Application of NMR based Metabonomics

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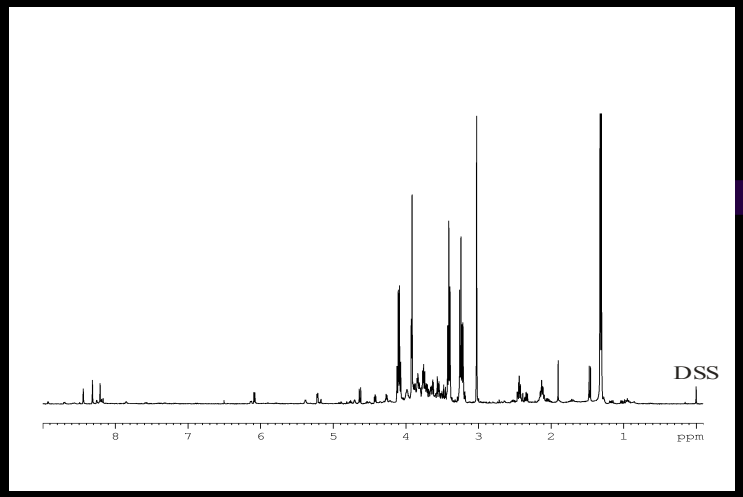
Metabonomics

Quantitative measurement of multivariate metabolic responses *of multicellular systems to pathophysiological stimuli or genetic modification*

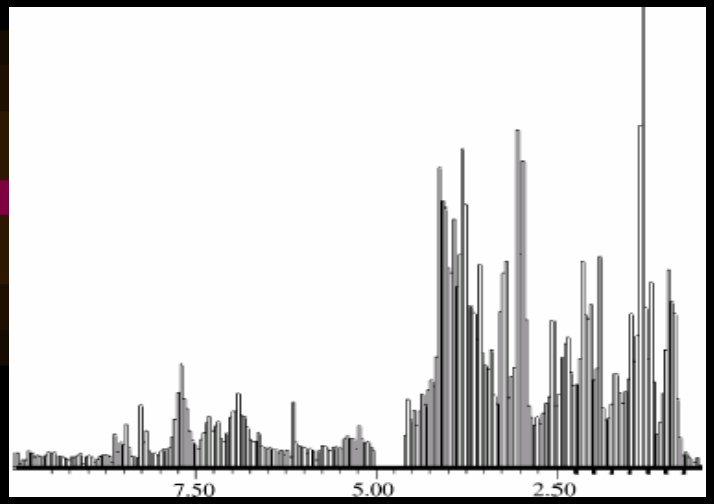
J.K. Nicholson 1999

NMR based Metabonomics



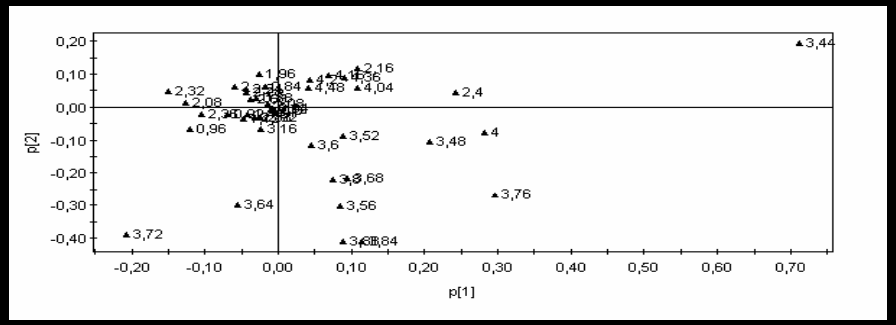
Tissue or biofluid sample

Measure the metabolite profile

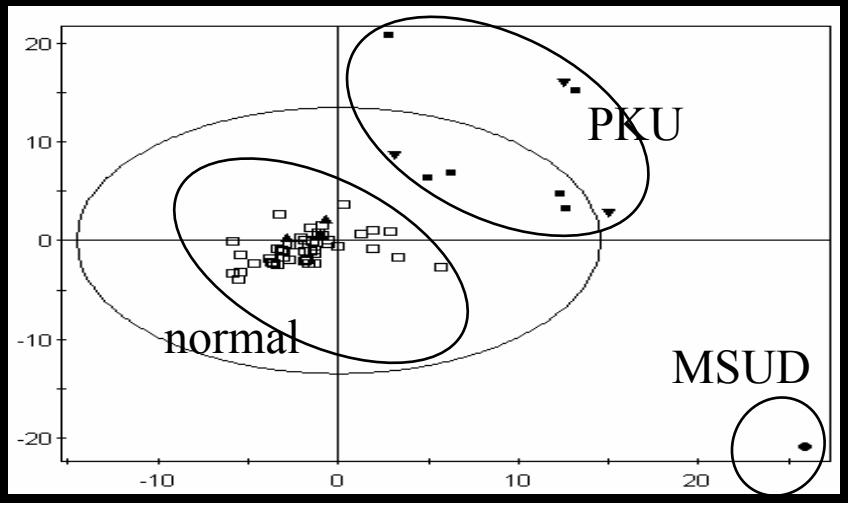


Explore metabolite profile to gain mechanistic insight

Treat metabolite profile as statistical 'object' for classification purposes



PCA



Application of NMR spectroscopy combined with principal component analysis in detecting inborn errors of metabolism using blood spots. A metabonomic approach
M.A. Constantinou, E. Papakonstantinou, M. Spraul, K. Shulpis, M.A. Koupparis, E. Mikros
Analytica Chimica Acta, 511, 303-312, 2004

Metabonomics

- Applications
  - Diagnosis
  - Drug toxicity
  - Phenotype variations

Metabonomics - Toxicity

- **Kidney cortical toxins**
  - mercury II chloride
  - *p*-aminophenol
  - uranyl nitrate
  - the anticancer drug ifosfamide
  - cephaloridine
  - the kidney medullary and papillary toxin, propylene imine
  - renal papillary toxin
  - 2-bromoethanamine hydrochloride
- **Liver toxins**
  - hydrazine
  - allyl alcohol
  - thioacetamide
  - 1-naphthylisothiocyanate
  - Allyl formate
  - galactosamine
  - bromobenzene
  - acetaminophen
  - carbon tetrachloride

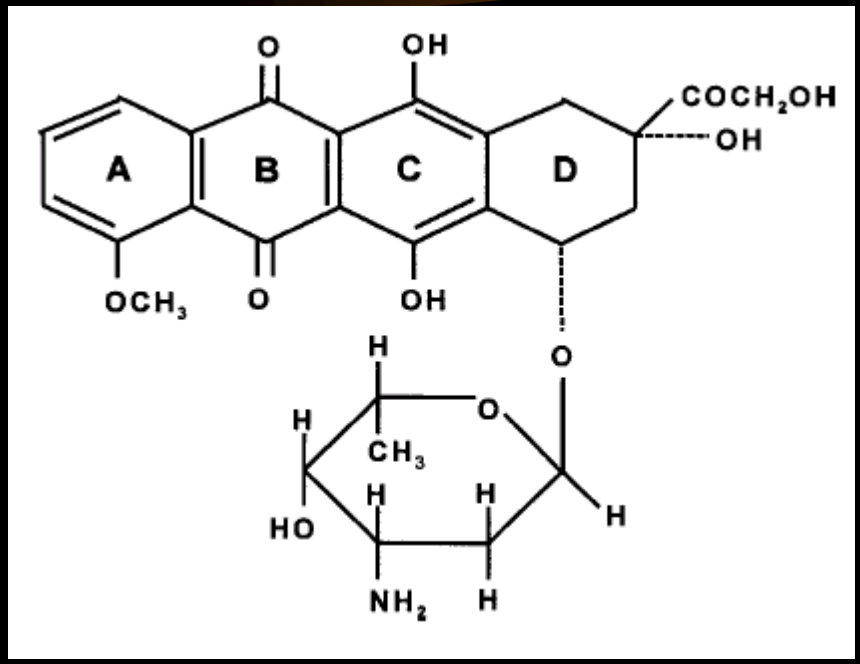
Prediction of Drug Toxicity

- Consortium for Metabonomic Toxicology COMET
  - **Metabonomic Toxicology Screening Approach**
    - five pharmaceutical companies and Imperial College, London, JK Nicholson's group
  - Construction of predictive and informative models of toxicity using NMR-based metabonomic data.
    - 147 model toxins and treatments
    - Curated databases of spectral (35 000 NMR spectra)
    - conventional (clinical chemistry, histopathology, etc.)
  - computer-based expert systems for toxicity prediction.

T.M. D. Ebbels, H. C. Keun, O.P. Beckonert, M. E. Bollard, J. C. Lindon, E. Holmes, and J. K. Nicholson
Journal of Proteome Research 2007

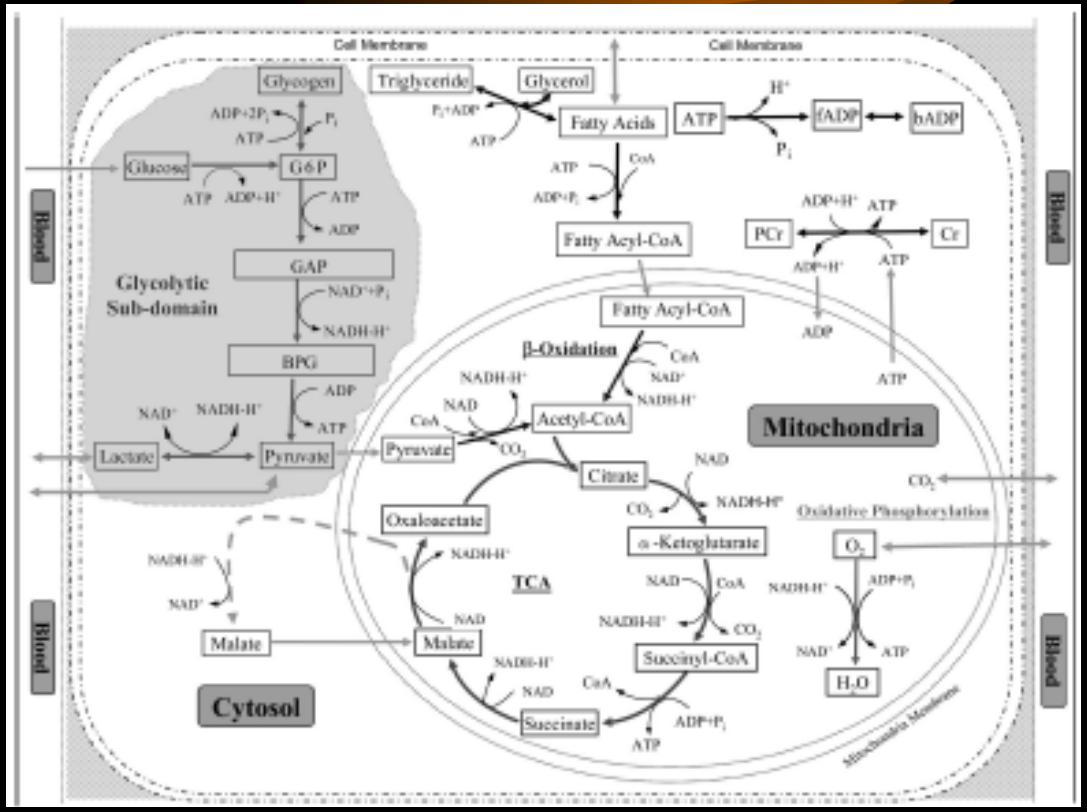
Adriamycin - DXR

Since the late 1960s, the anthracycline antibiotic doxorubicin (Adriamycin; DXR) has been one of the most largely prescribed chemotherapeutic drugs for the treatment of a variety of human cancers. Unfortunately, in addition to its potent antitumor effect, the use of DXR is associated with a number of unwanted side effects, especially with serious cardiac toxicity.

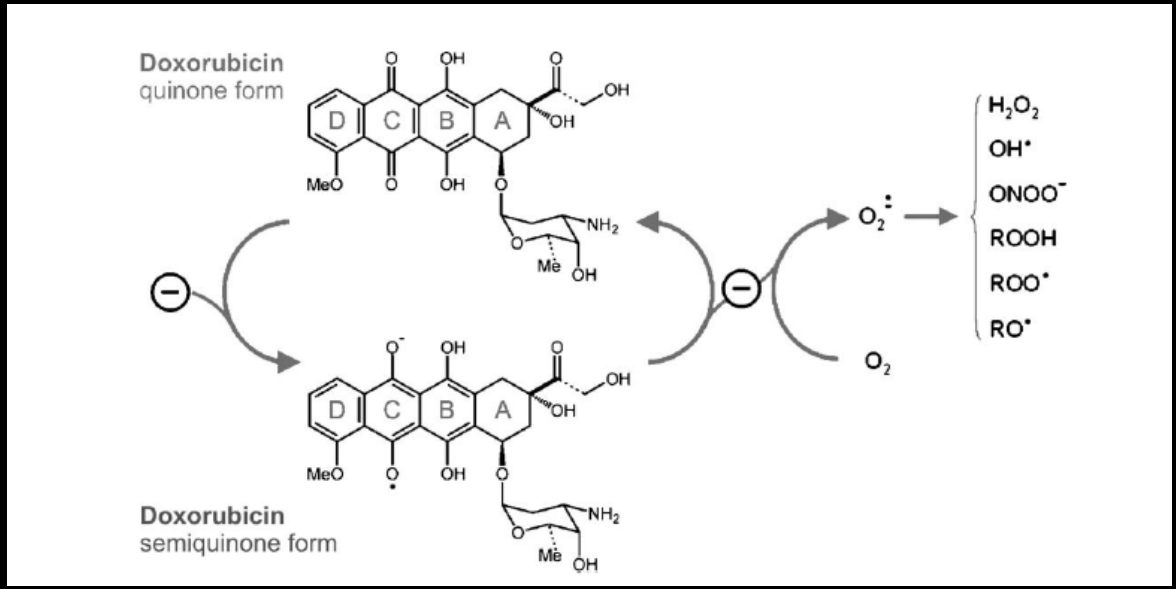


DXR and energy metabolism

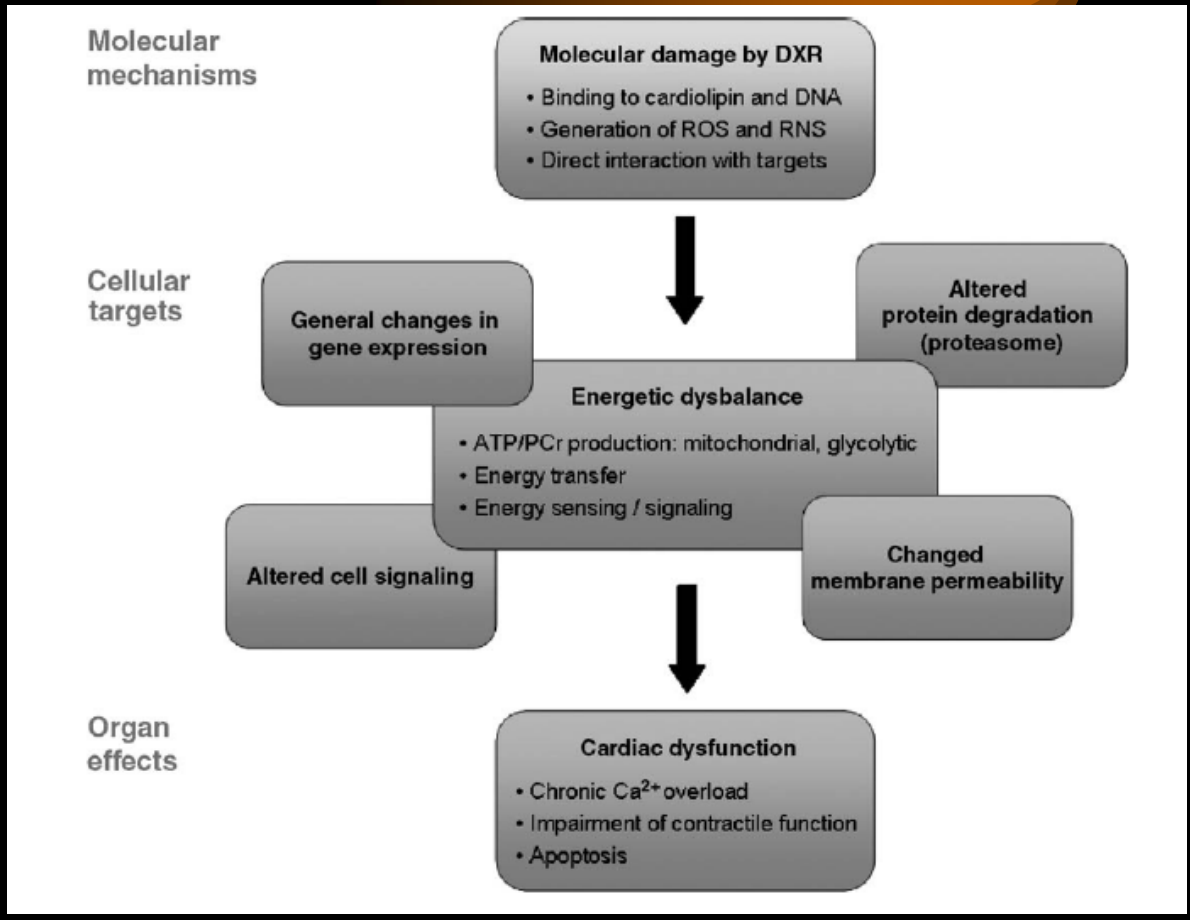
- DXR has been reported to diminish cardiac energy reserves, by reducing both ATP and PCr levels as well as the PCr/ATP ratio.
- Perturbed fatty acid metabolism with increased serum lipids, in particular free fatty acid levels, has been found in cell culture and animal models of DXR cardiotoxicity following DXR treatment



- Oxidative stress, is considered as the main mediator of DXR cardiotoxic action.
- DXR, a quinone containing drug, can be converted to the semiquinone form by one electron reduction.
- DXR generates free radicals and other related reactive oxygen and nitrogen species



- Further studies will be necessary to elucidate the relative impact of DXR on the different components of the cellular energy network and on cardiac function in general, and to clarify the onset of molecular damage in treated patients.
- testing protective strategies addressing specific energetic defects can be helpful to identify the critical steps that are affected by DXR.



M. Tokarska-Schlattner et al. / Journal of Molecular and Cellular Cardiology 41 (2006) 389–405

Protecting against anthracycline-induced myocardial damage

Table I. Concurrent therapies for reducing anthracycline toxicity.

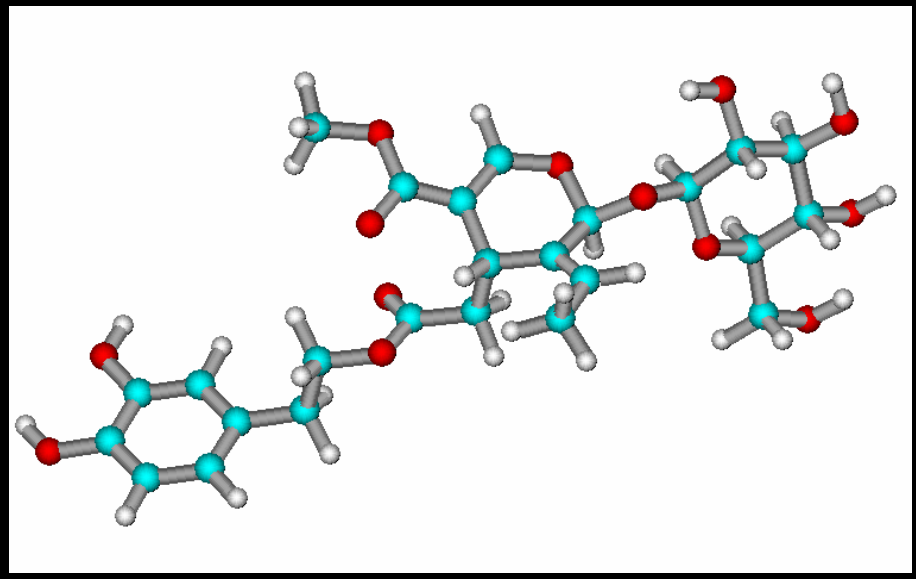
Agent	Class or action	Mechanism of action	Study subject
Dexrazoxane	Chelating agent	Prevents free radical formation; binds to iron; inhibits DNA topoisomerase	Humans
N-acetylcysteine	Mucolytic agent	Promotes endogenous antioxidant synthesis	Humans
Vitamin E	Nutrient	Antioxidant	Humans
Coenzyme Q10	Dietary supplement	Antioxidant	Humans
Carnitine	Dietary supplement	Antioxidant; transfer of long chain fatty acids into mitochondria	Humans
Probucol	Lipid-lowering drug	Promotes endogenous antioxidant synthesis	Animal model
Amifostine	Cytoprotective agent	Scavenges free radicals	Animal model
Carvedilol	β-Adrenergic antagonist	Prevents free radical formation; prevents depletion of endogenous antioxidants	Animal model
Vitamins A and C; carotenoids	Nutrient	Antioxidant	Animal model
Selenium	Trace element	Antioxidant; anticarcinogenic action	Animal model
Glutathione	Tripeptide thiol	Antioxidant	Animal model

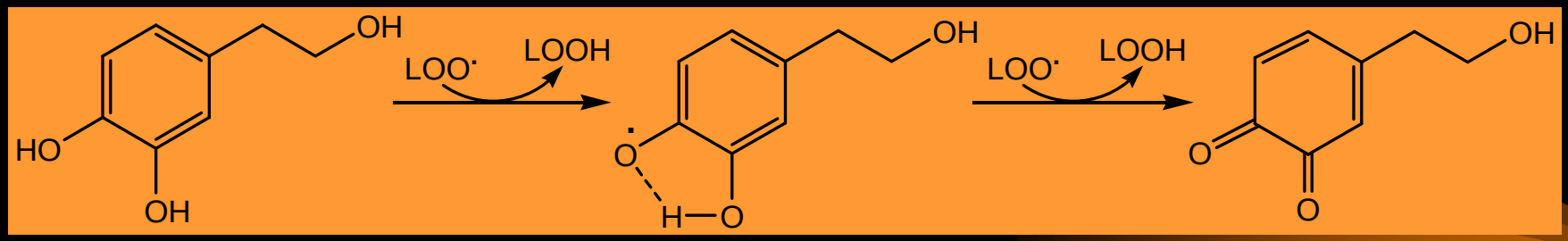
Karlijn A. Wouters, Leontien C. M. Kremer, Tracie L. Miller, Eugene H. Herman and Steven E. Lipshultz

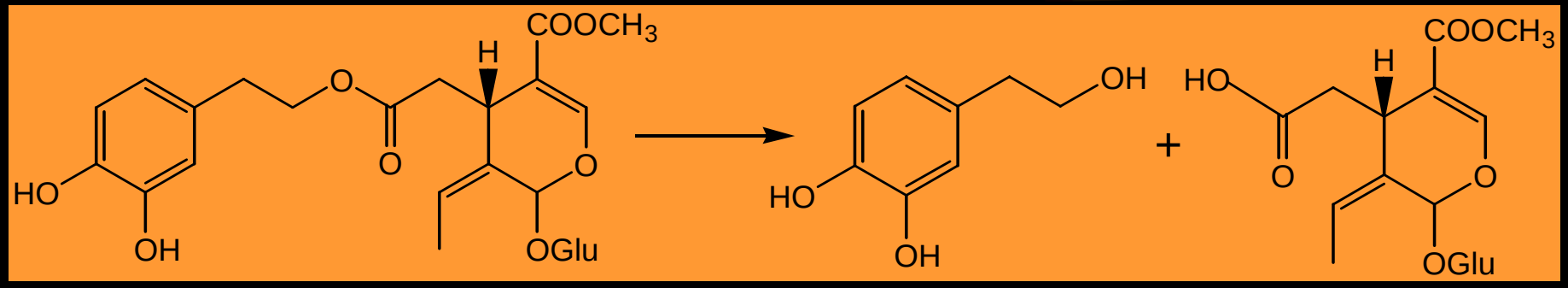
British Journal of Haematology, 2005 131, 561–578

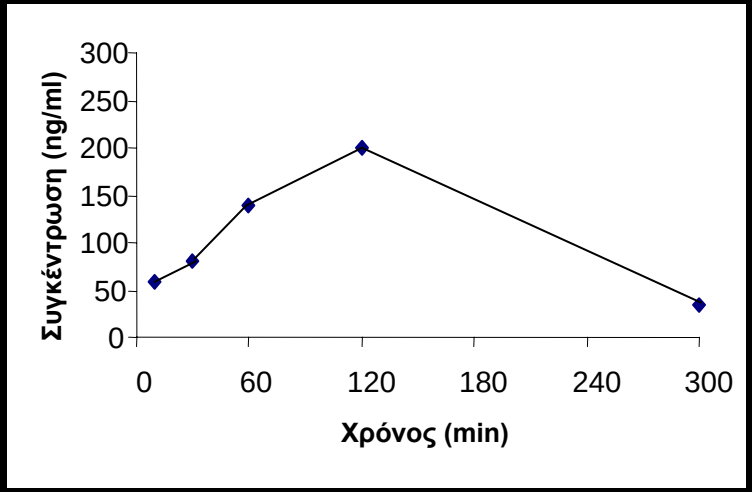
Oleuropein,

- The main constituent of olive leaf extract, is a complex phenol present in large quantities in olive tree leaves and in low quantities in olive oil.
- It possesses strong antioxidant as well as anti-inflammatory, antiatherogenic and anticancer properties.




OH
HO
OH
LOO·
LOOH
OH
O
H—O
LOO·
LOOH
OH
O
O

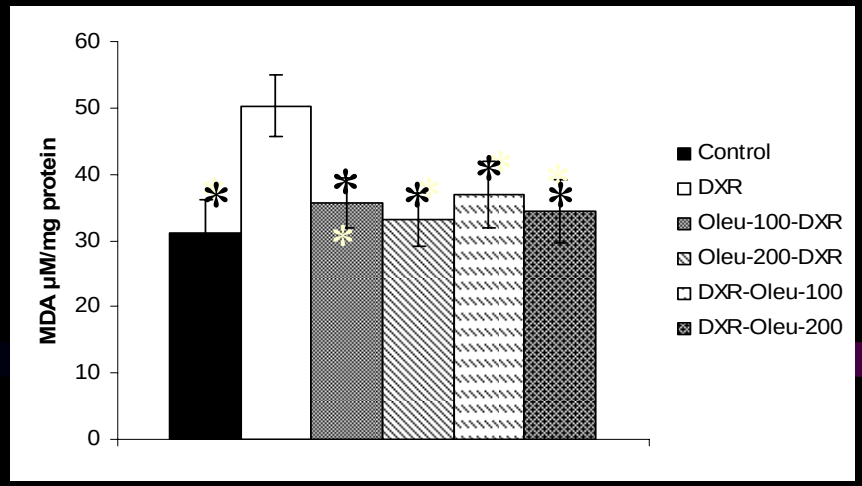

COOCH3
H
O
O
O
HO
OH
OGlu
OH
HO
OH
+
HO
COOCH3
H
O
O
OGlu

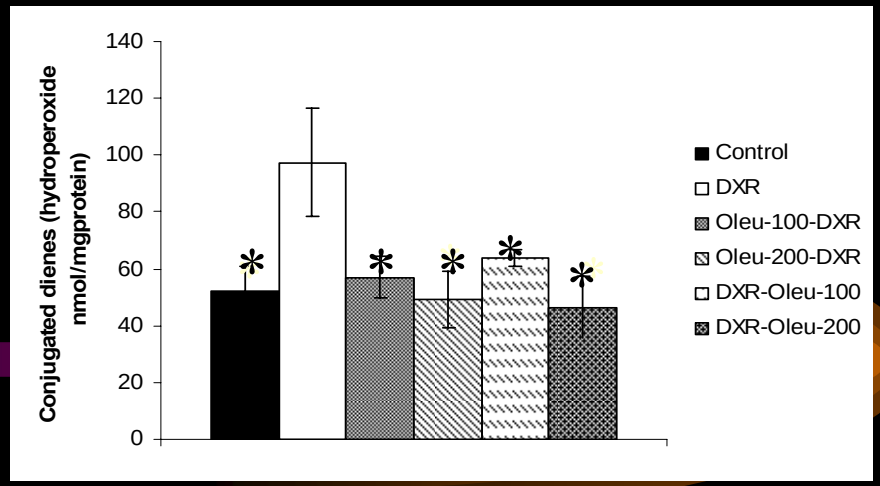

300
250
200
150
100
50
0
Συγκέντρωση (ng/ml)
0
60
120
180
240
300
Χρόνος (min)

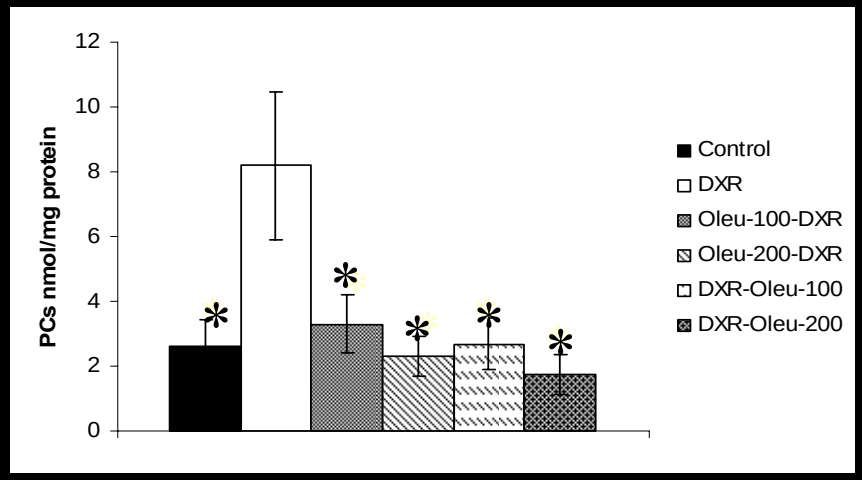
METHODS

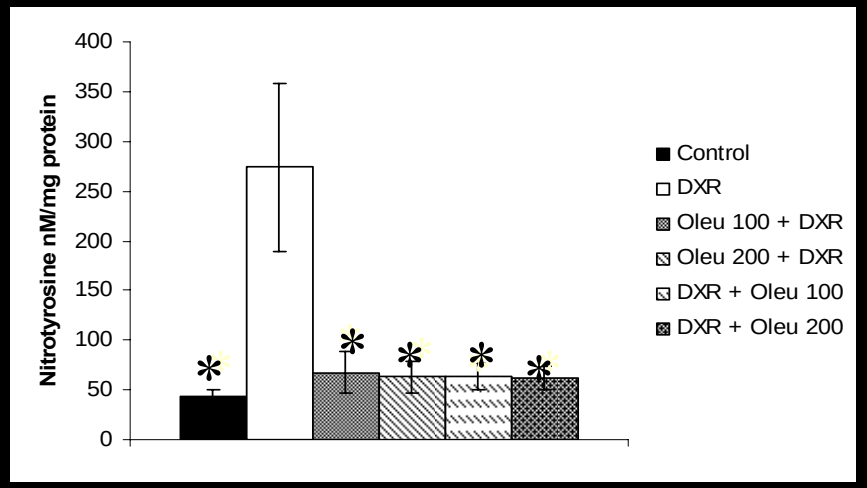
- Fifty Wistar rats were randomly divided into 6 groups:
- 1) Control group (CTL) (n=6): normal saline (2 ml, i.p)
- 2) DXR group (n=8): single dose of DXR, (20 mg/kg, i.p)
- 3) Oleu-100-DXR (n=9): Oleu (100 mg/kg/BW/day, i.p),
- 2 days before, on the same day and 3 days after DXR
- administration
- 4) Oleu-200-DXR (n=9): Oleu (200 mg/kg/BW/day, i.p),
- like in group 3
- 5) DXR-Oleu-100 (n=9): Oleu (100 mg/kg/BW/day, i.p),
- on the same day and 3 days after DXR administration
- 6) DXR-Oleu-200 (n=9): Oleu (200 mg/kg/BW/day, i.p),
- like in group 5

Animals were sacrificed three days after DXR administration and hearts were rapidly excised for ^{1}H-NMR Spectroscopy



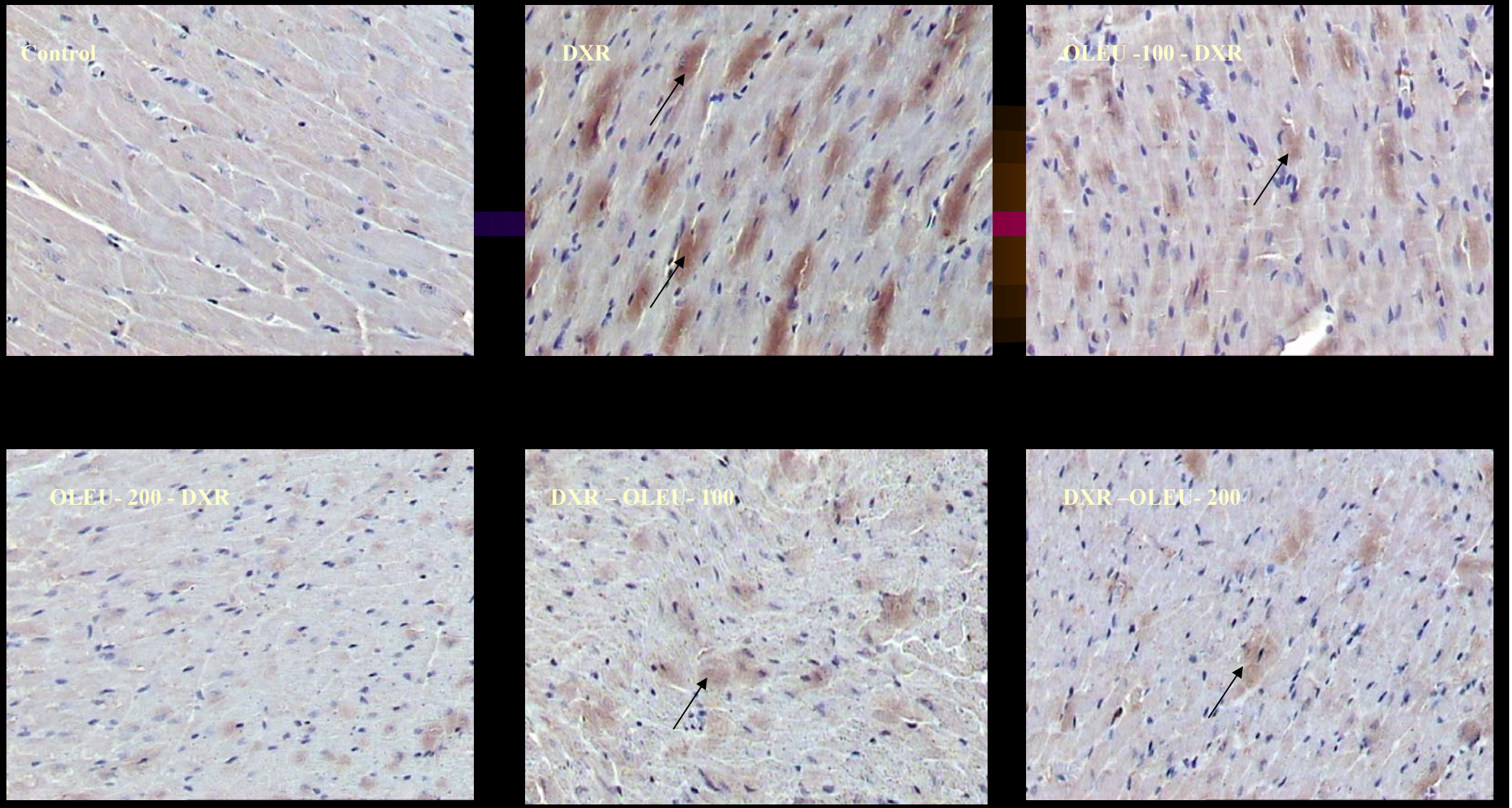






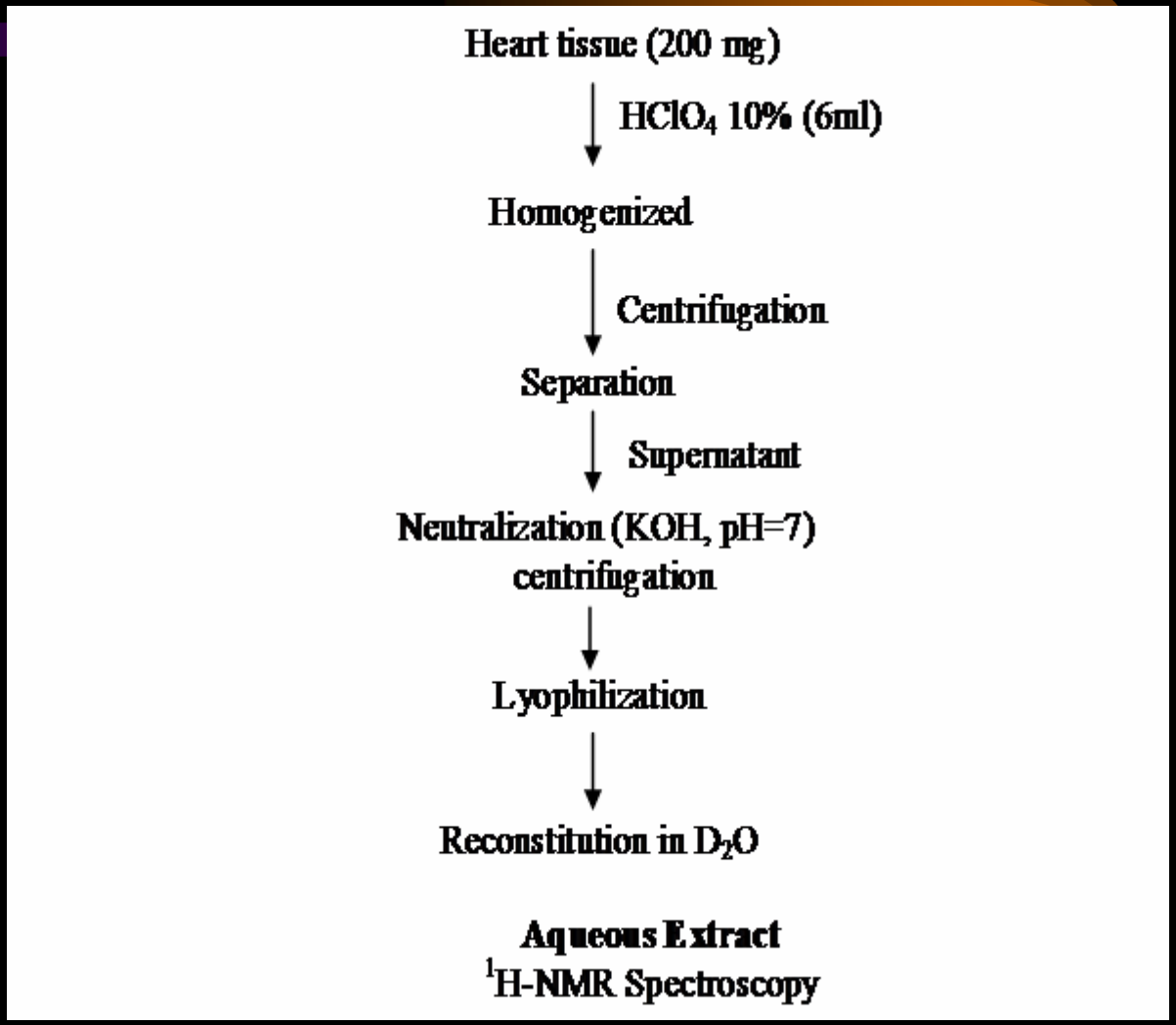
Acute doxorubicin cardiotoxicity is prevented and restored by the olive phenolic micronutrient oleuropein through oxidative and nitrosative stress suppression.

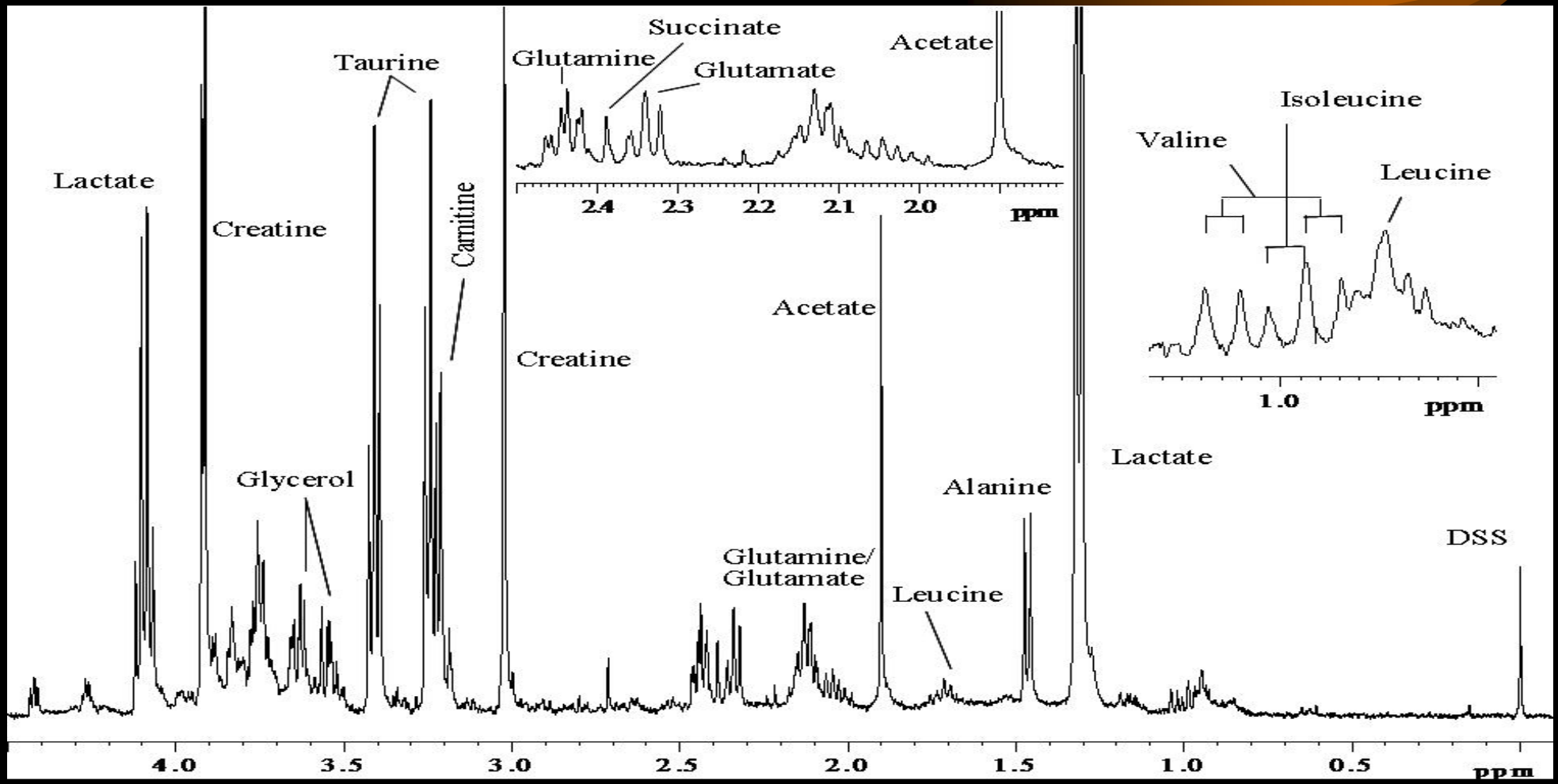
Andreadou et al J Mol Cell Cardiol, 42: 549-558, 2007

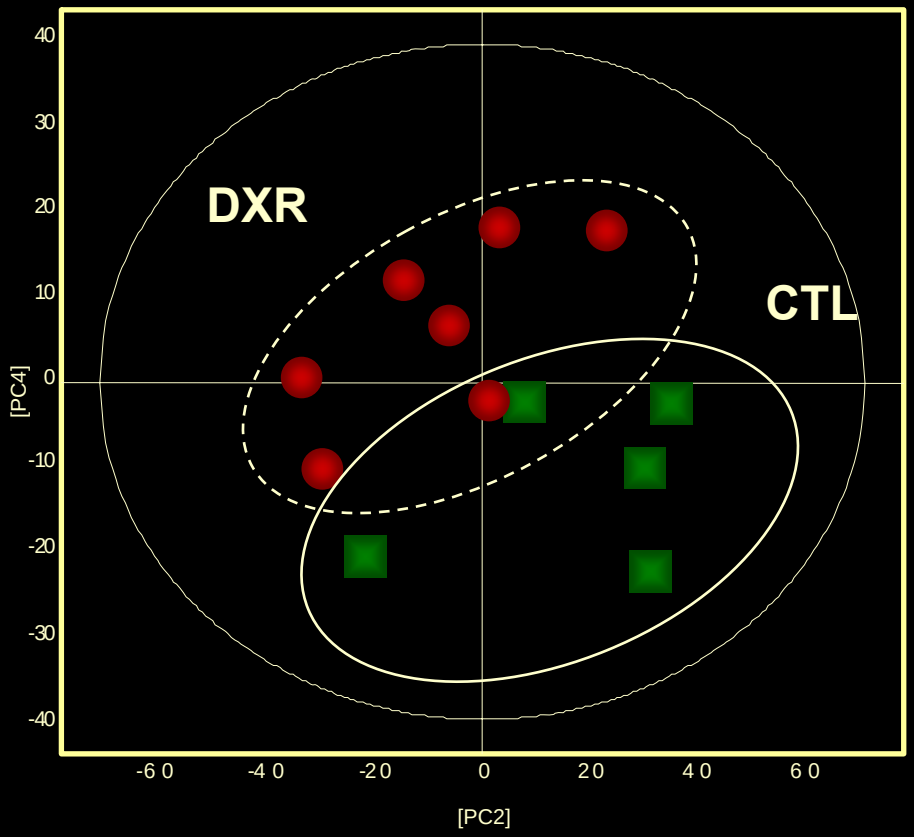


Representative immunohistochemistry analysis of iNOS in the experimental study groups. Arrows indicate iNOS positive cells (magnification x 200).

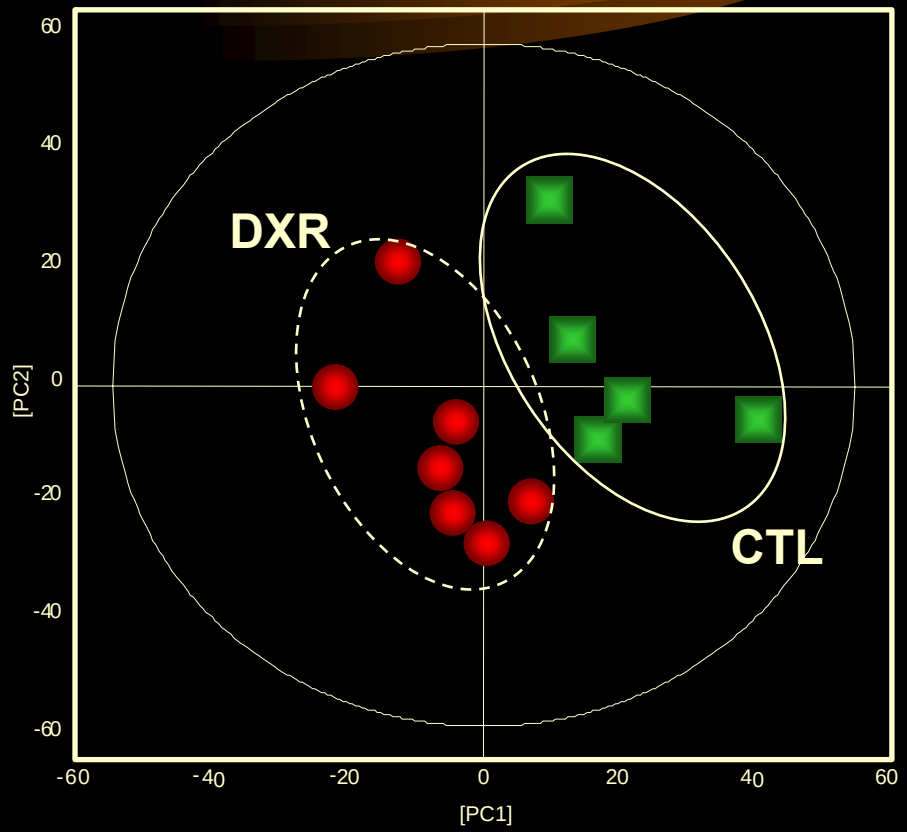
Tissue extraction



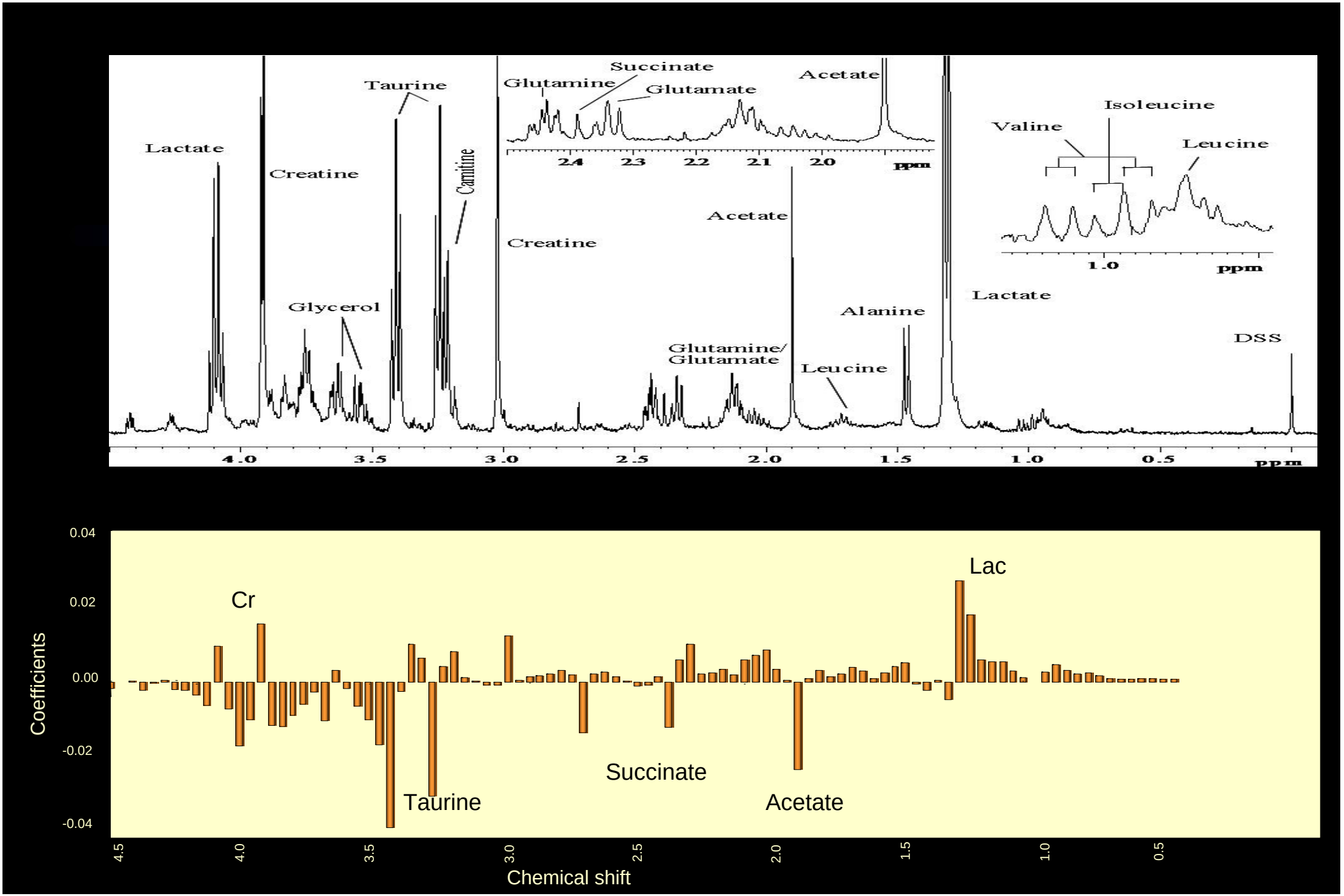

Lactate
Creatine
Taurine
Carnitine
Glycerol
Creatine
Acetate
Glutamine/
Glutamate
Leucine
Alanine
Lactate
DSS
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
Glutamine
Succinate
Glutamate
Acetate
2.4
2.3
2.2
2.1
2.0
ppm
Valine
Isoleucine
Leucine
1.0
ppm

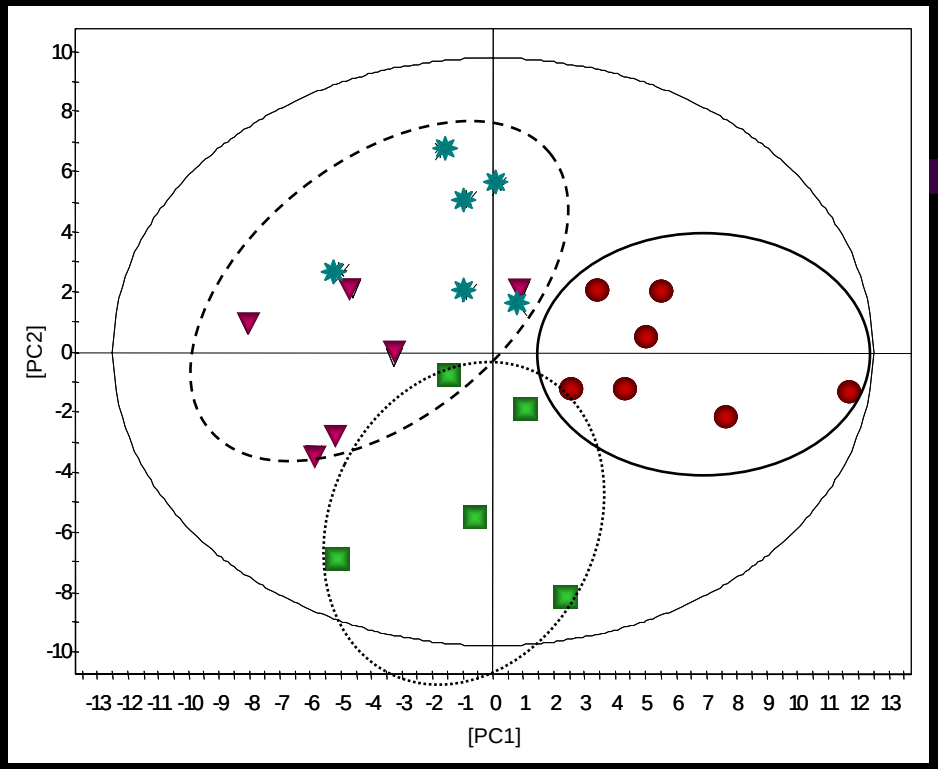

DXR
CTL
[PC4]
[PC2]
40
30
20
10
0
-10
-20
-30
-40
-60
-40
-20
0
20
40
60

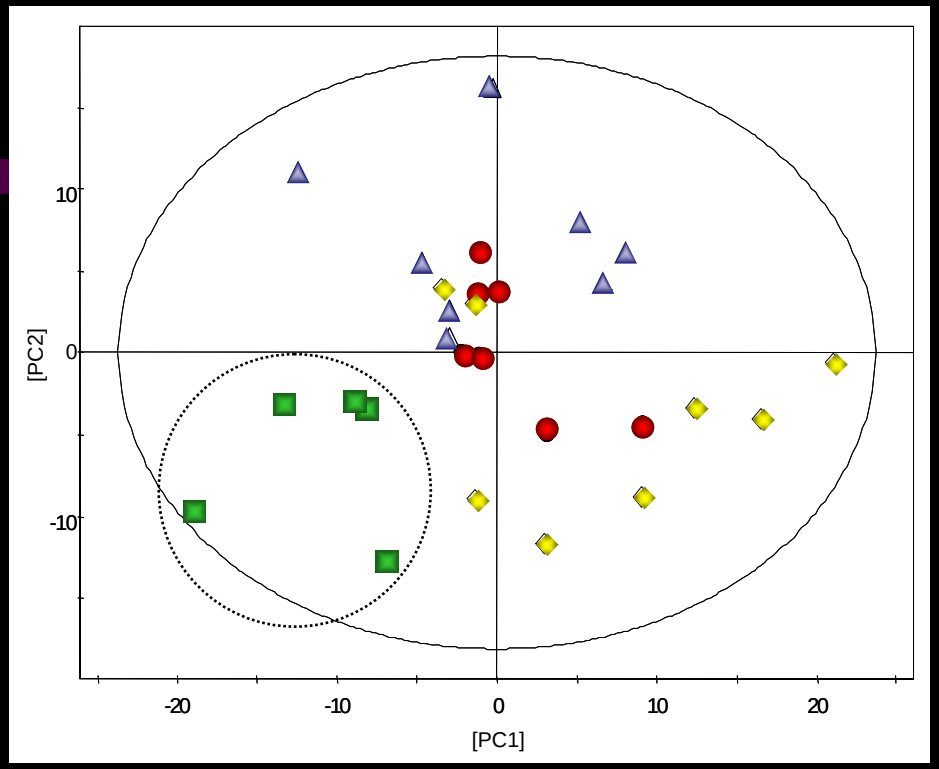
PCA


DXR
CTL
[PC2]
[PC1]
60
40
20
0
-20
-40
-60
-60
-40
-20
0
20
40
60

PLS-DA


Lactate
Creatine
Taurine
Carnitine
Glycerol
Glutamine
Succinate
Glutamate
Acetate
Creatine
Acetate
Glutamine/
Glutamate
Leucine
Alanine
Lactate
DSS
Valine
Isoleucine
Leucine
ppm
0.04
0.02
0.00
-0.02
-0.04
Coefficients
Cr
Taurine
Succinate
Acetate
Lac
Chemical shift



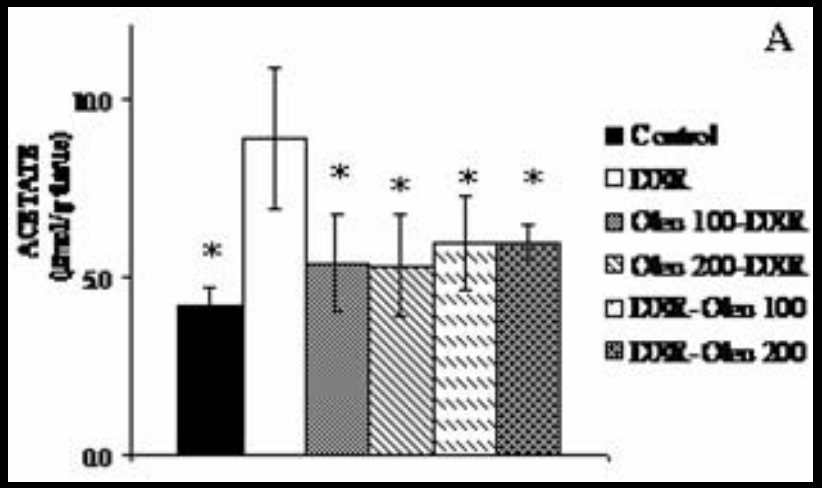


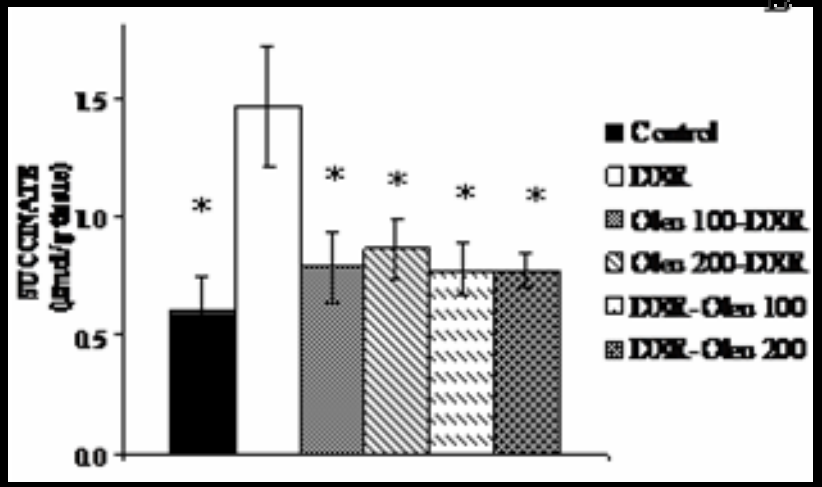
Dose-dependent discrimination.

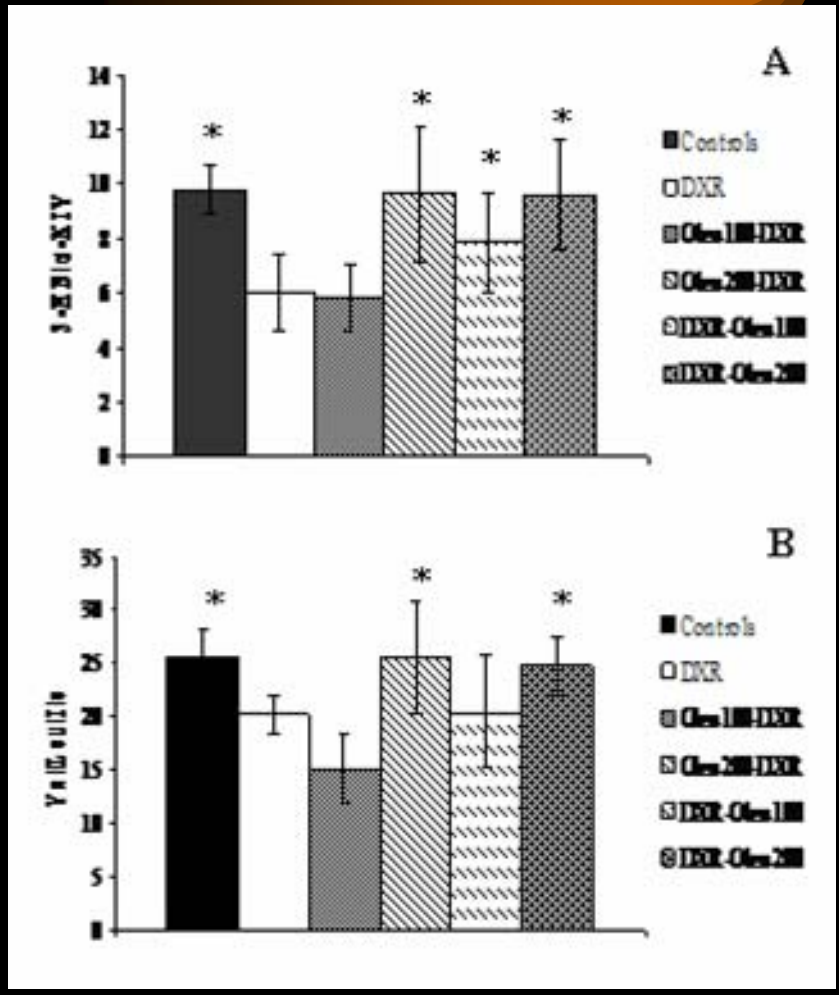
Myocardial levels of metabolites in different study groups.

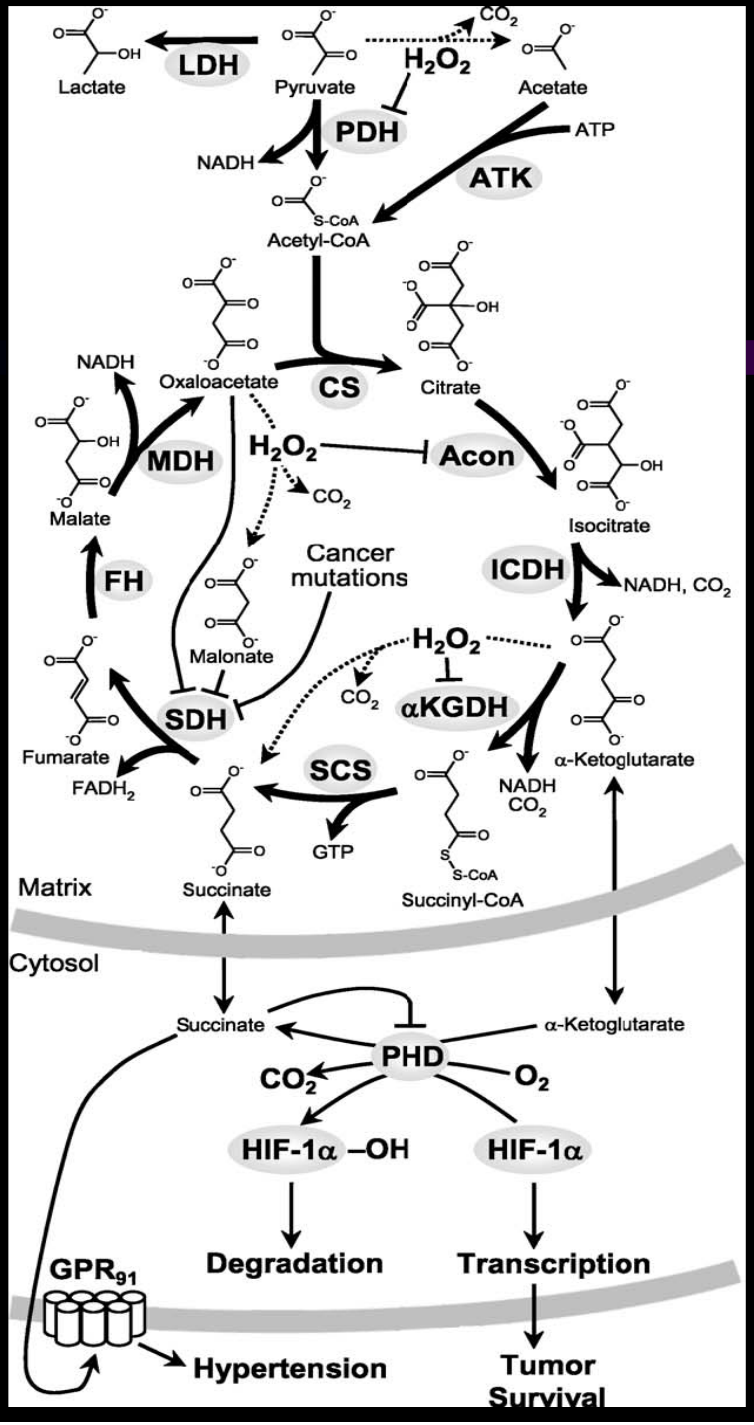
	Control	DXR	Oleu 100-DXR	Oleu 200-DXR	DXR-Oleu100	DXR-Oleu200
Lactate	28.94±3.81	31.12±3.14	28.79±2.51	19.16±2.60*	25.55±1.47	23.60±0.41*
Alanine	2.94±0.29*	3.97±0.25	2.71±0.19*	2.47±0.39*	2.18±0.14*	2.49±0.32*
Glutamate	7.31±0.84	6.24±0.82	4.58±0.45	5.23±0.96	4.88±0.22	4.69±0.23
Glutamine	9.65±1.14	11.89±1.71	10.65±0.67	6.61±1.29*	9.79±0.96	8.19±0.73*
Glucose	1.21±0.22	1.49±0.23	2.31±0.68	0.87±0.24	1.48±0.24	1.67±0.44
Succinate	0.60±0.14*	1.46±0.26	0.78±0.15*	0.86±0.13*	0.77±0.11*	0.78±0.07*
Acetate	4.14±0.55*	10.07±1.64	5.35±1.38*	5.29±1.43*	5.88±1.31*	5.95±0.47*
Creatine	8.33±0.79	8.20±0.87	8.85±1.08	6.19±0.87	6.21±0.38	6.20±0.39
Taurine	14.31±0.27	18.40±2.12	19.57±1.85	13.01±2.42	14.65±0.92	16.53±2.04

Data are means ± SEM (μmol/g myocardial tissue).
*$P<0.05$, compared to DXR group


A
ACETATE
(μmol/g tissue)
10.0
5.0
0.0
Control
DXR
Oleo 100-DXR
Oleo 200-DXR
DXR-Oleo 100
DXR-Oleo 200


B
SUCCINATE
(μmol/g tissue)
1.5
1.0
0.5
0.0
Control
DXR
Oleo 100-DXR
Oleo 200-DXR
DXR-Oleo 100
DXR-Oleo 200


A
14
12
10
8
6
4
2
0
Controls
DXR
B
35
30
25
20
15
10
5
0
Controls
DXR



Nonezymatic formation of succinate in mitochondria under oxidative stress

Fedotcheva, N. I. Sokolov, A. P. Kondrashova, M. N.

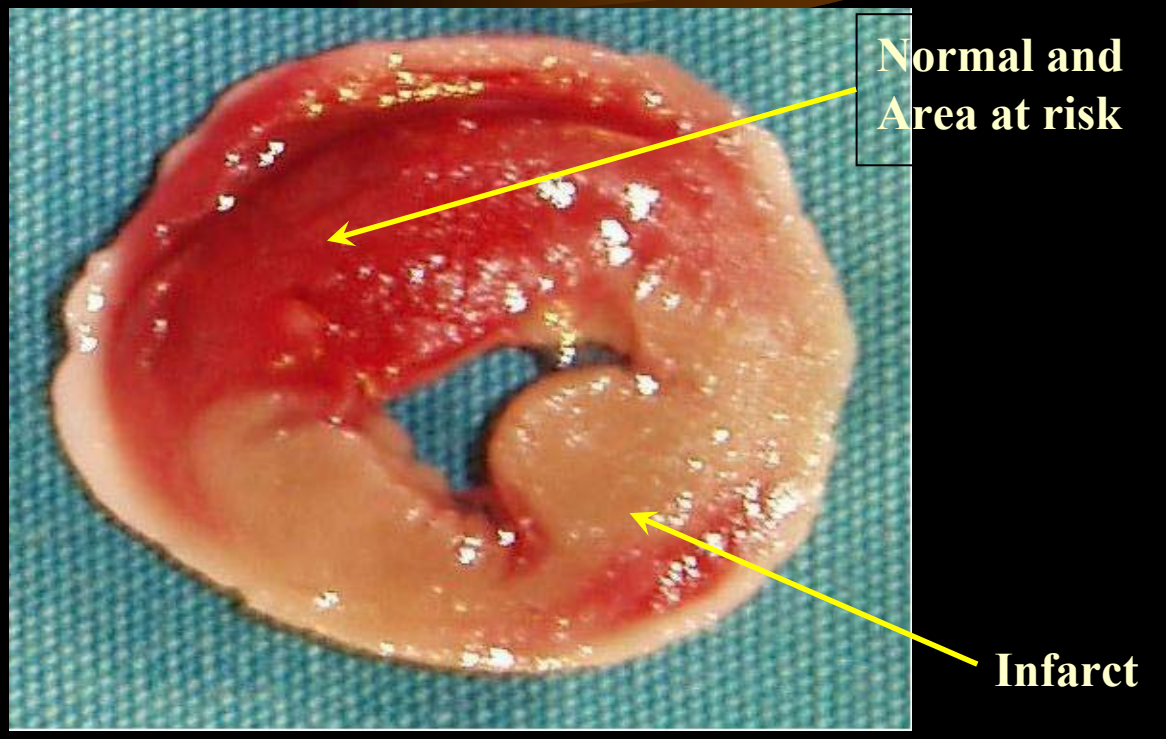
Free Radic.Biol. Med.; 2006.

In summary, the results of Fedotcheva et al. force a major reappraisal of the very structure of the TCA cycle, and the roles of its intermediates in cell signaling, physiology, and pathology. **Krebs and Johnson may be, quite literally, turning in their graves.**

P.S. Brookes et al. / Free Radical Biology & Medicine 41 (2006) 41–45

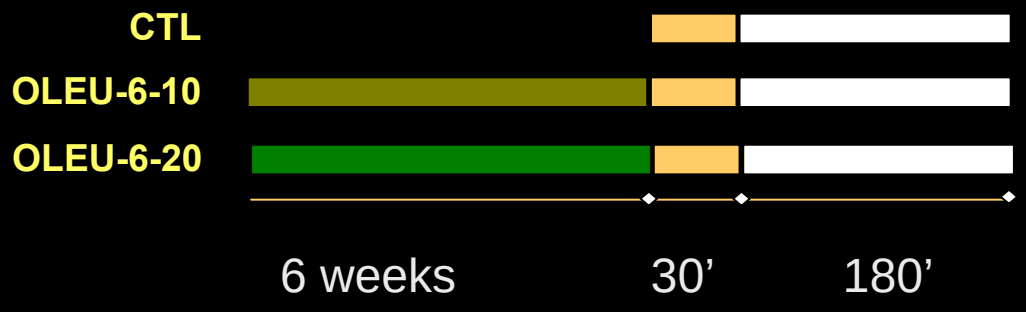
Ischemia - Reperfusion

- A heart attack occurs when a blood clot forms in a coronary artery depriving blood flow from a region of the heart, a condition termed ischemia. Current therapy is to reopen the artery but blood flow is seldom restored before a significant amount of the heart muscle has died.

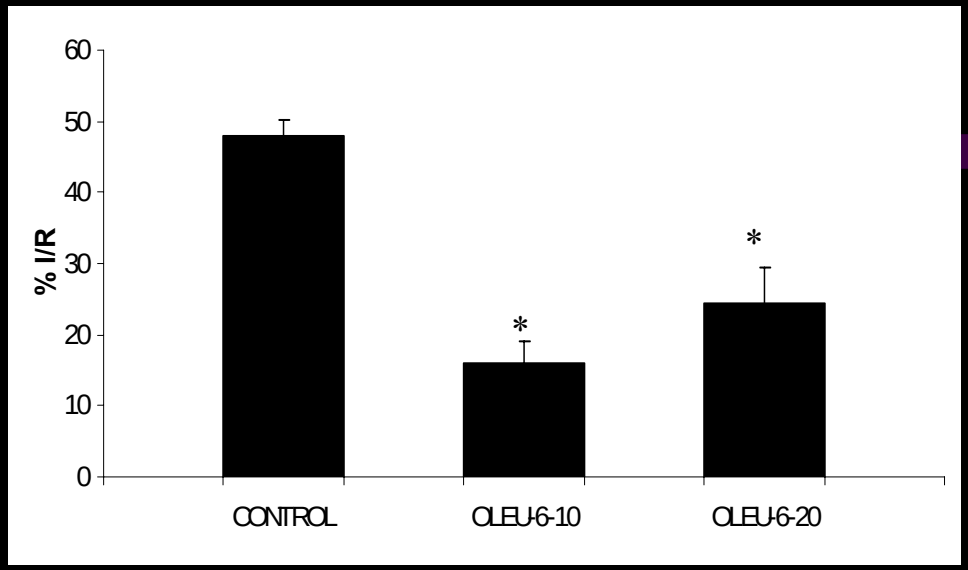


Oleuropein – Ischemia Reperfusion

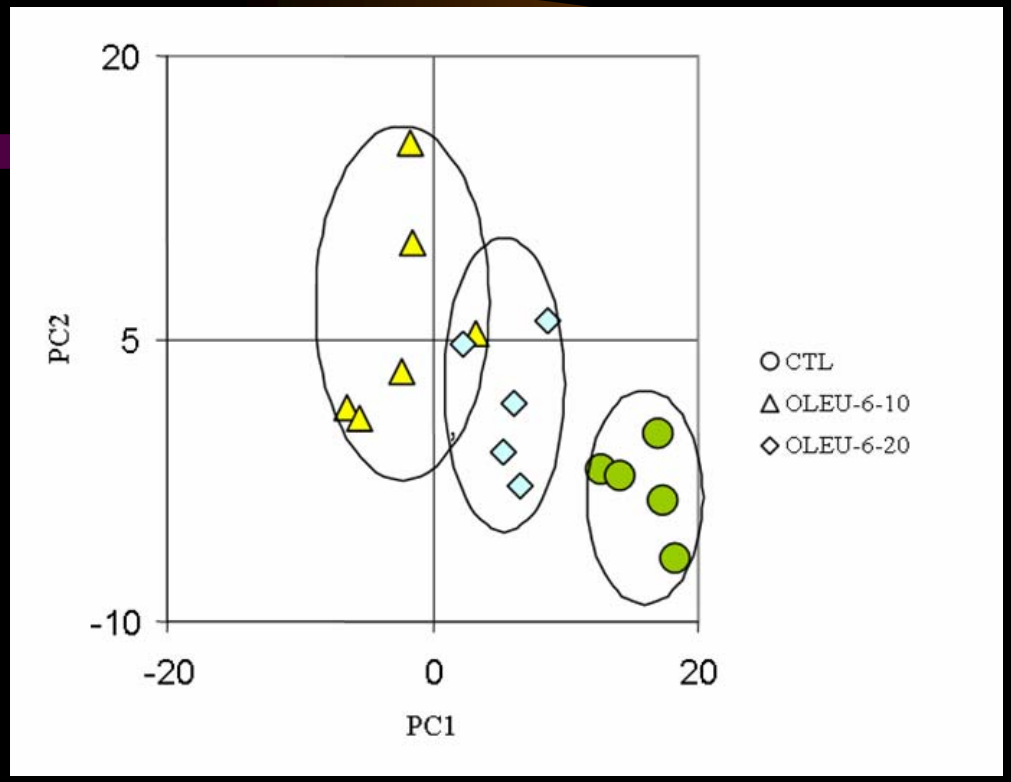
Oleuropein Exhibits Anti-Ischemic, Antioxidative, Hypolipidemic Effects in Anesthetized Rabbits



Andreadou et al The Journal of Nutrition and Disease 136: 2213-2219, 2006.

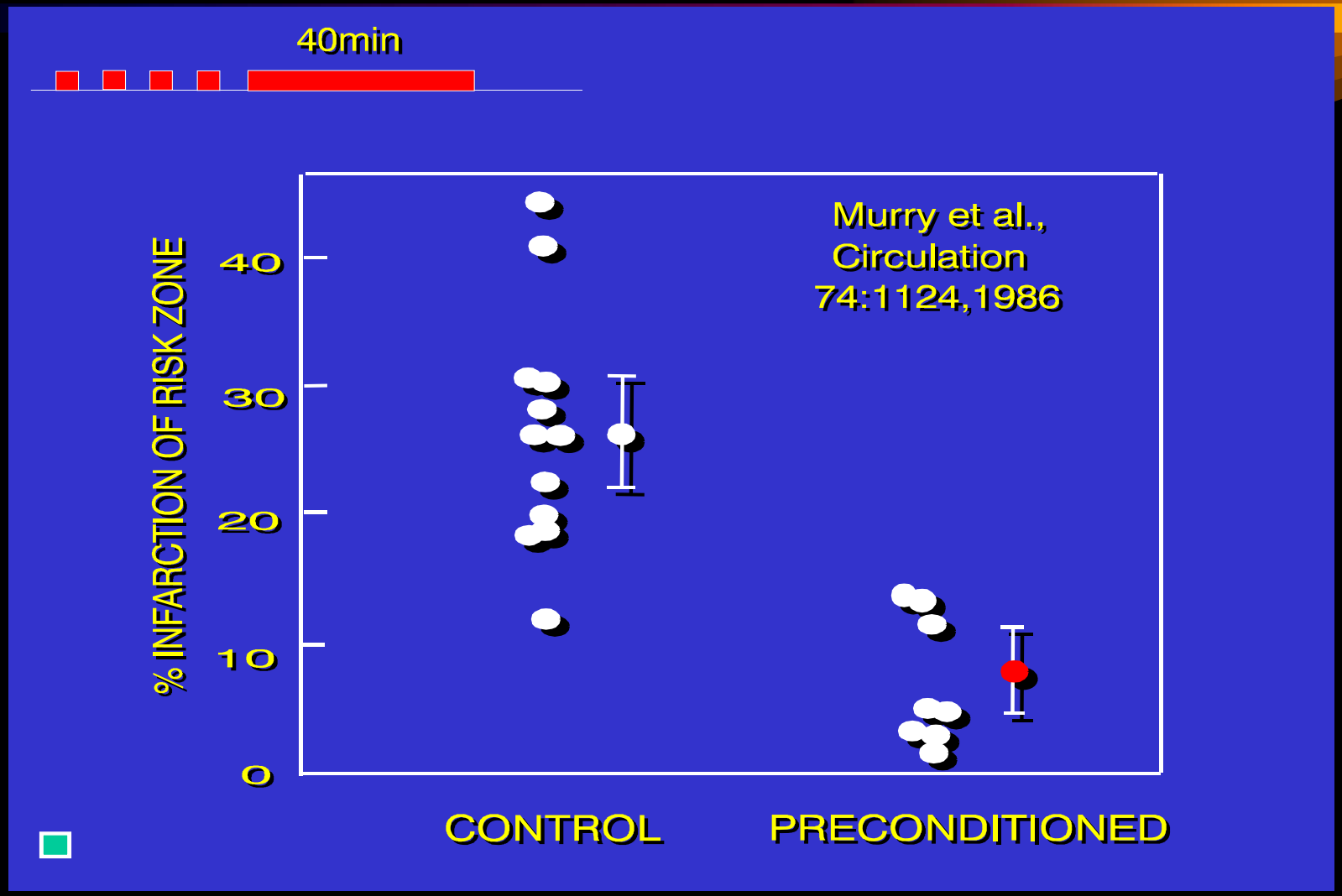


The effect of various interventions on infarct size (expressed as a percent of risk zone) in rabbit heart

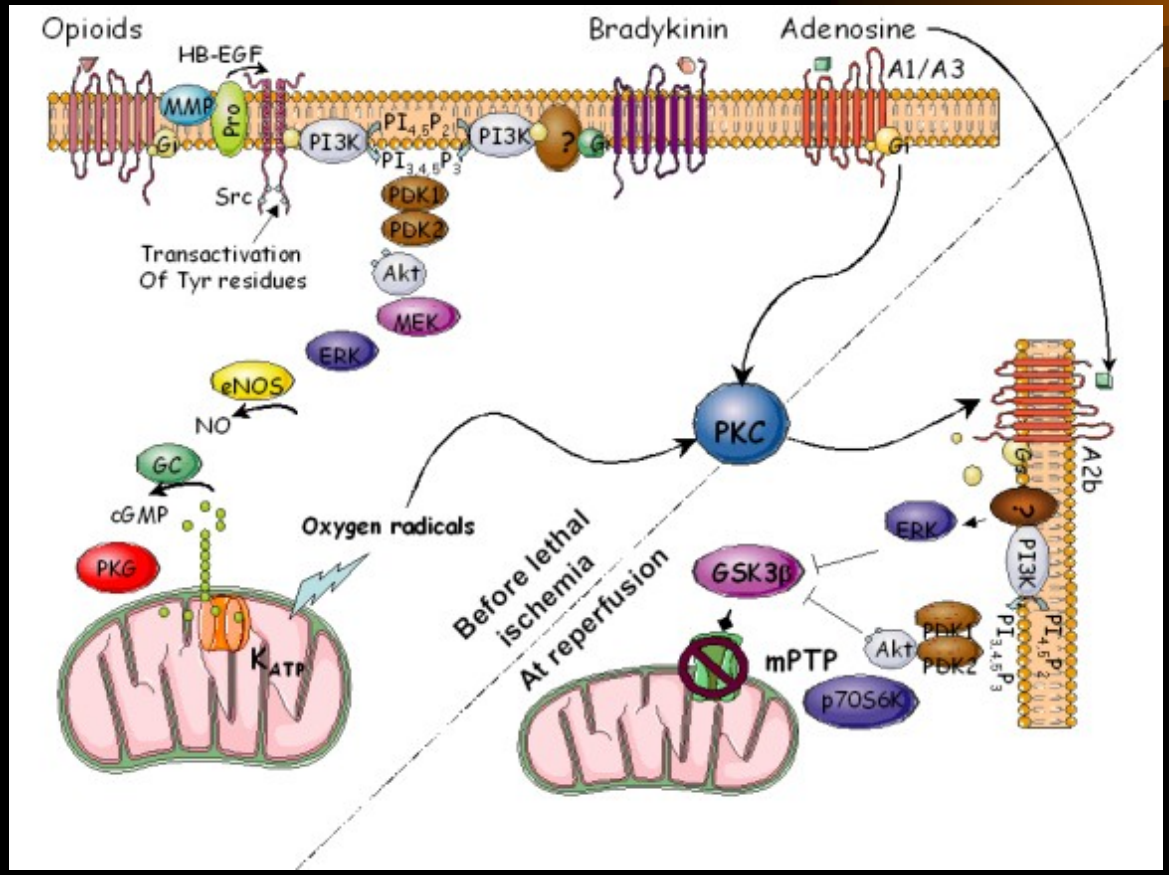


PCA of CPMG 1H-NMR plasma samples

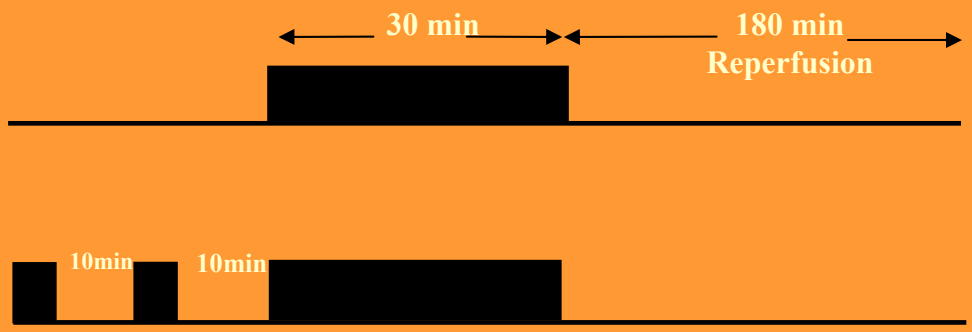
Ischemic Preconditioning

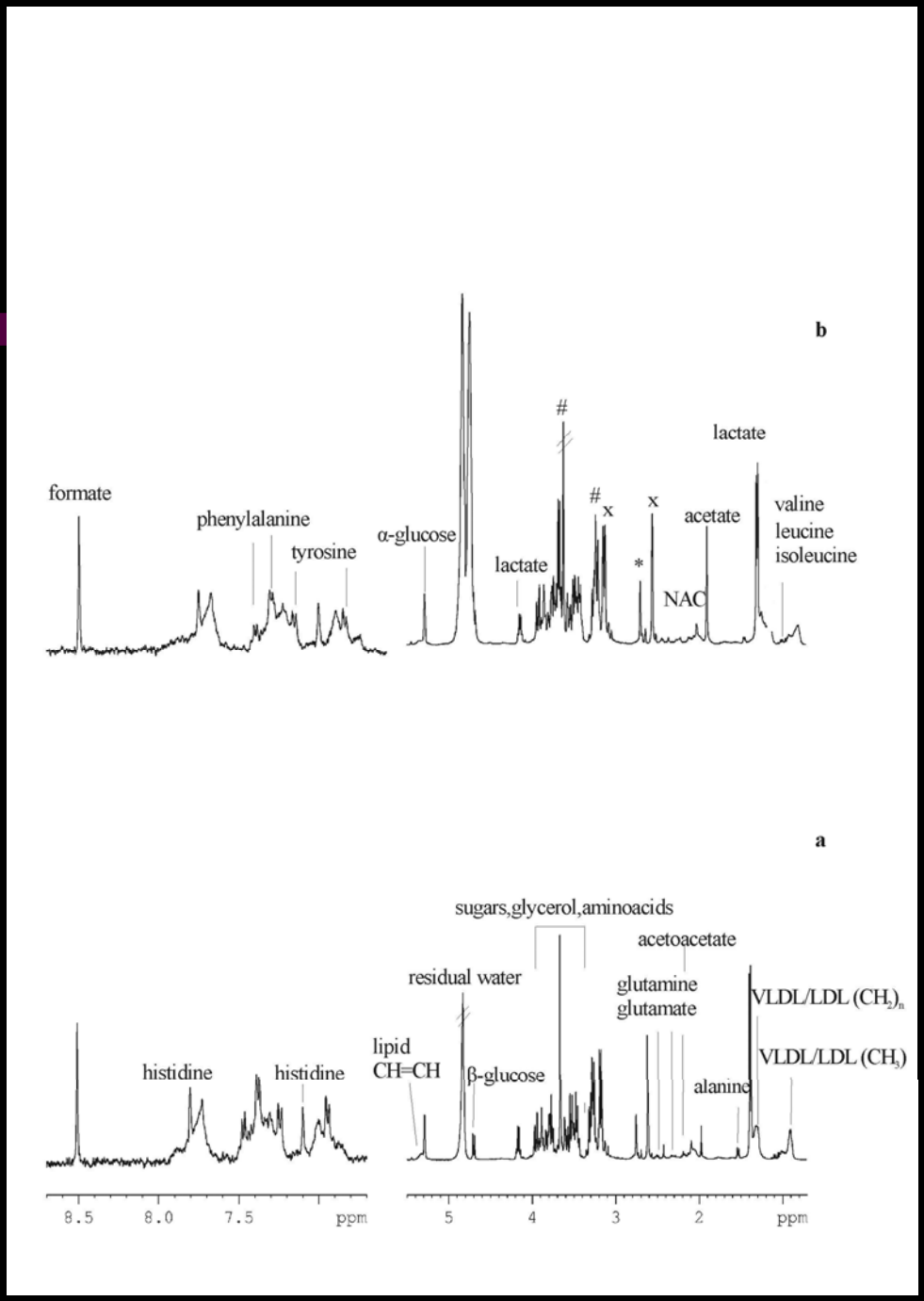


Preconditioning

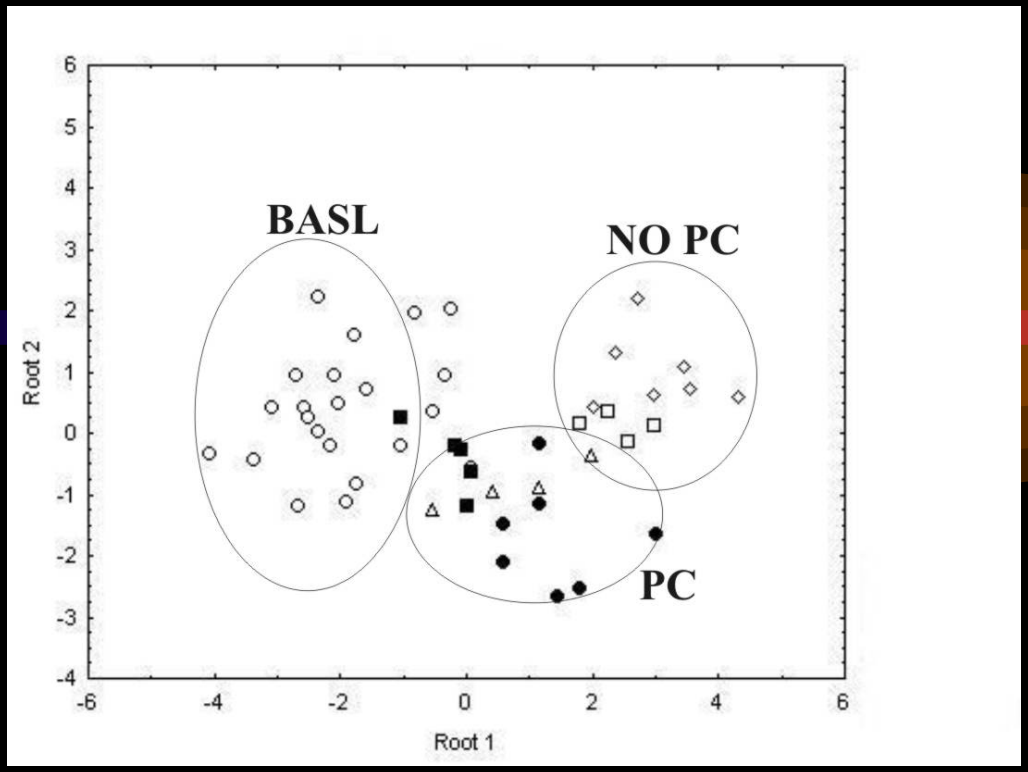


Ischemic preconditioning in rabbits





M. A. Constantinou et al, *Eur. J. Pharm. Sc.* 30, 303–314, 2007



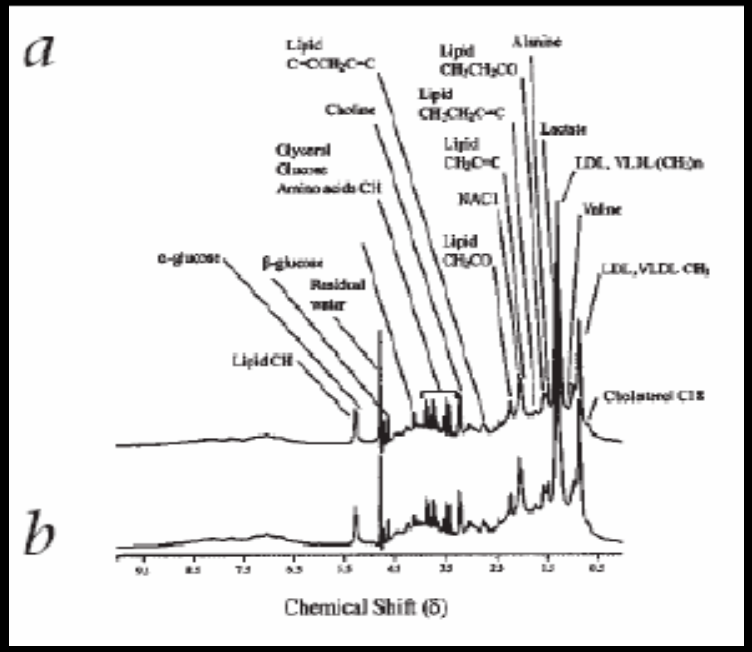
^{1}H NMR signal intensity ratios of lactate/glucose and lactate+alanine/acetate as Means±SE for the studied groups along with the percentage of infarcted-to-risk zone ratio (%I/R) detected in whole heart[e].

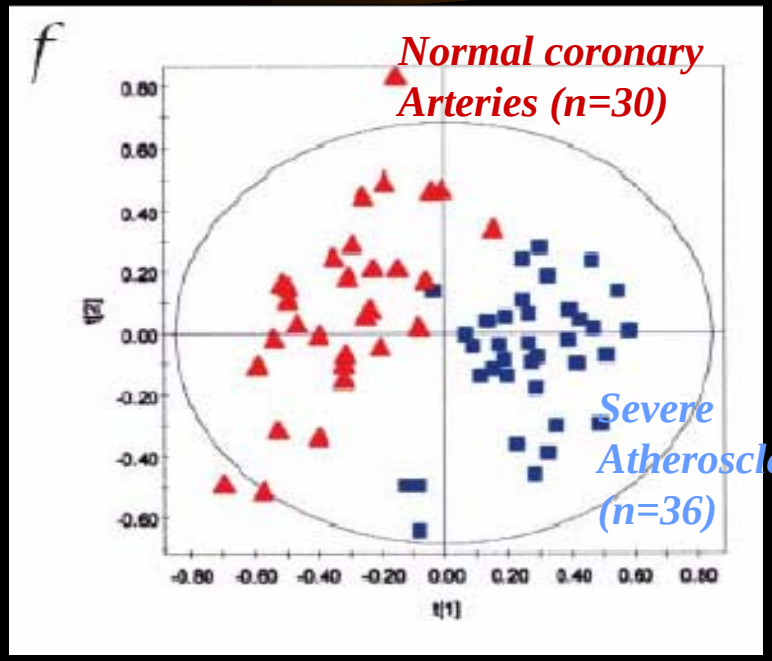
Sample groups	N	Lactate/glucose	Lactate+alanine/acetate	% I/R
Basl	20	2.76±0.60	6.17±0.52	
Rep	6	4.89±0.17[a]	4.50±0.71	46.4±4.9[e]
IpC-Rep	7	3.62±0.44	8.82±1.51	14.0±1.7[e]

[a]$P<0.05$ vs basl; [e]Andreadou et al. 2004

Metabonomics and Clinical Diagnosis

Predicting Coronary Artery Disease In Humans





Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomics
Joanne T. Brindle, Henrik Antti, Elaine Holmes, George Tranter, Jeremy K. Nicholson, Hugh W.L. Bethell, Sarah Clarke, Peter M. Schofield, Elaine McKilligin, David E. Mosedale & David J. Grainger Nature Medicine 8, 1439 - 1445 **(2002)**

Disease Diagnosis via NMR (140+ Detectable Conditions)

- Adenine Phosphoribosyltransferase Deficency
- Adenylosuccinase Deficiency
- Alcaptonuria
- α-Aminoadipic Aciduria
- β-Aminoisobutyric Aciduria
- α-Aminoketoadipic Aciduria
- Anorexia Nervosa
- Argininemia
- Argininosuccinic Aciduria
- Aspartylglycosaminuria
- Asphyxia
- Biopterin Disorders
- Biotin-responsive Multiple Carboxylase Deficiency
- Canavan's Disease
- Carcinoid Syndrome
- Carnosinemia
- Cerebrotendinous Xanthomatosis/sterol 27-hydroxylaseDeficiency
- Citrullinemia
- Cystathioninemia
- Cystinosis
- Cystinuria (Hypercystinuria)
- Diabetes
- Dibasic Aminoaciduria
- Dicarboxylic Aminoaciduria
- Dichloromethane Ingestion
- Dihydrolipoyl Dehydrogenase Deficiency
- Dihydropyrimidine Dehydrogenase Deficiency
- Dimethylglycine Dehydrogenase Deficiency
- Essential Fructosuria
- Ethanolaminosis
- Ethylmalonic Aciduria
- Familial Iminoglycinuria
- Fanconi's Syndrome
- Folate Disorder
- Fructose Intolerance
- Fulminant Hepatitis
- Fumarase Deficiency
- Galactosemia
- Glucoglycinuria
- Glutaric Aciduria Types 1 & 2
- Glutathionuria
- Glyceroluria (GKD)
- D-Glyceric Aciduria
- Guanidinoacetate-Methyltransferase Deficiency
- Hartnup Disorder
- Hawkinsinuria
- Histidinemia
- Histidinuria
- Homocystinsufonuria
- Homocystinuria
- 4-Hydroxybutyric Aciduria
- 2-Hydroxyglutaric Aciduria
- Hydroxykynureninuria
- Hydroxylysinemia
- Hydroxylysinuria
- 3-Hydroxy-3-methylglutaric Aciduria
- 3-Hydroxy-3-methylglutaryl-Co A Lyase Deficiency
- Hydroxyprolinemia
- Hyperalaninemia
- Hyperargininemia (Argininemia)
- Hyperglycinuria
- Hyperleucine-Isoleucinemia
- Hyperlysinemia
- Hyperornithinemia
- Hyperornithinemia-Hyperammonemia-Homocitrullinuria Syndrome (HHH)
- Hyperoxaluria Types I & 2
- Hyperphenylalaninemia
- Hyperprolinemia
- Hyperthreoninemia

gastric and colon cancer

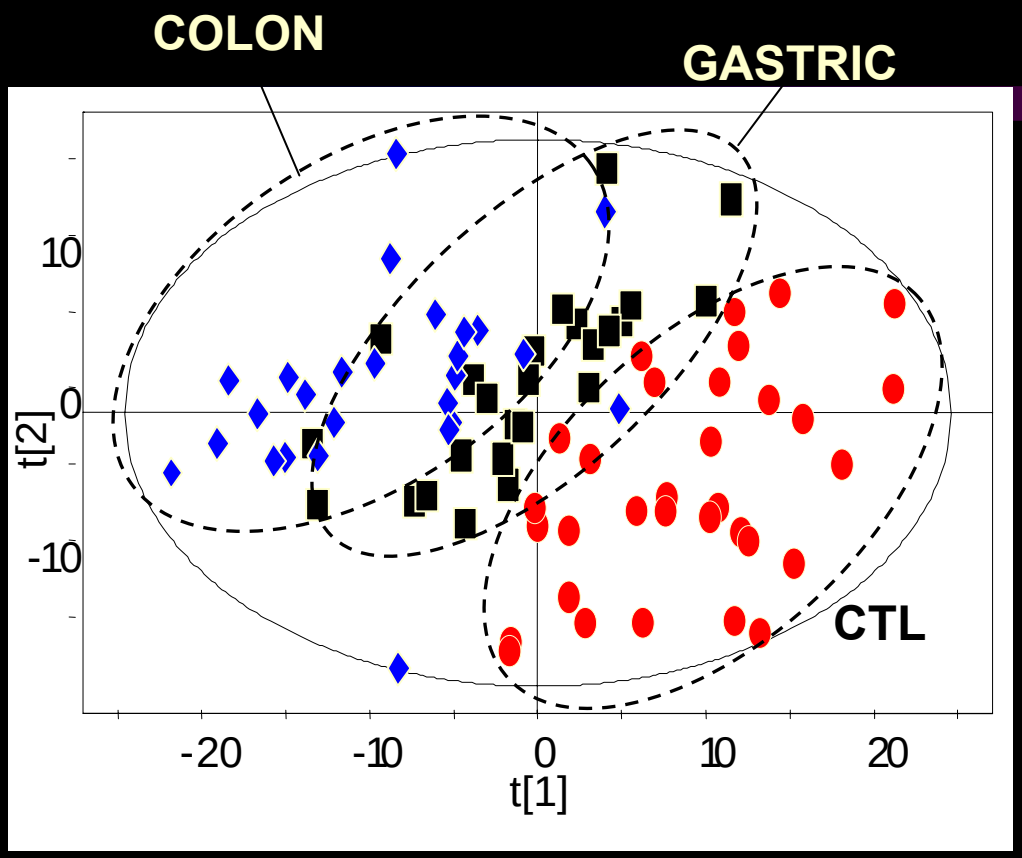
- **Sample collection**
  - The present study comprised 3 groups:
    - Group 1: 35 healthy volunteers (controls)
    - Group 2: 26 patients with gastric cancer
    - Group 3: 27 patients with colon cancer.

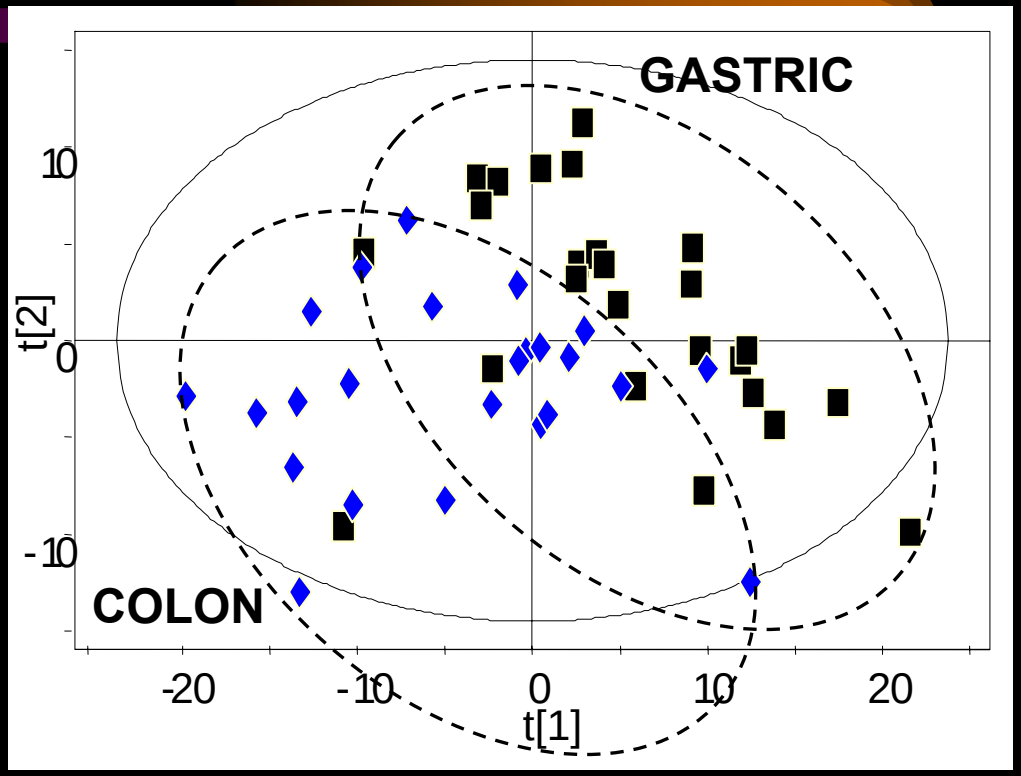
- Serum samples were collected from healthy volunteers and cancer patients, in the morning before surgery. In all cases cancer was pathologically confirmed

gastric and colon cancer

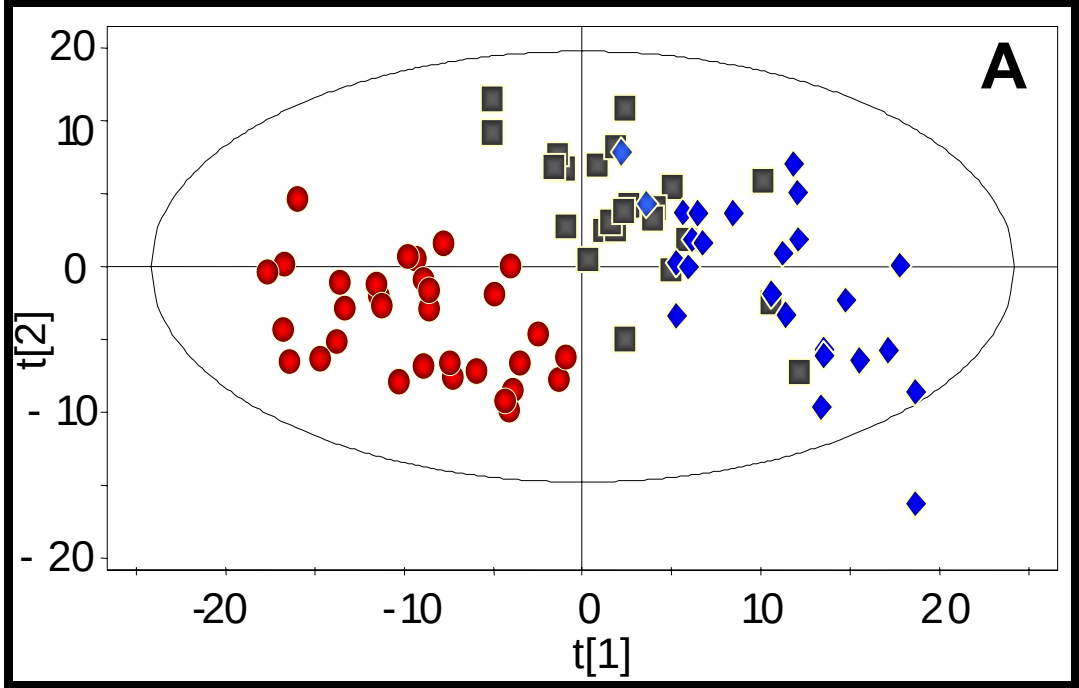
Clinicopathological characteristics	Gastric Cancer
Patients	26
Age (mean ± SD)	64.6 ± 12.4
Gender Male Female	 21 5
TNM classification T	 T1 - 5 T2 - 2 T3 - 13 T4 - 6
N	N0 - 11 N1 - 15
M	M0 - 24 M1 - 2
Grade of differentiation well moderately poorly	 2 10 14
Lauren classification intestinal type difused type	 14 11

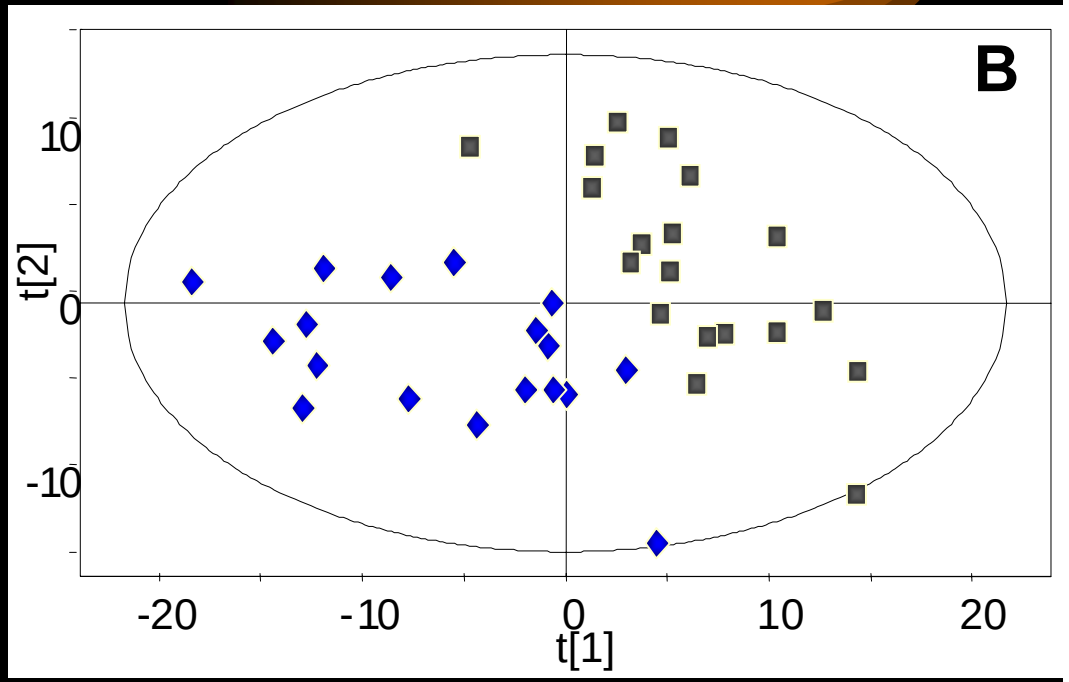
Clinicopathological characteristics	Colon Cancer
Patients	27
Age (mean ± SD)	65.7 ± 8.6
Gender Male Female	 14 13
Grade of differentiation well moderately poorly	 1 21 5
Astler-Coller stage	 A - 2 B1 - 1 B2 - 11 B3 - 1 C1 - 1 C2 - 11


COLON
GASTRIC
CTL
t[2]
t[1]
10
0
-10
-20
-10
0
10
20

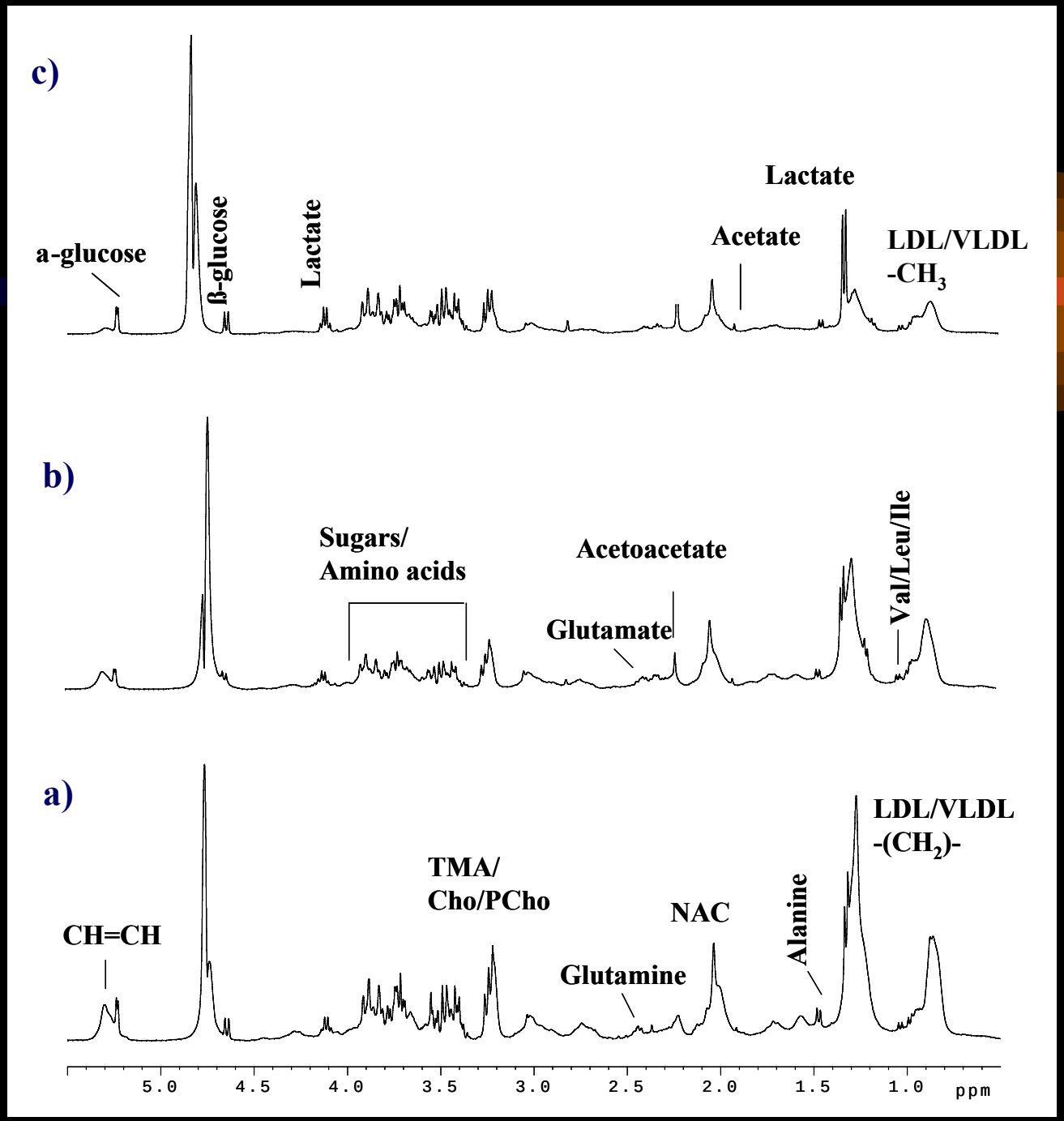

GASTRIC
COLON
t[2]
t[1]
10
0
-10
-20
-10
0
10
20

PCA





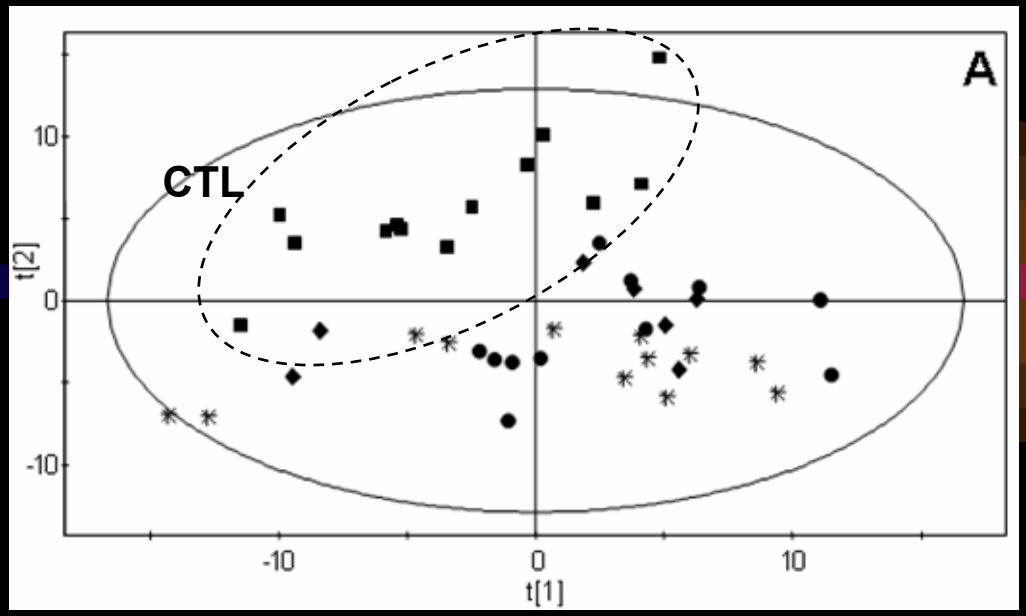
PLS-DA


c)
a-glucose
ß-glucose
Lactate
Acetate
Lactate
LDL/VLDL
-CH3
b)
Sugars/
Amino acids
Acetoacetate
Glutamate
Val/Leu/Ile
a)
CH=CH
TMA/
Cho/PCho
Glutamine
NAC
Alanine
LDL/VLDL
-(CH2)-
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

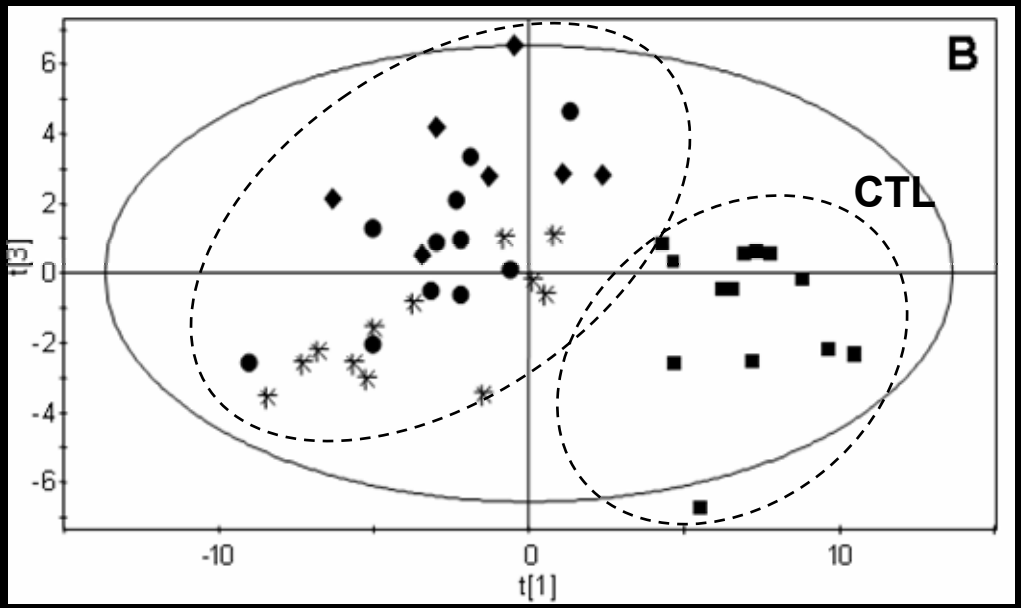
Renal Cell Carcinoma

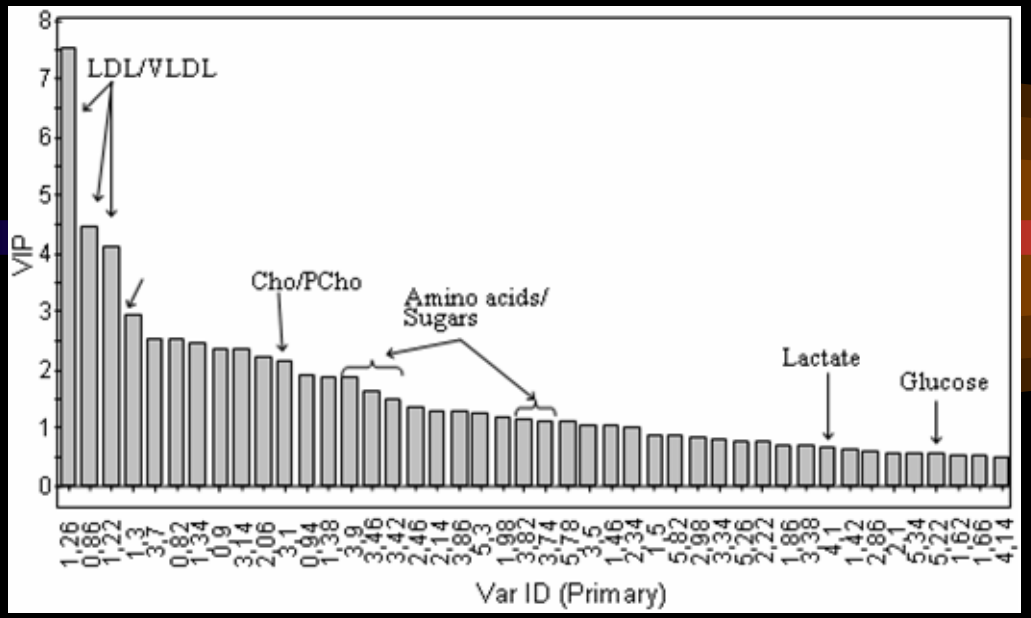
Clinicopathological characteristics	**RCC**
Patients	36
Age (mean ± SD)	63.33± 12.15
Gender	
Male	25
Female	11
Size	
Histological Grade	
1	2
2	10
3	14
Histological Type	
1	
2	
Stage	
I	5
II	6
III	8
IV	7

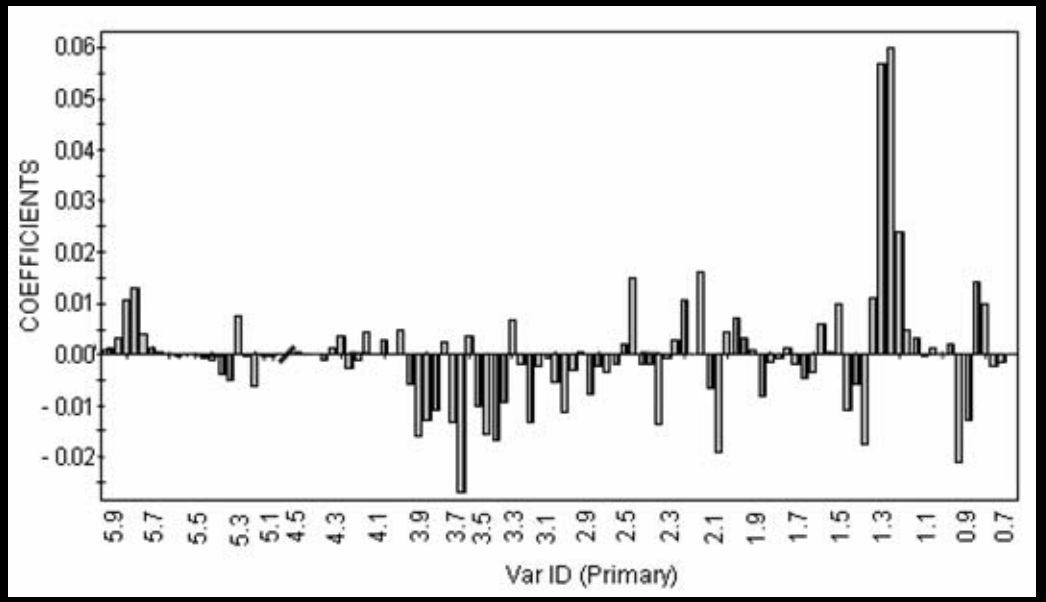
PCA

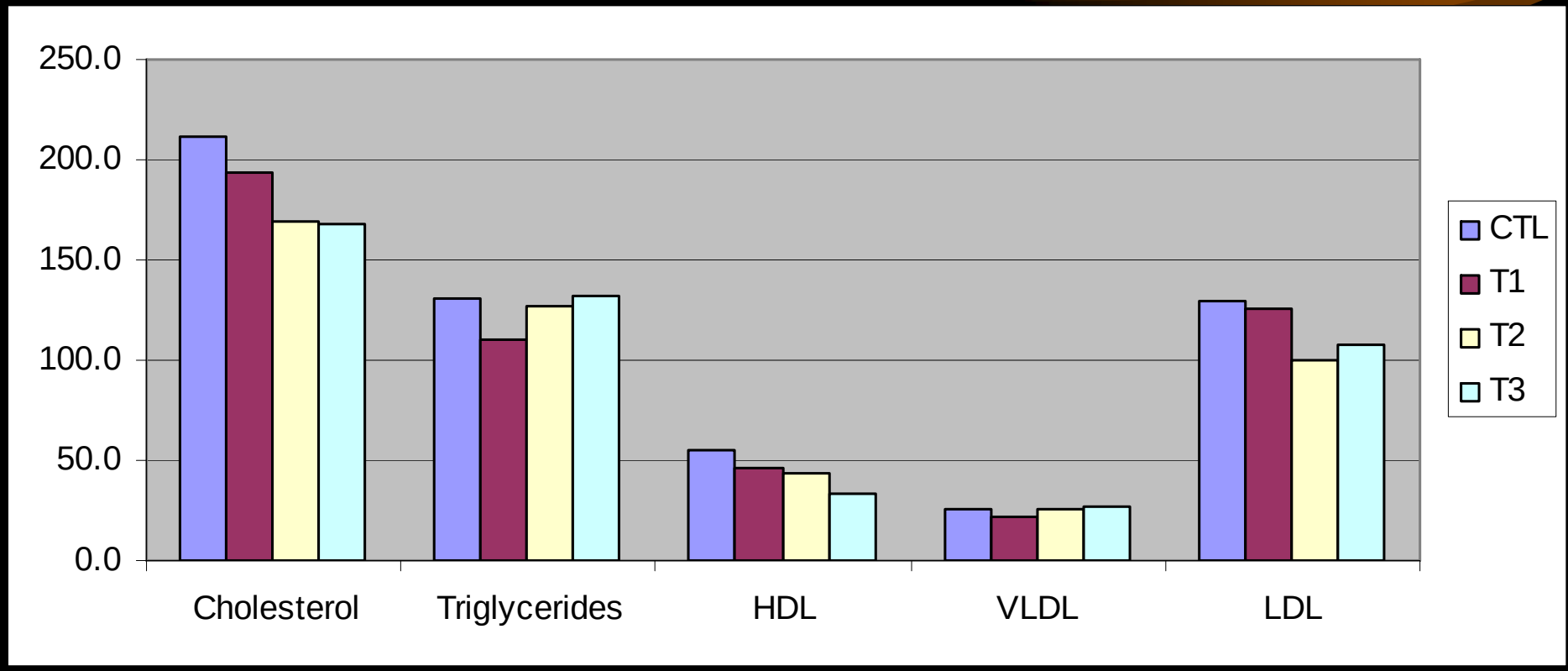


PLS-DA



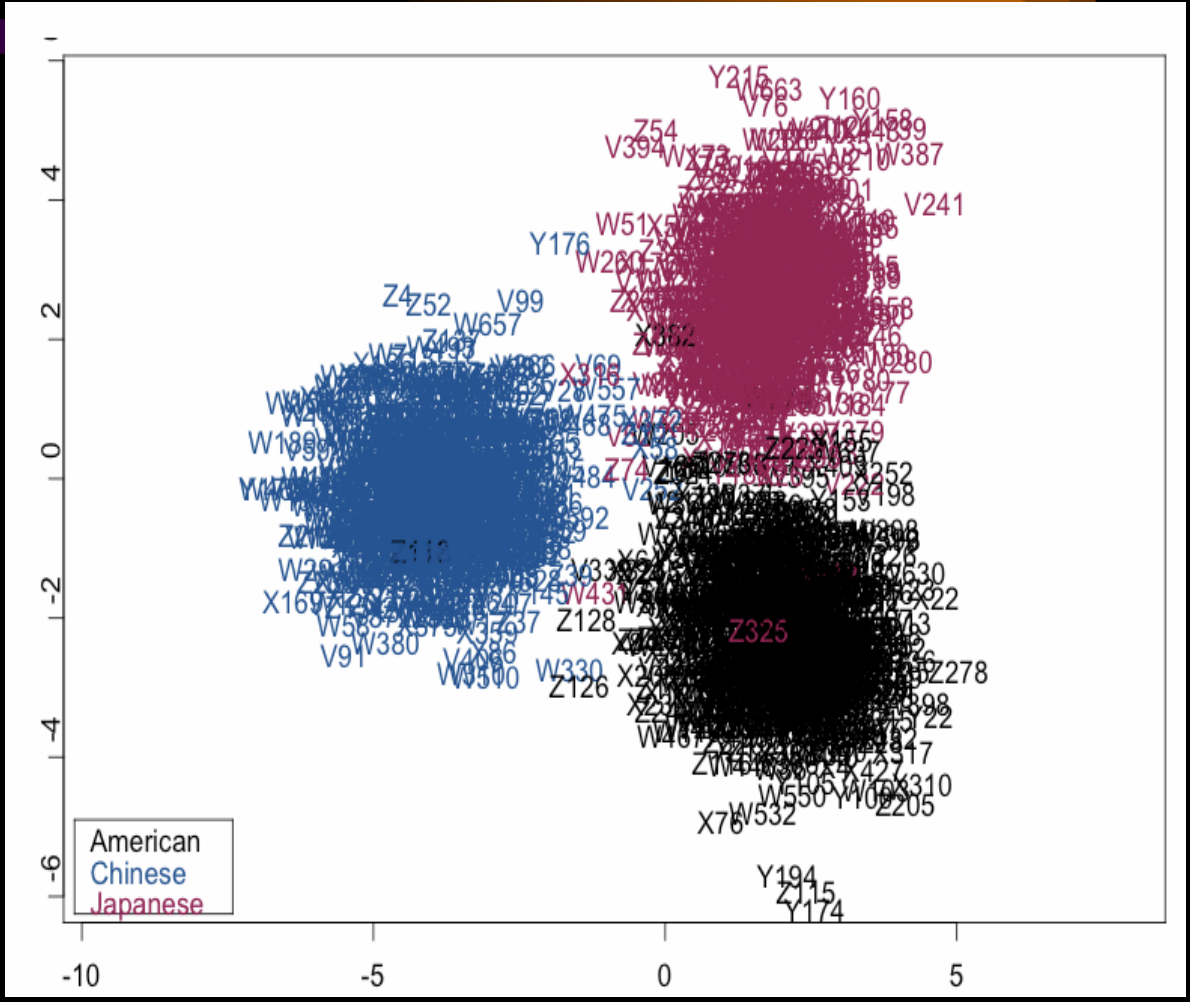

VIP
LDL/VLDL
Cho/PCho
Amino acids/
Sugars
Lactate
Glucose
Var ID (Primary)


COEFFICIENTS
Var ID (Primary)


250.0
200.0
150.0
100.0
50.0
0.0
Cholesterol
Triglycerides
HDL
VLDL
LDL
CTL
T1
T2
T3

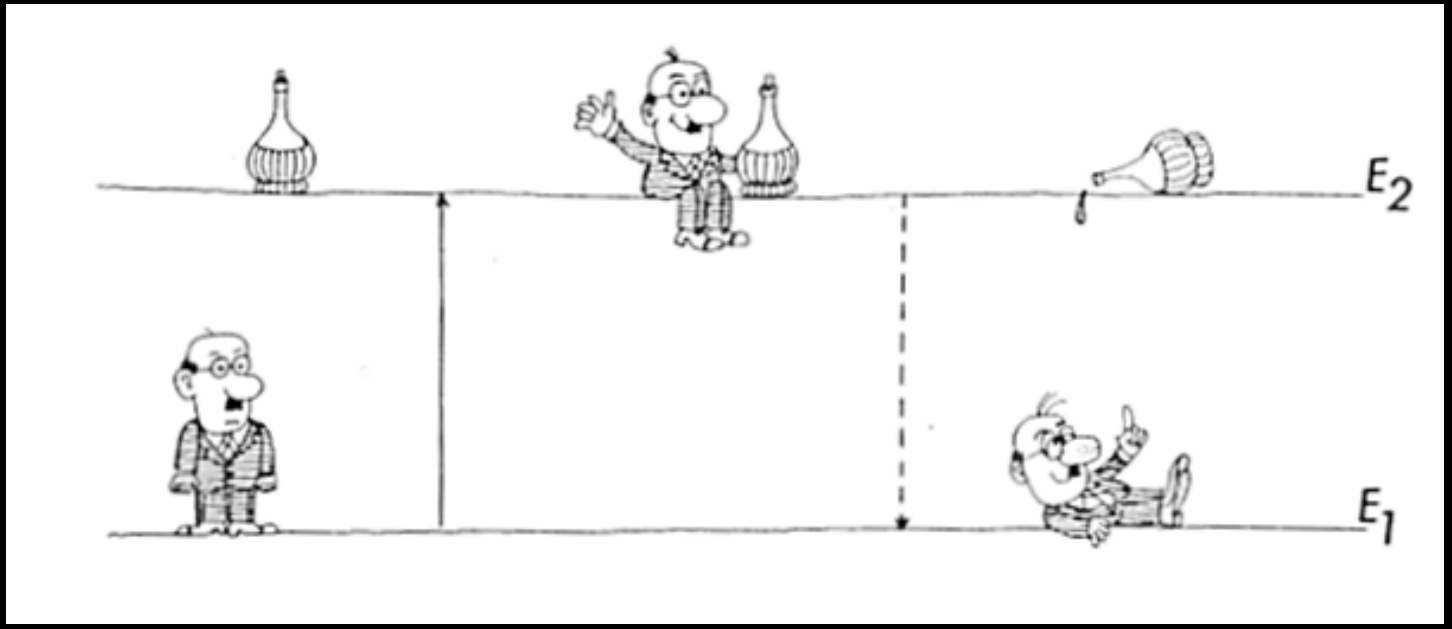
Metabotype Variability

- Is it possible to discriminate pathological to physiological states?
- INTERMAP
  - Nicholson and coworkers



Plant Metabonomics

Wine authentication

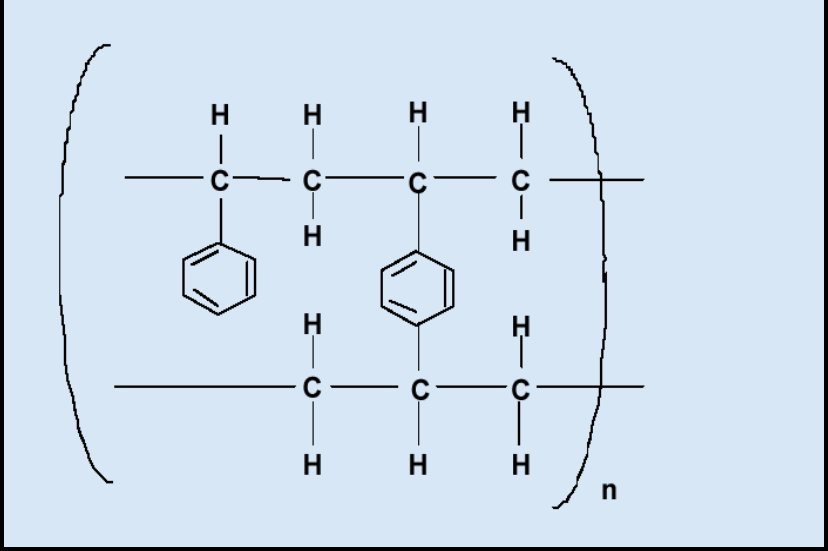


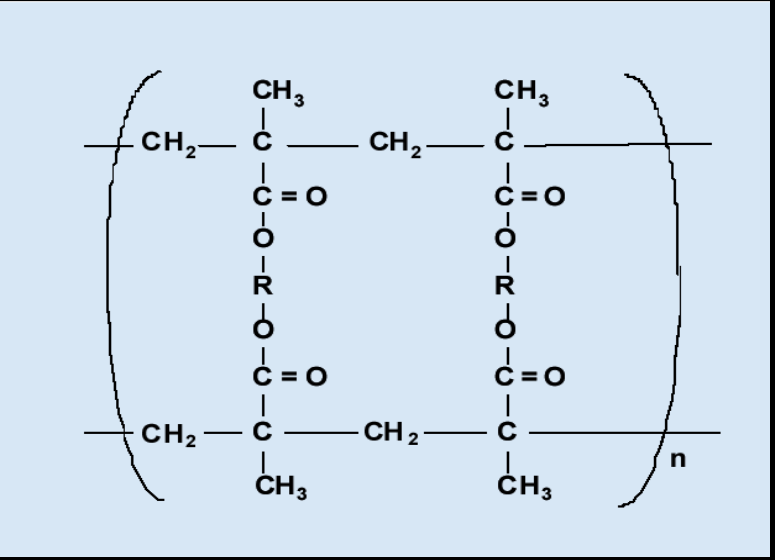
Wine Classification

- Polyphenol extraction-XAD technology
- ^{1}H 1D NMR
- Multivariate analysis

ADSORPTION RESINS

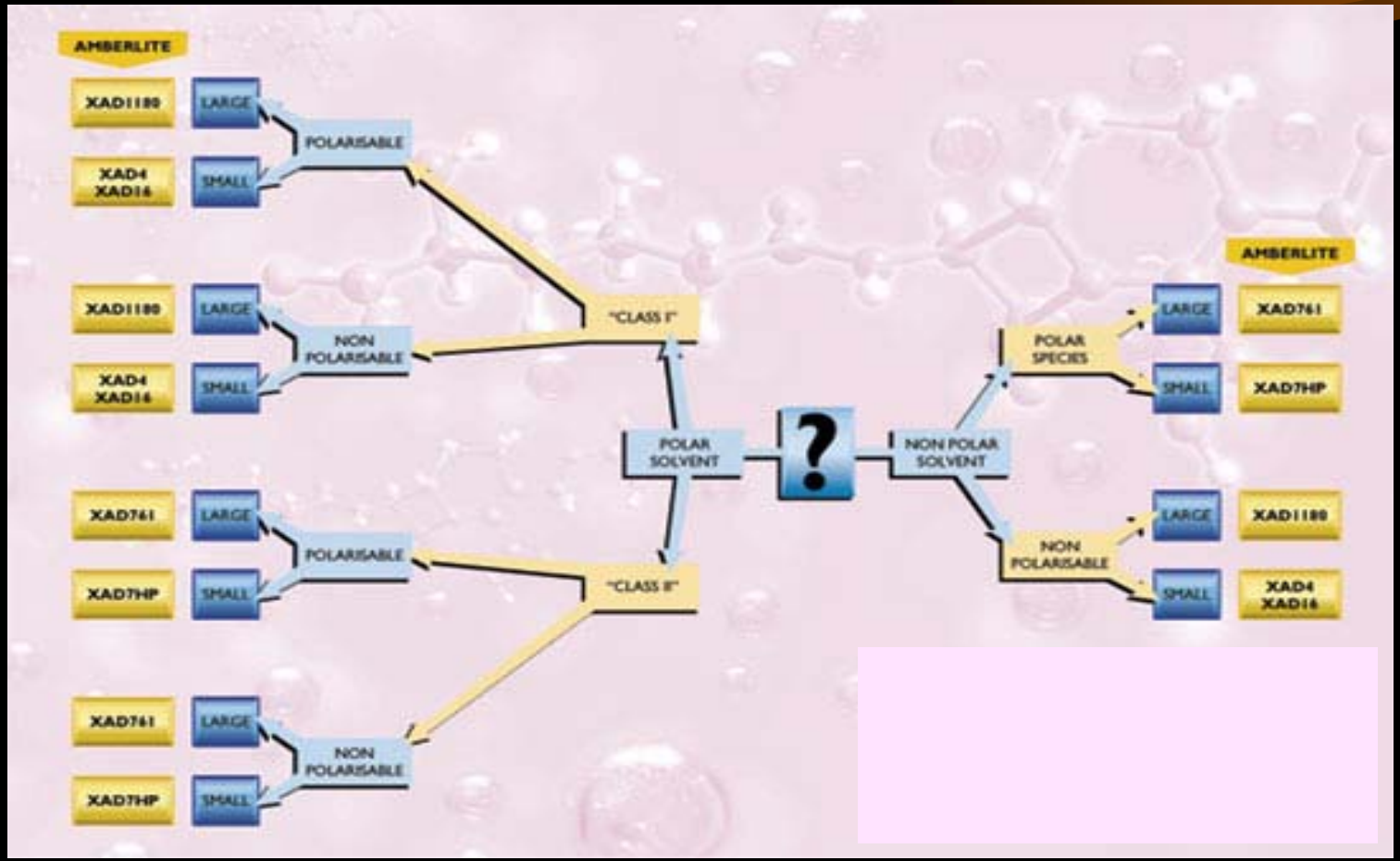
- Polymeric adsorbents are highly porous structures, mainly of styrenic or acrylic type, whose internal surfaces can adsorb mainly by π-π interactions and then desorb a wide variety of different chemical substances depending on the solvent with which they are used.





ADSORPTION RESINS

- They present an increased affinity for molecules with conjugated double bonds or planar aromatic systems and this property makes them very useful for the purification of several types of natural products especially polyphenols or for the enrichment of plant extracts.



Pilot Scale Extraction

- **Sample Preparation**
- **Wine samples were collected from the principal red and white varieties cultivated in the appellation of Nemea in South Greece and in Santorini, a volcanic island in the Southern Aegean Sea with extreme weather conditions and limited rainfall, for two successive vintages.**
- **All samples were provided from the local wineries, and care was taken, that multiple wine samples were collected from different tanks, to ensure that the samples were representative of the certain variety and geographical origin.**
- **Polyphenols were isolated from 150 mL wine using the XAD adsorption resins technology.**
- **The polyphenolic fraction was collected with elution of the column with EtOH and lyophilisation. The solutions were reconstructed using 700 μL of MeOD for NMR analysis.**

Variety and origin of the 66 analyzed wines

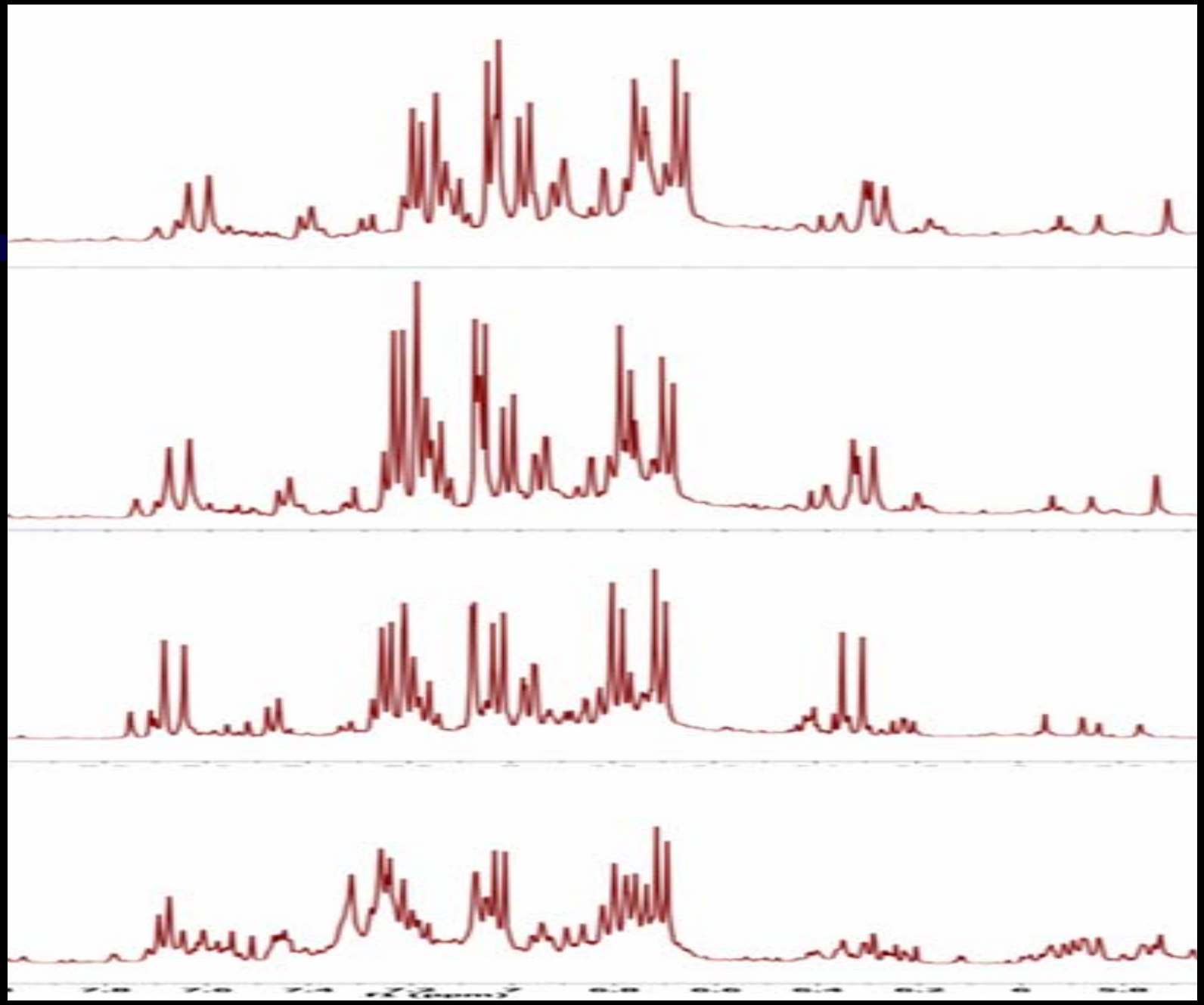
Groups	Vine variety	Region	Year of production	Number of samples	Wine Color
1	Agiorgitiko	Nemea	2006	22	Red
2	Agiorgitiko	Nemea	2005	12	Red
3	Moschofilero	Nemea	2006	3	White
4	Mandilaria	Santorini	2005	3	Red
5	Mandilaria	Santorini	2006	3	Red
6	Asyrtiko	Santorini	2005	10	White
7	Asyrtiko	Santorini	2006	13	White

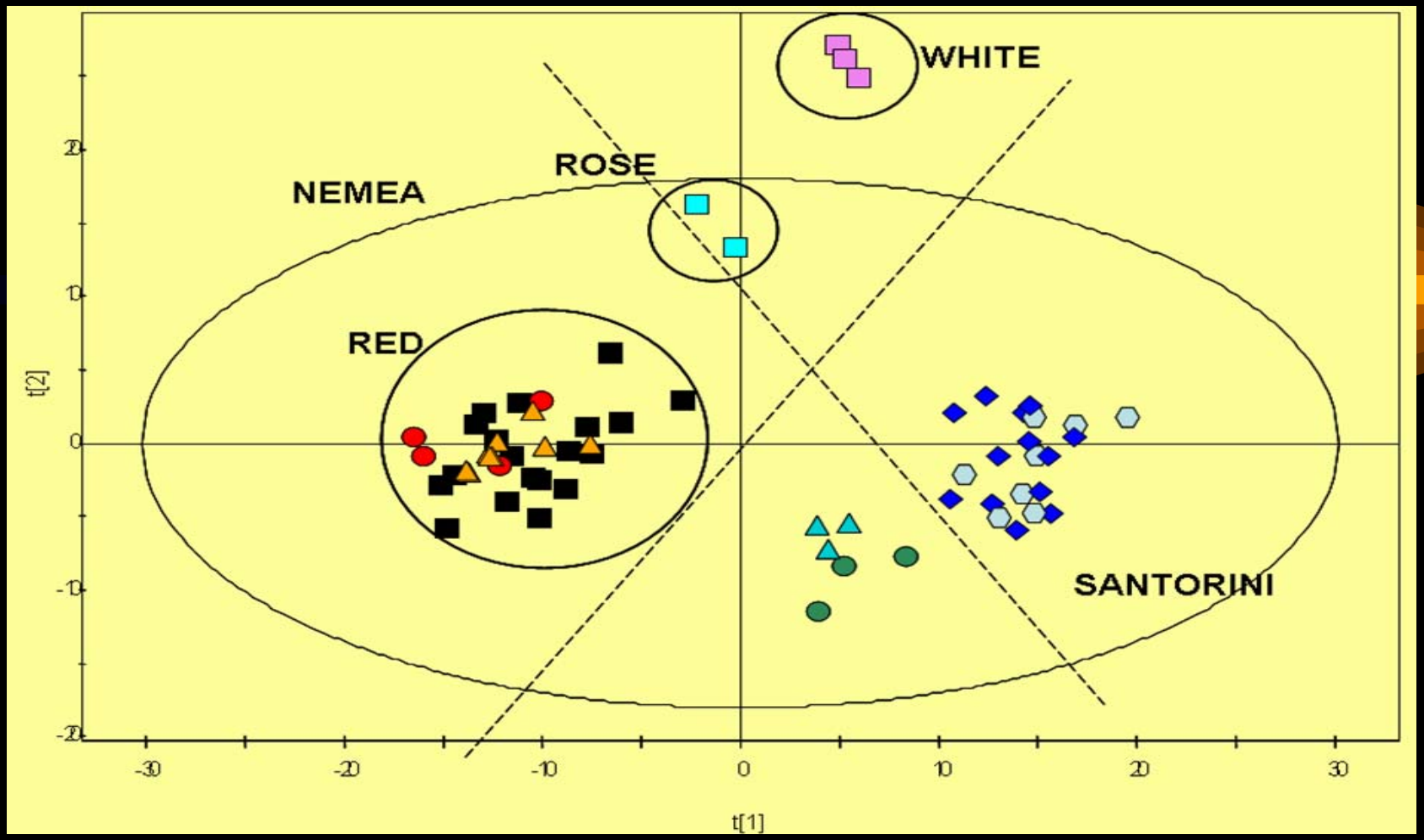
Agiorgitiko

Mandilaria

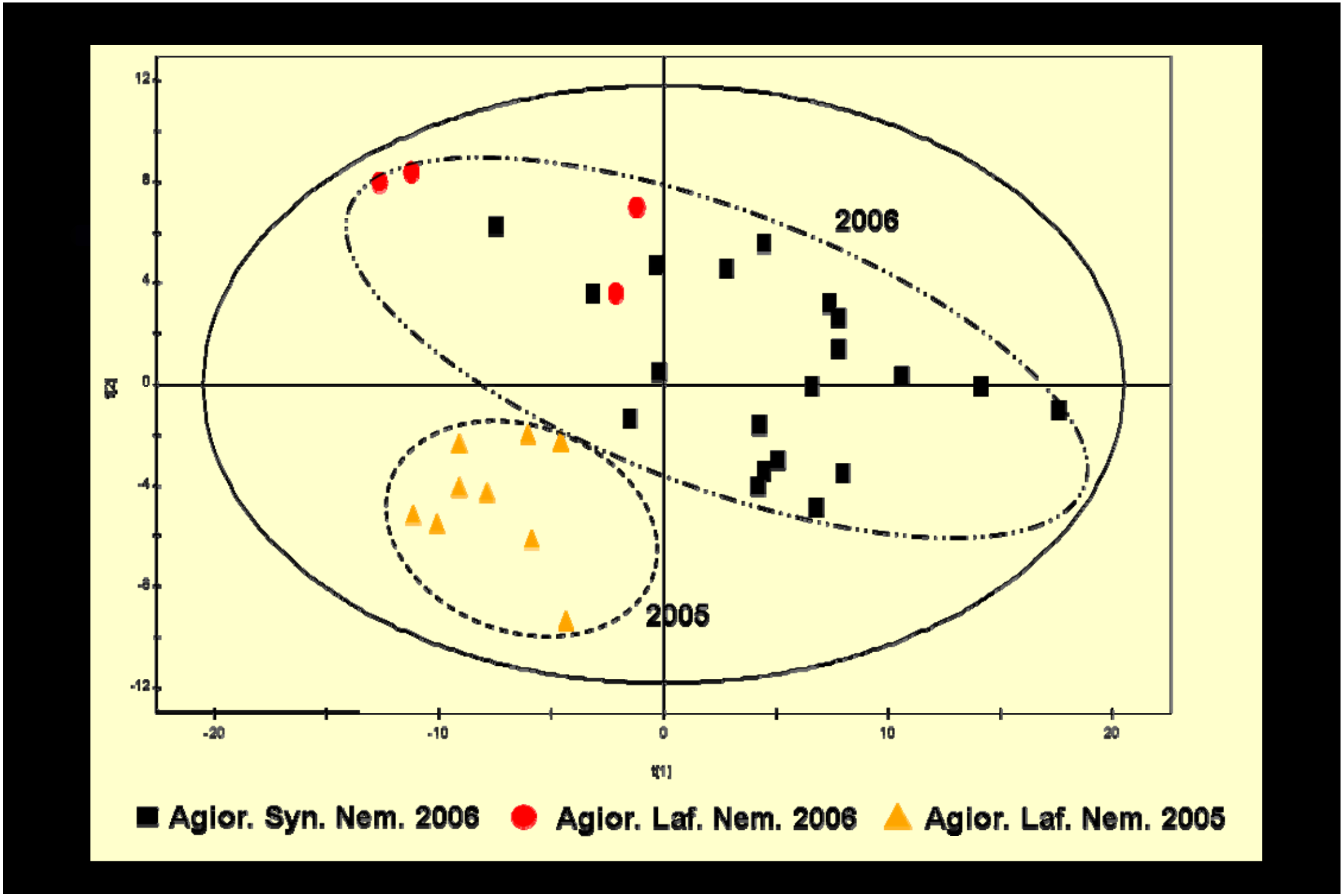
Asyrtiko

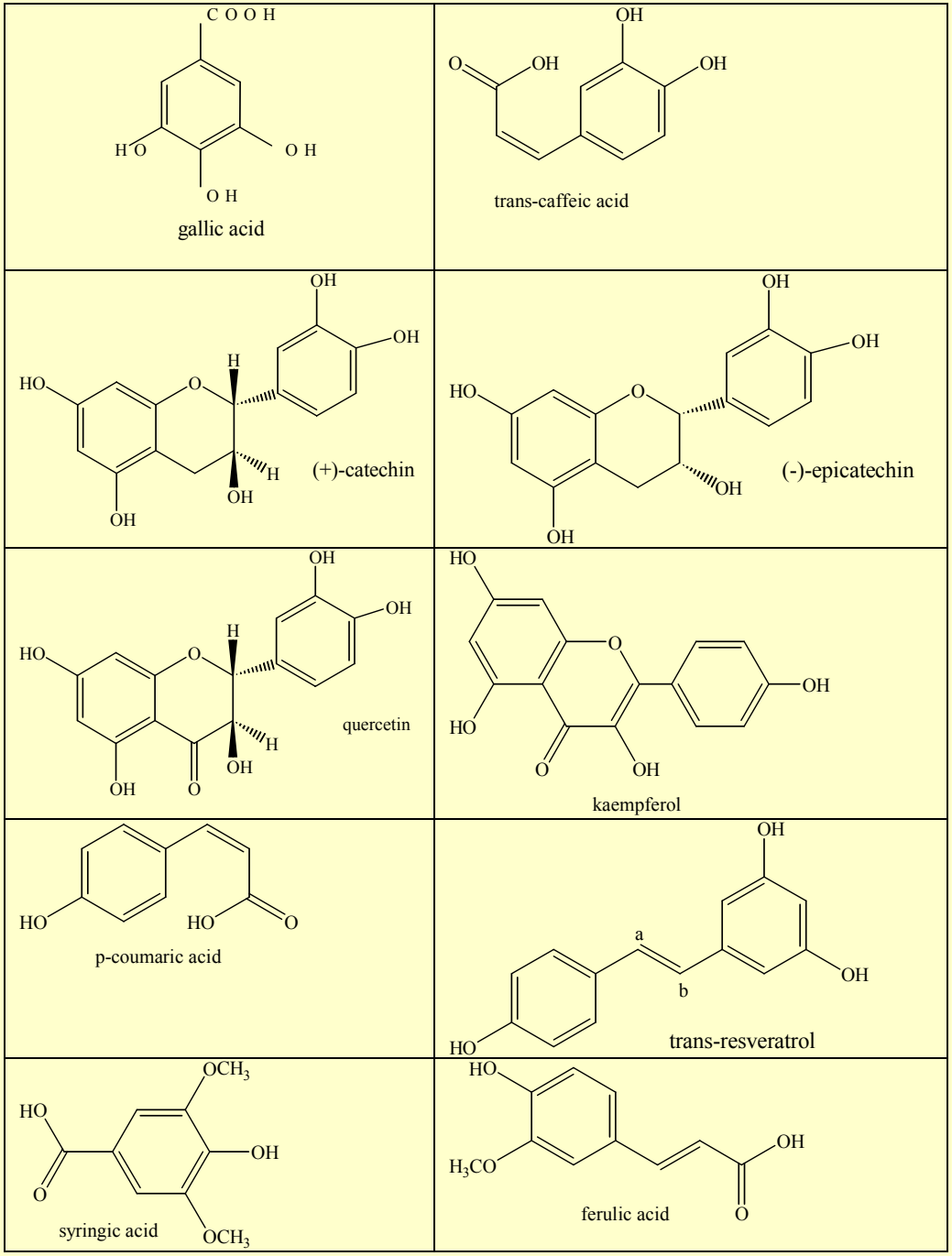
Moshofilero

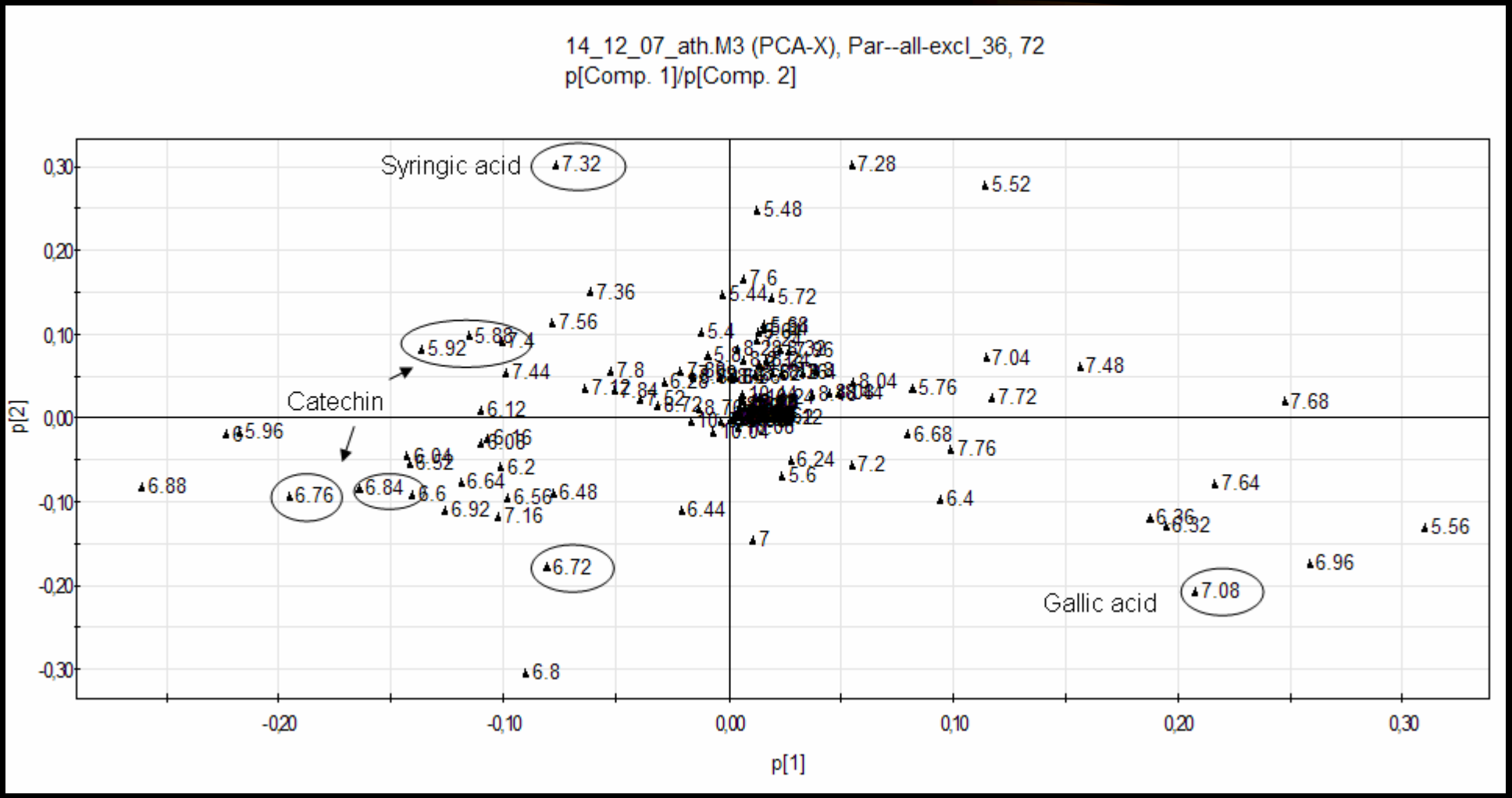




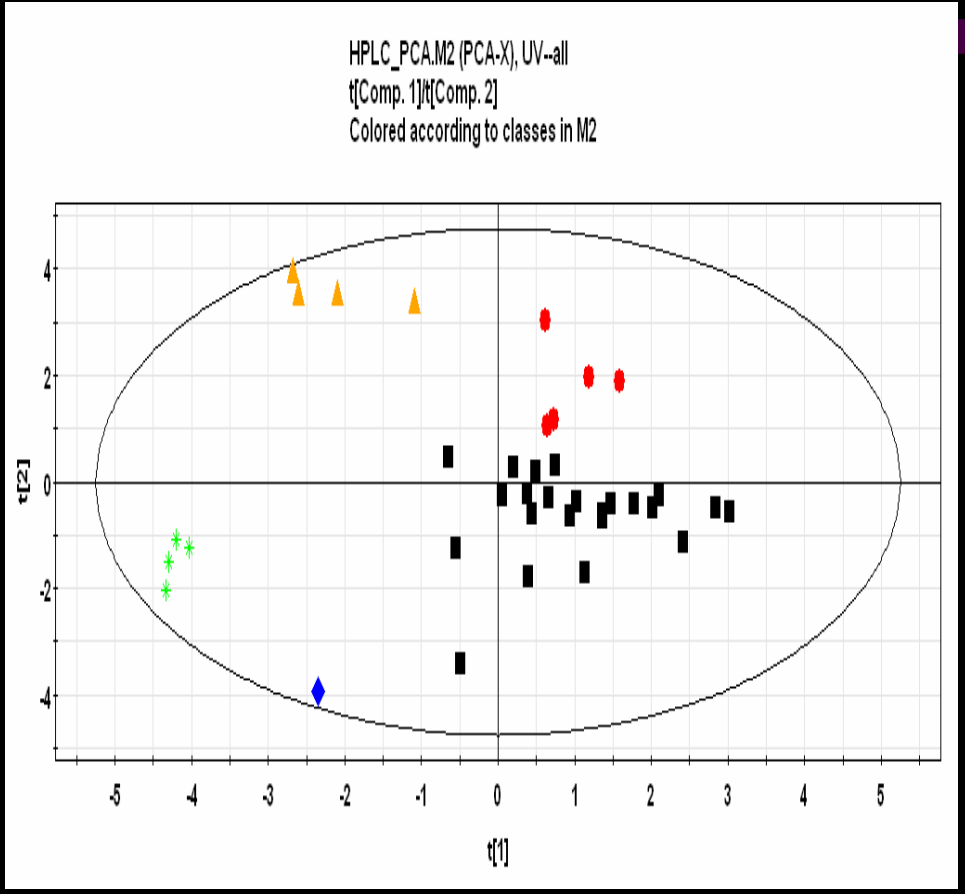
- Agior. Syn. Nem. 2006
- Agior. Laf. Nem. 2006
- Asyrt. Santo. 2006
- Asyrt. Santo. 2005
- Agior. Laf. Nem. 2005
- Mosx. Laf. 2006
- Mandil. Santo. 2005
- Mandil Santo 2006

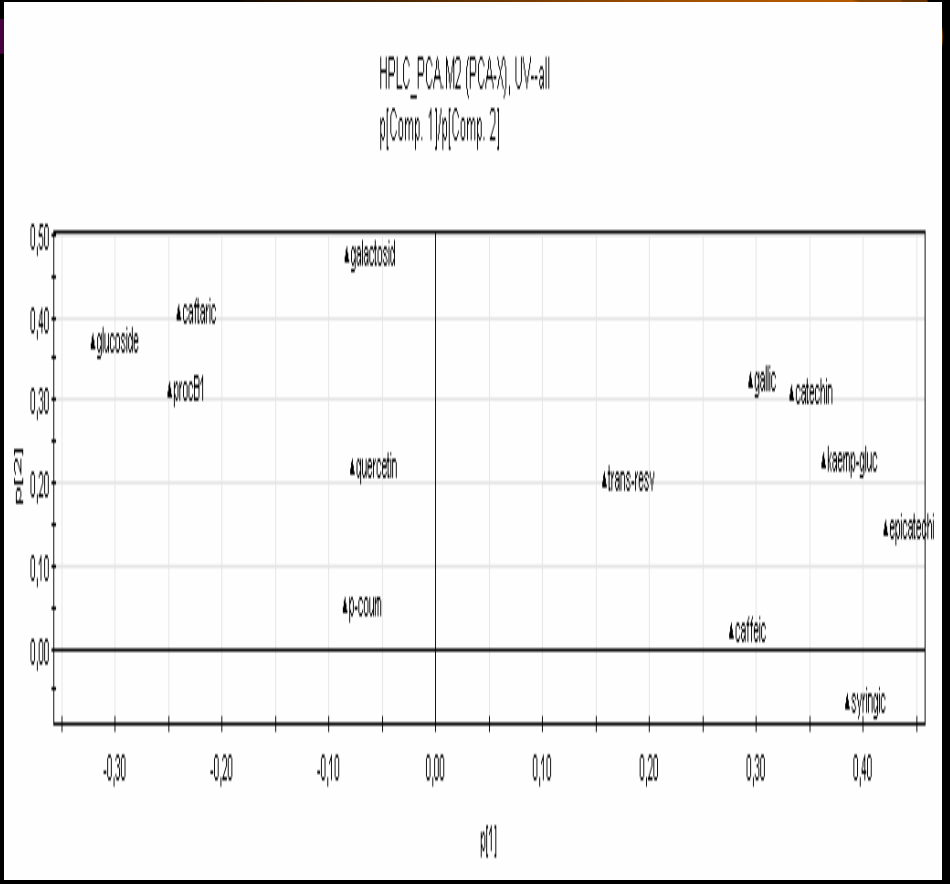

12
8
4
0
-4
-8
-12
t[2]
-20
-10
0
10
20
t[1]
2006
2005
Agior. Syn. Nem. 2006
Agior. Laf. Nem. 2006
Agior. Laf. Nem. 2005


gallic acid
trans-caffeic acid
(+)-catechin
(-)-epicatechin
quercetin
kaempferol
p-coumaric acid
trans-resveratrol
syringic acid
ferulic acid


14_12_07_ath.M3 (PCA-X), Par--all-excl_36, 72
p[Comp. 1]/p[Comp. 2]
Syringic acid
Catechin
Gallic acid
7.32
7.08
6.72
6.76
6.84
5.92
5.88
7.4
7.44
7.36
7.56
6.12
5.96
6.88
6.64
6.2
6.6
6.56
6.48
6.92
7.16
6.8
7.28
5.52
5.48
7.6
5.44
5.72
5.4
7.8
7.12
7.84
6.28
7.52
8.04
5.76
7.72
7.04
7.48
7.68
6.68
7.76
6.24
7.2
5.6
6.4
6.44
7
7.64
6.36
6.32
5.56
6.96
p[2]
p[1]
0,30
0,20
0,10
0,00
-0,10
-0,20
-0,30
-0,20
-0,10
0,00
0,10
0,20
0,30

HPLC - PCA


HPLC_PCA.M2 (PCA-X), UV--all
t[Comp. 1]/t[Comp. 2]
Colored according to classes in M2
t[2]
t[1]


HPLC_PCA.M2 (PCA-X), UV--all
p[Comp. 1]/p[Comp. 2]
galactosid
caftaric
glucoside
procB1
gallic
catechin
quercetin
trans-resv
kaempg-gluc
epicatechi
p-coum
caffeic
syringic
p[2]
p[1]

Aknowlegments

- Anna Tsantili
- Maria Constantinou
- Athina Zira
- Maria Papaefthimiou
- Elena Niotaki
- Maria Anastasiadi

- Ioanna Andreadou
- Efstathios Iliodromitis
- Dimitrios Kremastinos

- Stamatis Theocharis

- Leandros Skaltsounis
- Prokopios Magiatis