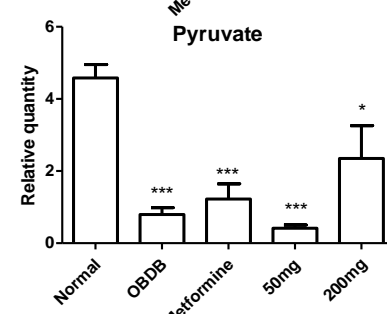
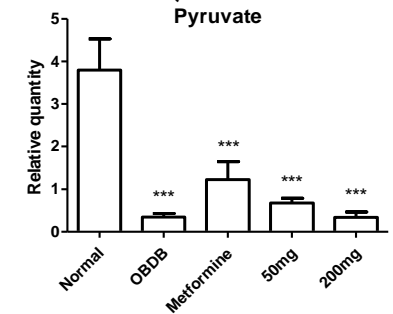
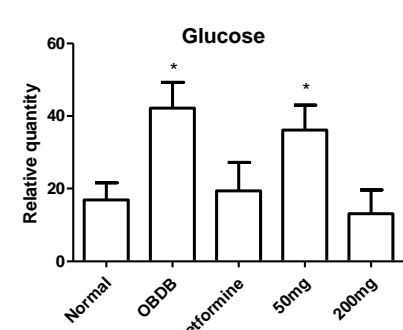
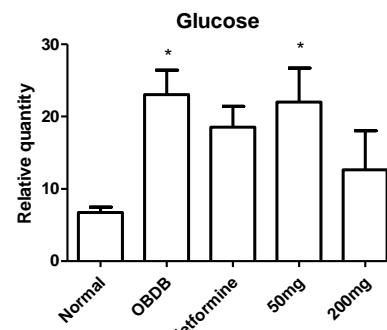
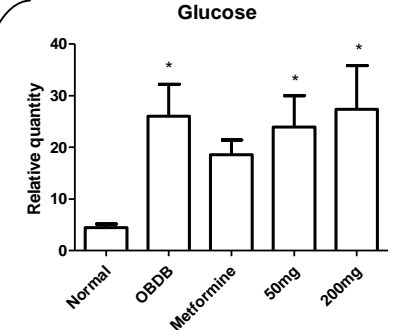
Relative quantification of discriminative metabolites

Base

2 week/Middle

Final week

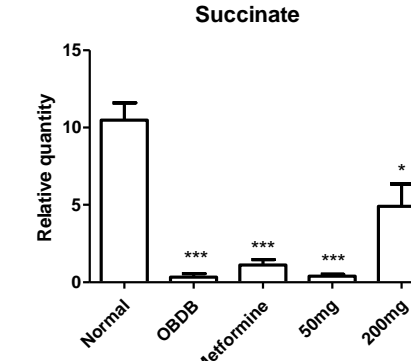
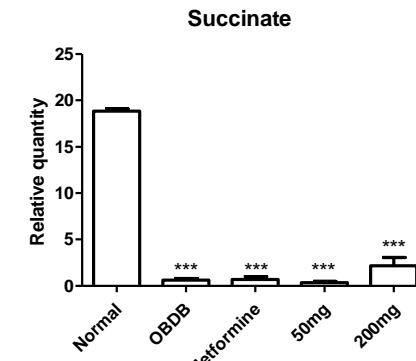
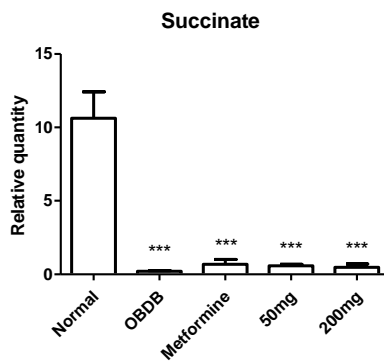
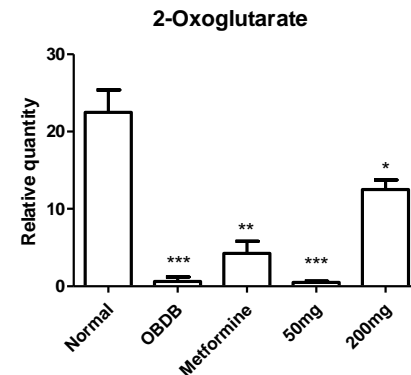
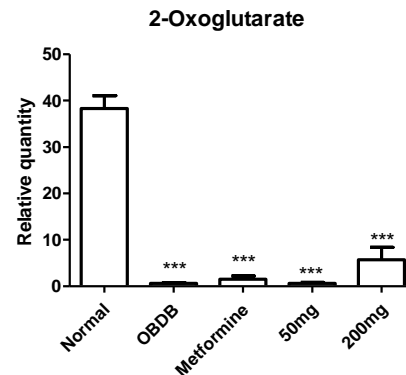
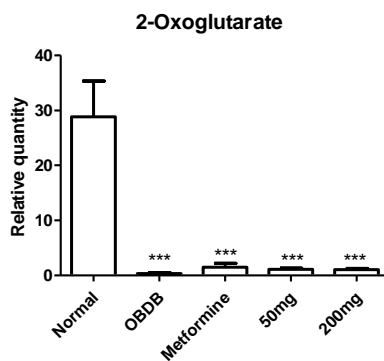
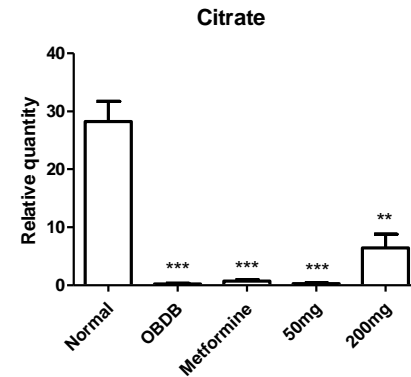
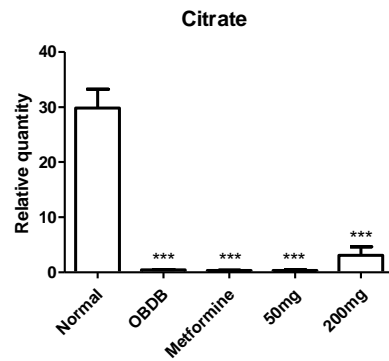
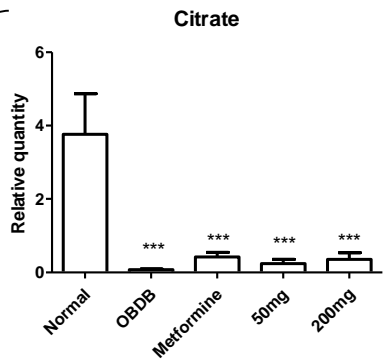
**Glucose
Metabolism**



Base

2 week/Middle

Final week



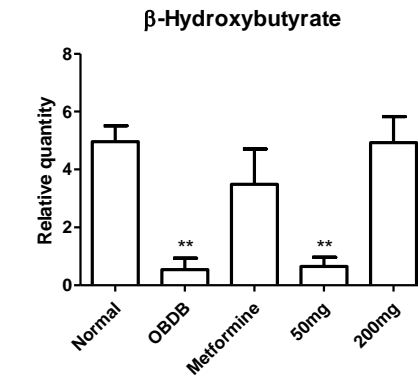
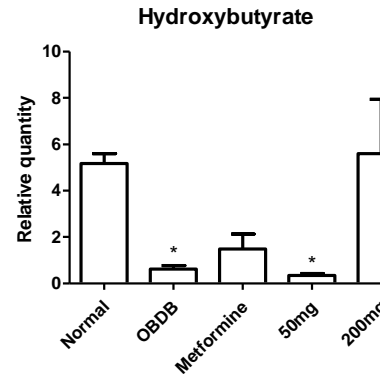
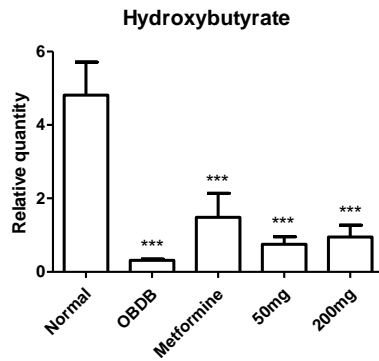
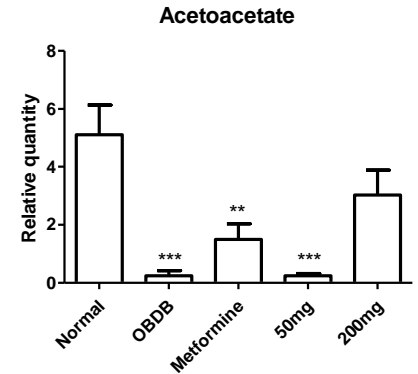
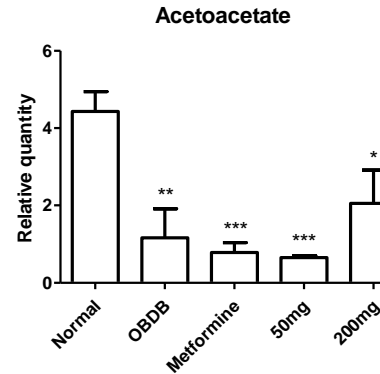
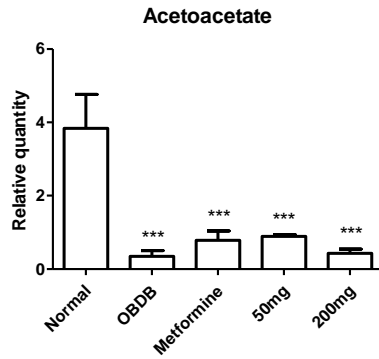
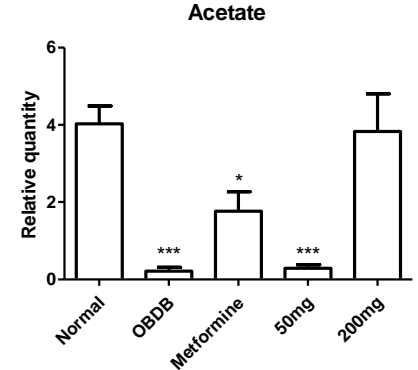
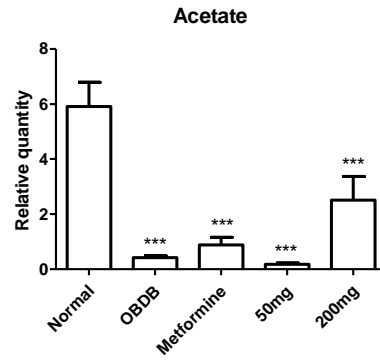
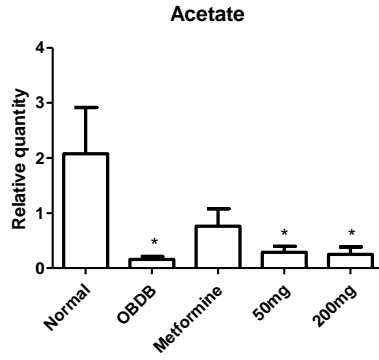
TCA cycle

Base

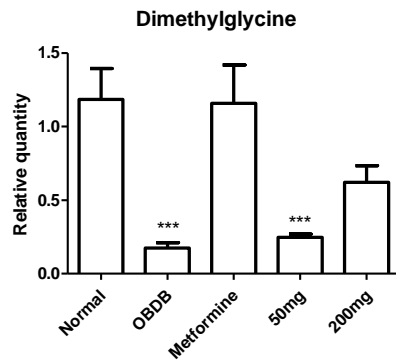
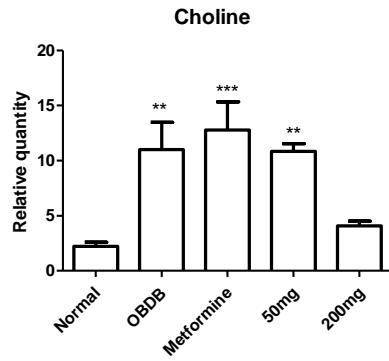
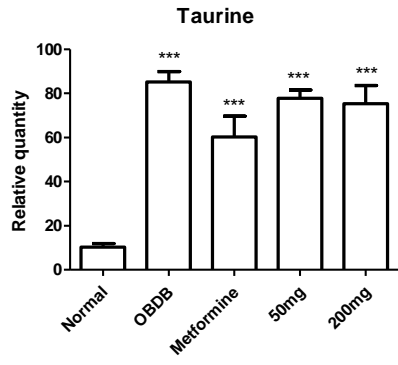
2 week/Middle

Final week

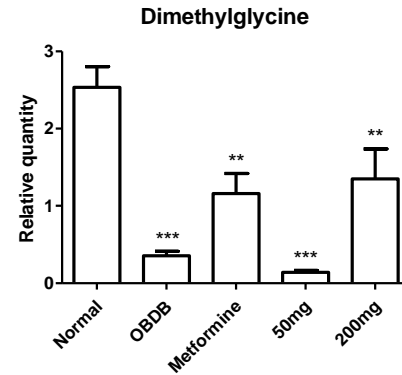
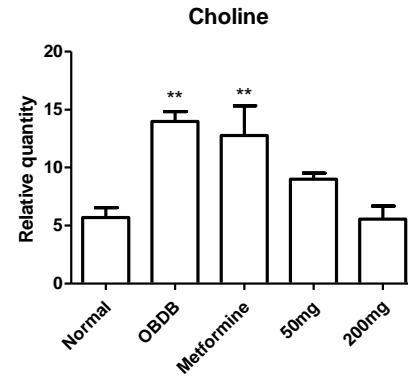
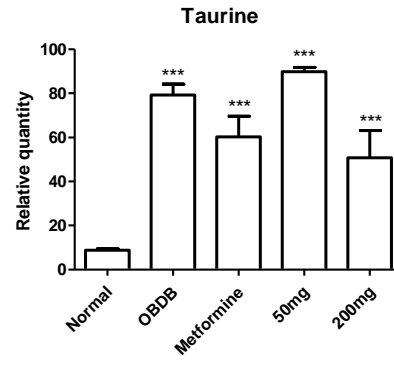
Fatty Acid Metabolism



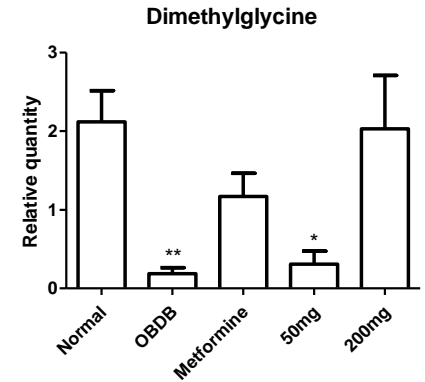
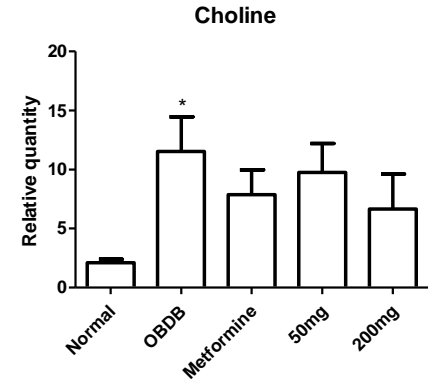
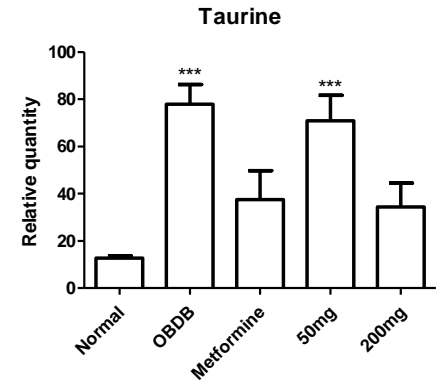
Base



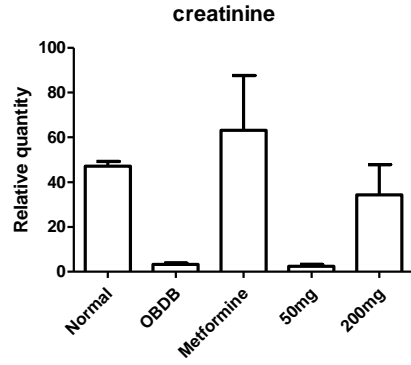
2 week/Middle



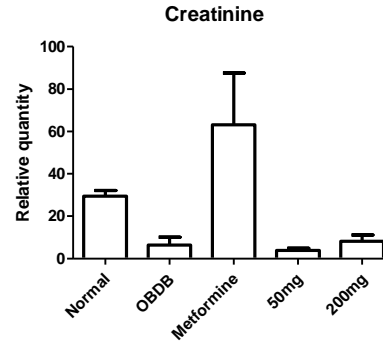
Final week



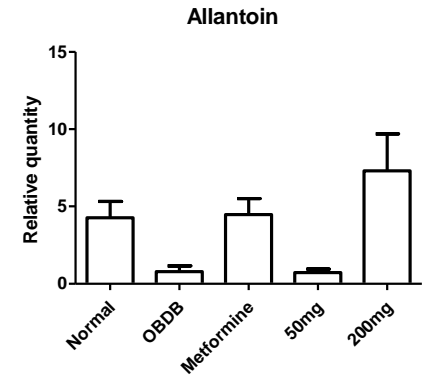
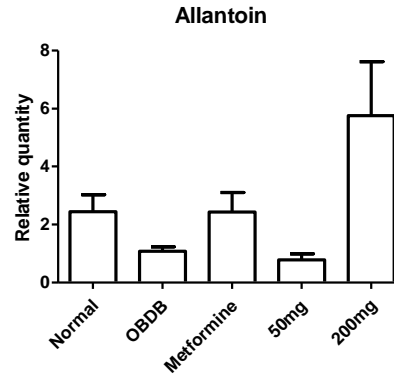
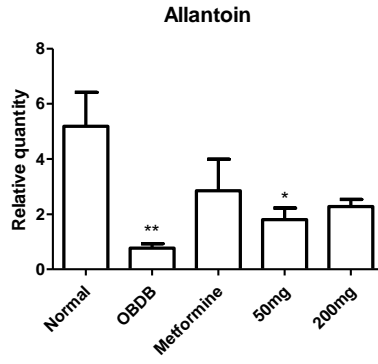
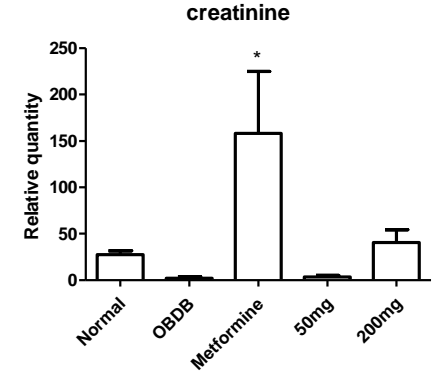
Base



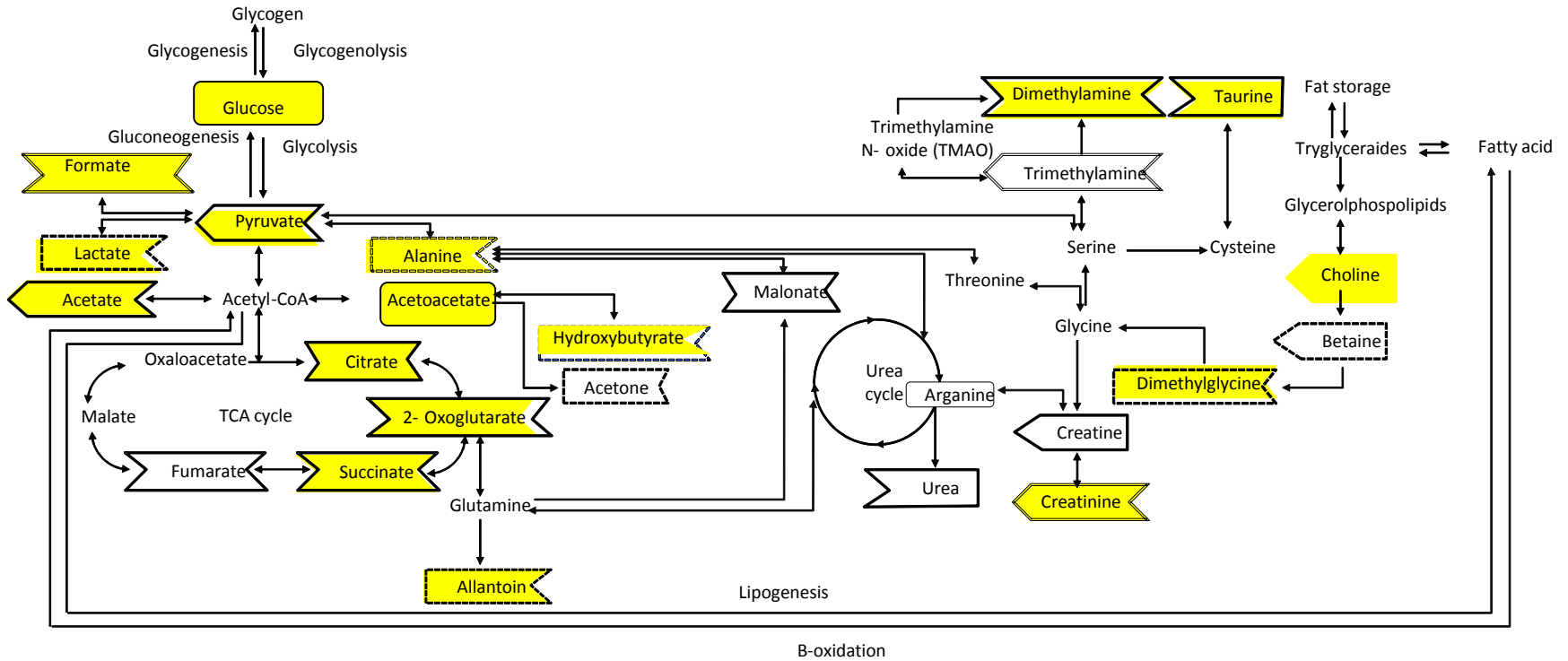
2 week/Middle



Final week



Metabolic pathway network in obese and obese diabetes



Significantly effected by 200mg of *A. paniculata* plant extract

- ✓ *A. paniculata* leaf water extract contains the four main diterpene lactones (AG, DAG, DDAG and NAG) as the bioactive constituents
- ✓ There was no anti-obesity activity was found when obese rats were treated with leaf water extract of *A. paniculata*
- ✓ Treatment with *A. paniculata* leaf water extract (at a dose of 200 mg/kg BW) was able to partially restore the disturbed metabolism of an obese-diabetic animal model back towards normal conditions

This study is still on-going, pending metabolomics results on serum samples and histopathological analysis of animal tissue samples



THANK YOU