National and Kapodistrian
UNIVERSITY OF ATHENS

INVESTIGATING THE USE OF OLEUROPEIN AS PROPHYLACTIC AND THERAPEUTIC TREATMENT IN CARDIOVASCULAR DISEASES.

Ioanna Andreadou PhD

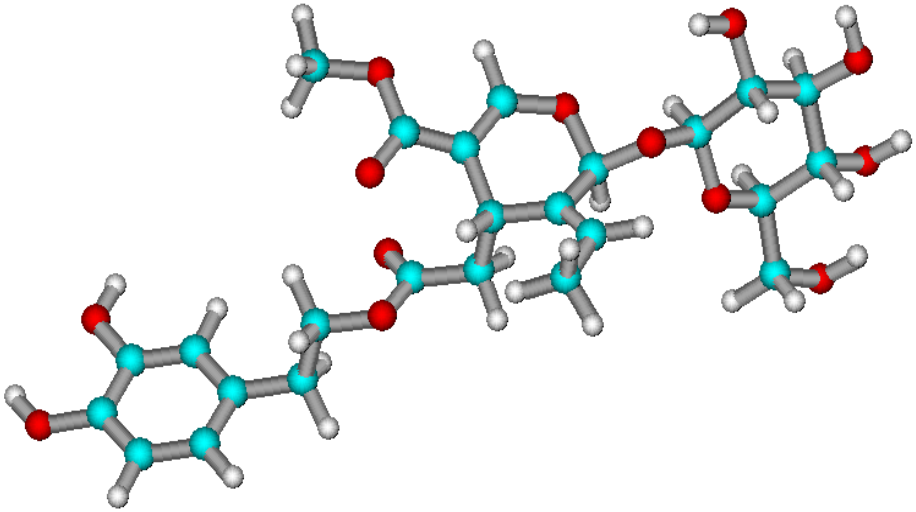
Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, Greece

Data from clinical studies indicate that compounds present in the olive oil are exhibiting antioxidant activities and can view additional beneficial effects as pharmacological intervention in the management of patients with coronary artery disease

Olea europaea L. leaf constituents possess proven beneficial results on myocardial oxidative stress and atherosclerosis. **Basic research is mandatory to evaluate the cardioprotective effects of olive leaf constituents in order to investigate plant origin compounds as pharmacological tools for the prevention and the protection of heart diseases.**

Efentakis P., et al., Planta Medica, 81:648-54, 2015

OLEUROPEIN



- Polyphenolic compound
- Compound of olives and olive oil (Olea europaea)
- **Effect:** Antioxidant

OLEUROPEIN

PREVENTIONAL AGENT

Nutritional dose on myocardial infarct size limitation and atherosclerosis

THERAPEUTICAL AGENT

Pharmaceutical dose on myocardial infarct size limitation-
Molecular mechanisms

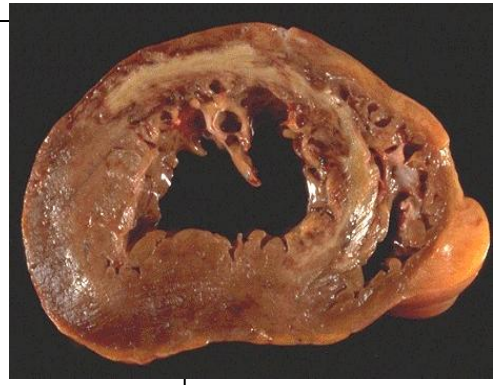
PREVENTIONAL AND THERAPEUTICAL AGENT

Acute and chronic cardiomyopathy (Doxorubicin induced)

Plaque rupture or erosion

Acute thrombosis

Necrosis



Thrombolysis or PCI

Flow restoration

COST

Cell Survival

Reperfusion Injury

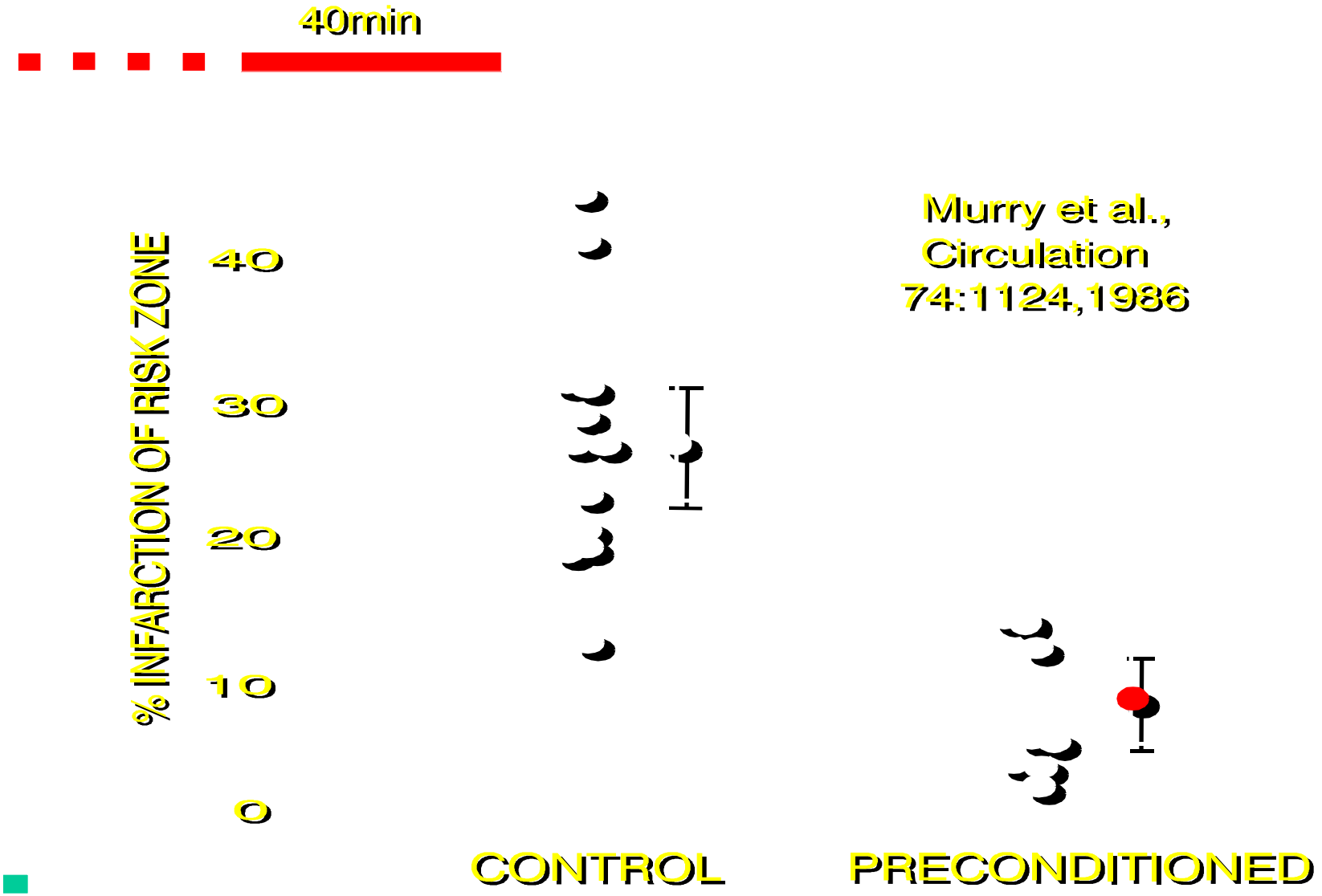
Cell Death

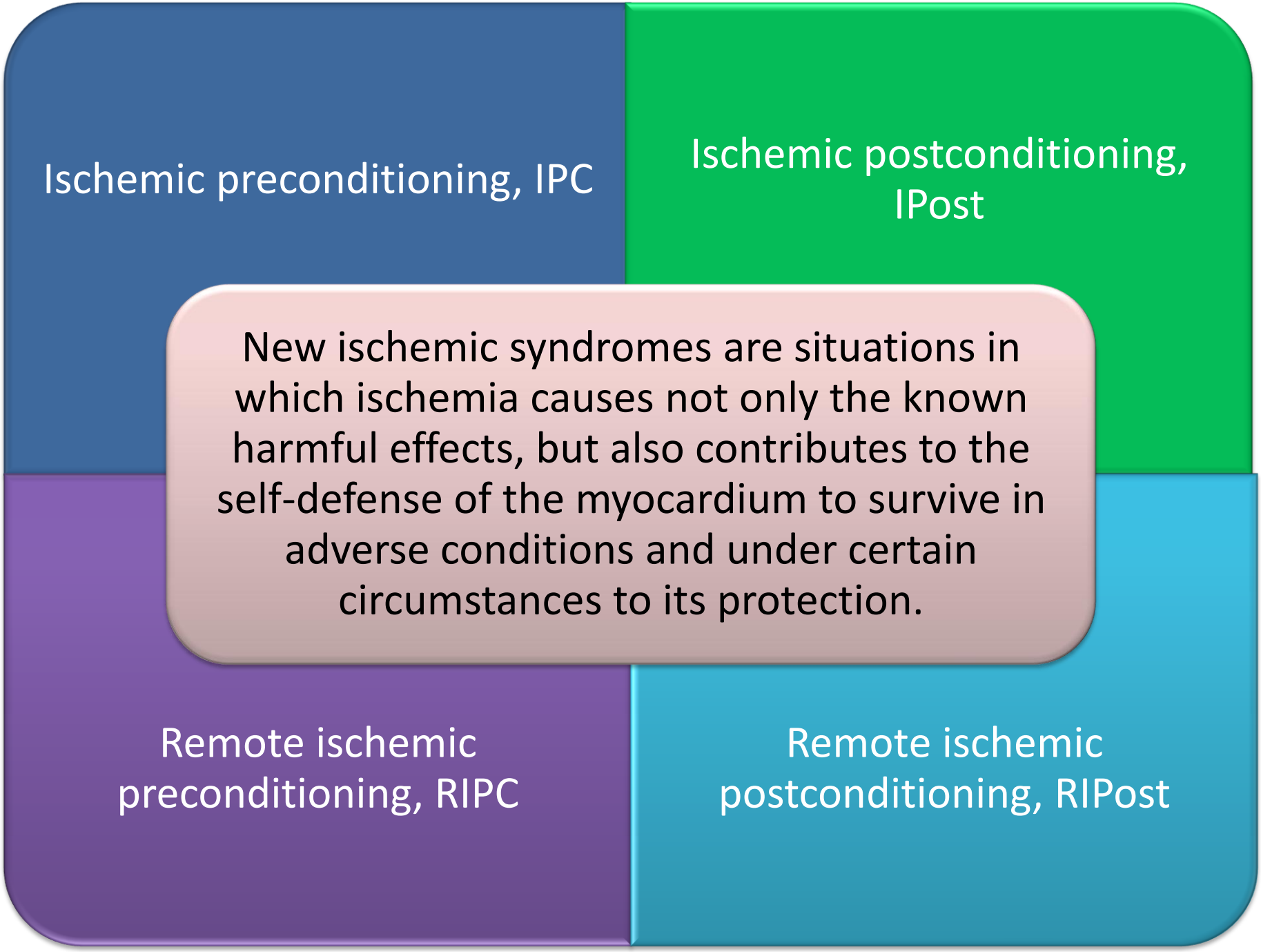
Endothelial dysfunction

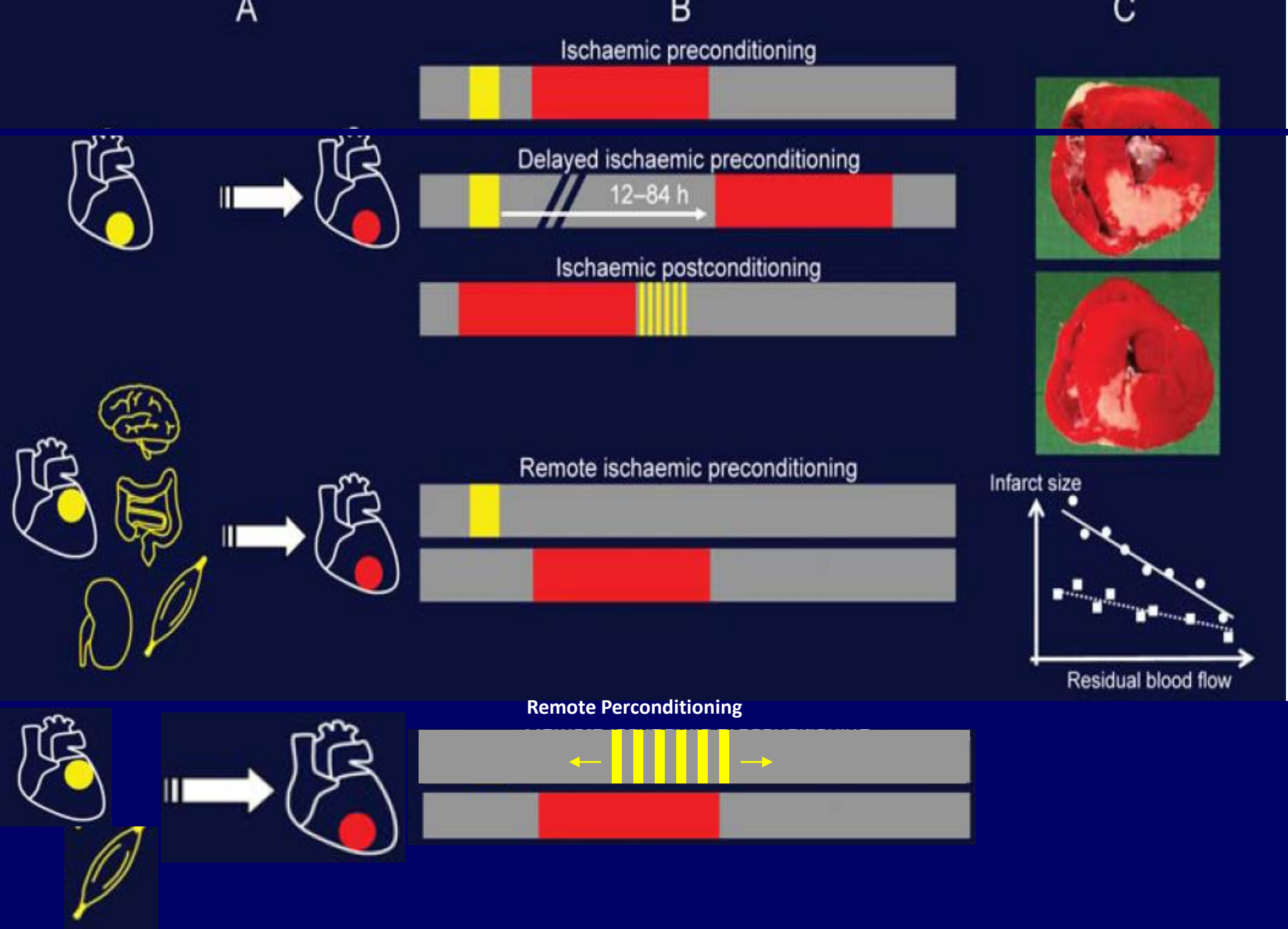
Arrhythmias

Calcium overload
Free radicals

The biggest progress on the comprehension of the mechanism of cell survival is noted on 1986.



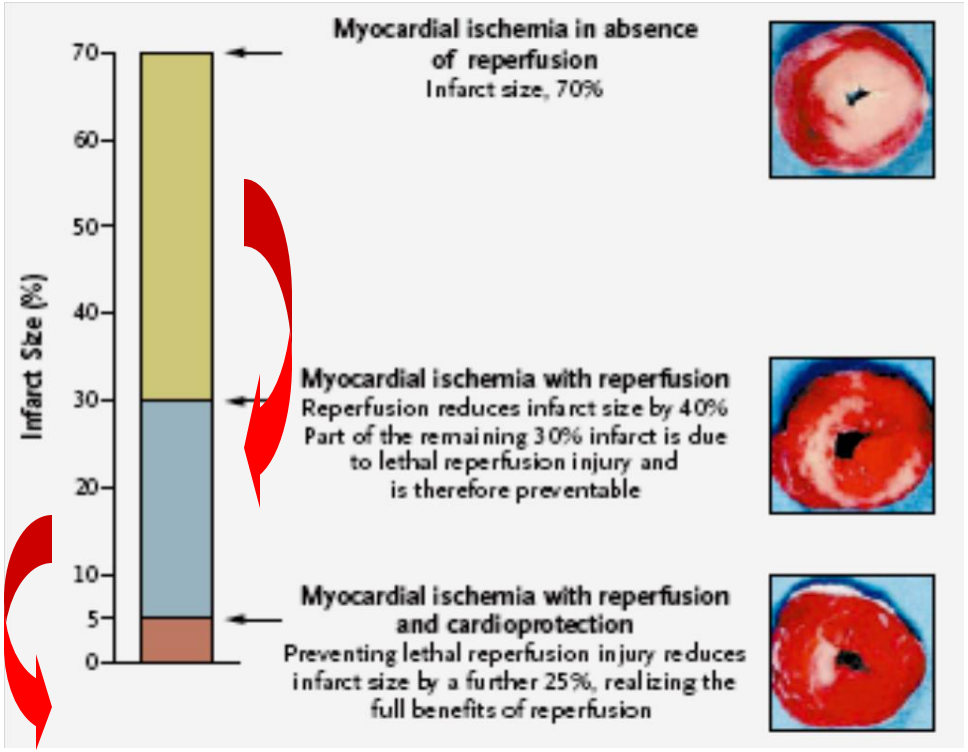

Ischemic preconditioning, IPC
Ischemic postconditioning, IPost
New ischemic syndromes are situations in which ischemia causes not only the known harmful effects, but also contributes to the self-defense of the myocardium to survive in adverse conditions and under certain circumstances to its protection.
Remote ischemic preconditioning, RIPC
Remote ischemic postconditioning, RIPost



Remote Perconditioning

Reperfusion injury is an entity separate from ischemic injury

- Cell Death
- Stunning
- Endothelial dysfunction
- Arrhythmias
- Apoptosis

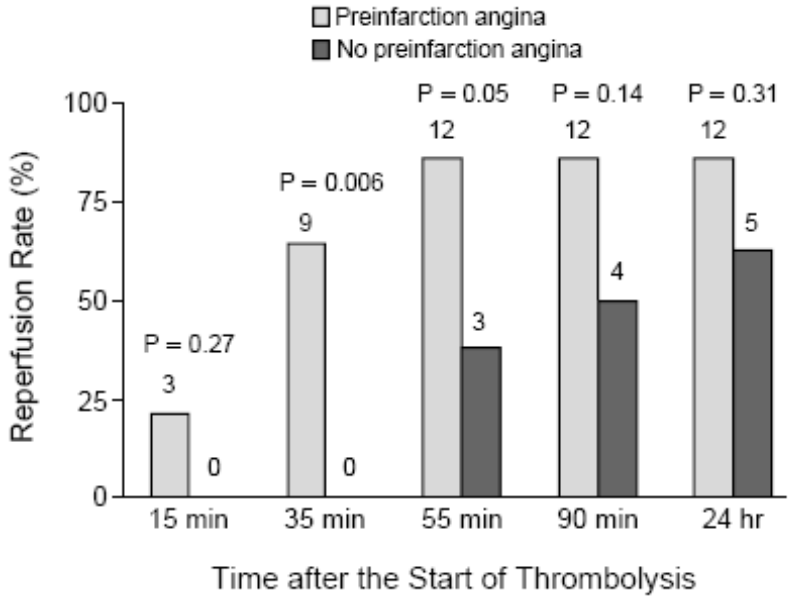


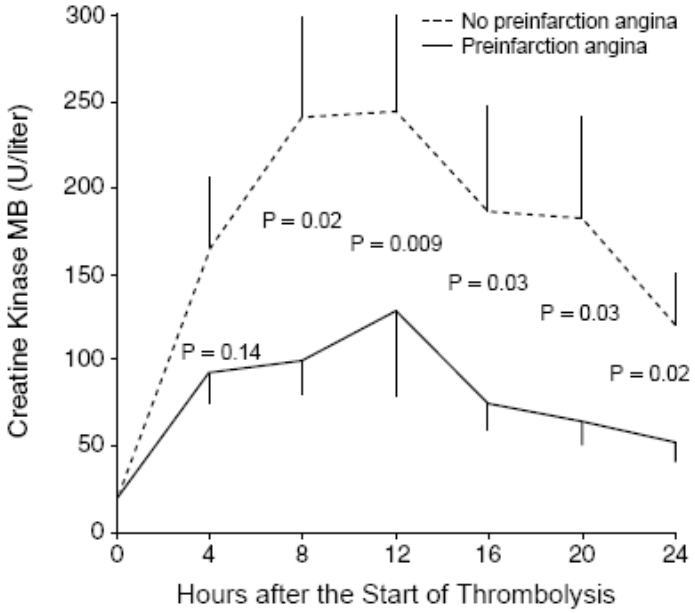
PC and PostC protect from *R.I.*

Yellon DM, Hausenloy DJ: New Engl J Med 2007;357:1121-1135

PREINFARCTION ANGINA AS A PREDICTOR OF MORE RAPID CORONARY THROMBOLYSIS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

FELICITA ANDREOTTI, M.D., PH.D., VINCENZO PASCERI, M.D., DAVID R. HACKETT, M.D., GRAHAM J. DAVIES, M.D., AGHA W. HAIDER, M.D., AND ATTILIO MASERI, M.D.





It has been suggested that the benefit of preinfarction angina with respect to infarct size is determined by ischemic preconditioning. We propose that this benefit may depend on faster coronary thrombolysis, in addition to or instead of myocardial preconditioning.

N Engl J Med 1996;334:7-12

Pharmacological preconditioning and postconditioning thus represents an ideal alternative to preconditioning and greater effort needs to be exerted to invent pharmacological tools that mimic high-grade preconditioning

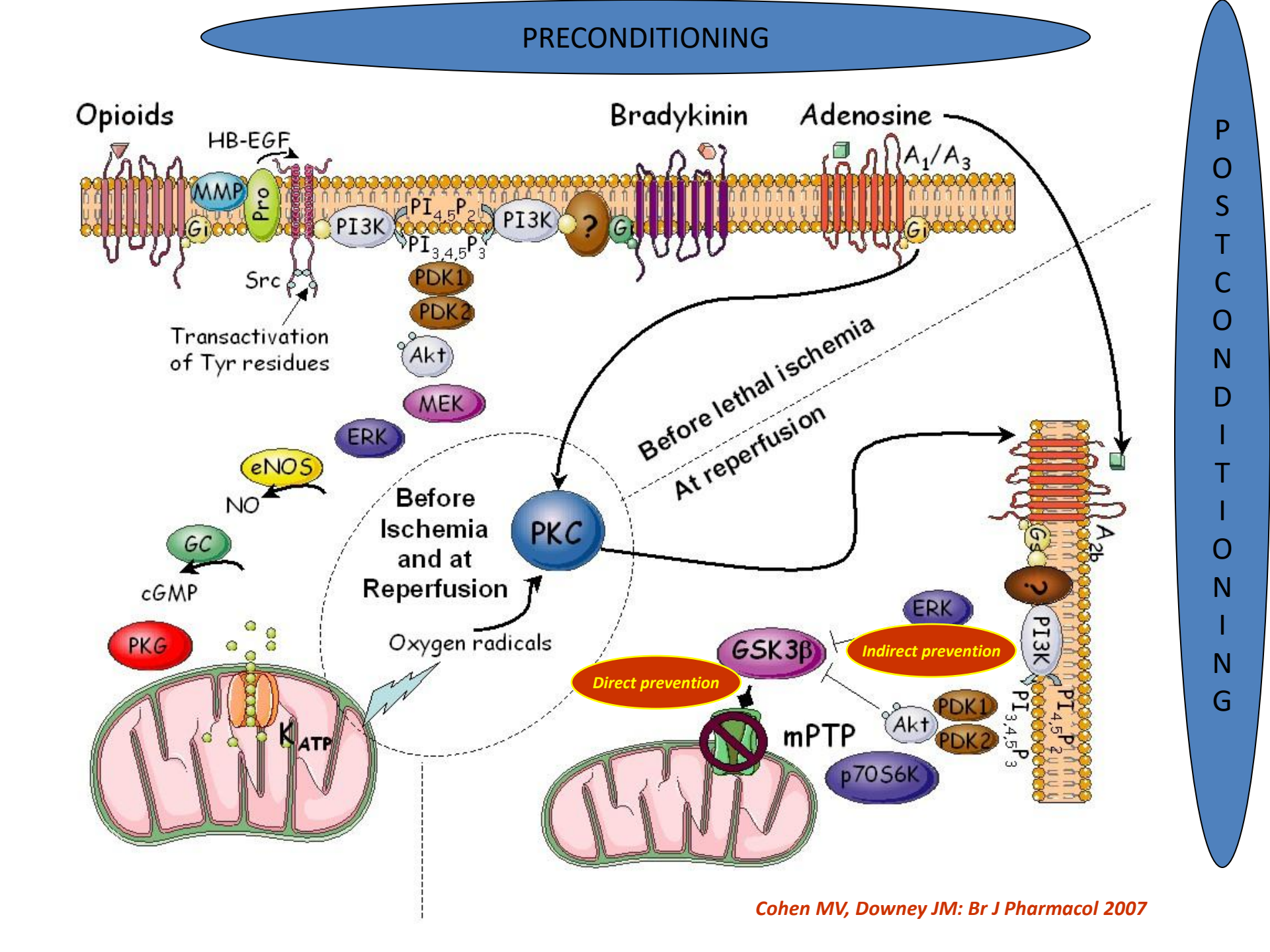
- Development of novel drugs that mimic ischemic preconditioning may result
- in increased tolerance to effort angina,
- may limit the infarct size in acute coronary syndromes and
- may better preserve the left ventricular function after PCI or open heart surgery.

Andreadou I, Iliodromitis EK,. Mini Rev Med Chem, 15: 1304-1313, 2008.
Andreadou I, Iliodromitis EK, et al., Curr Med Chem, 15: 1304-1313, 2008.

NUTRITIONAL PRECONDITIONING

- The nutritional intervention that provides equivalent cardioprotection to IPC by prior exposure.
- Mimic the signal transduction of ischemic preconditioning

Abdukeyum GG, Owen AJ, McLennan PL, J Nutrition, 138: 1902-9, 2008

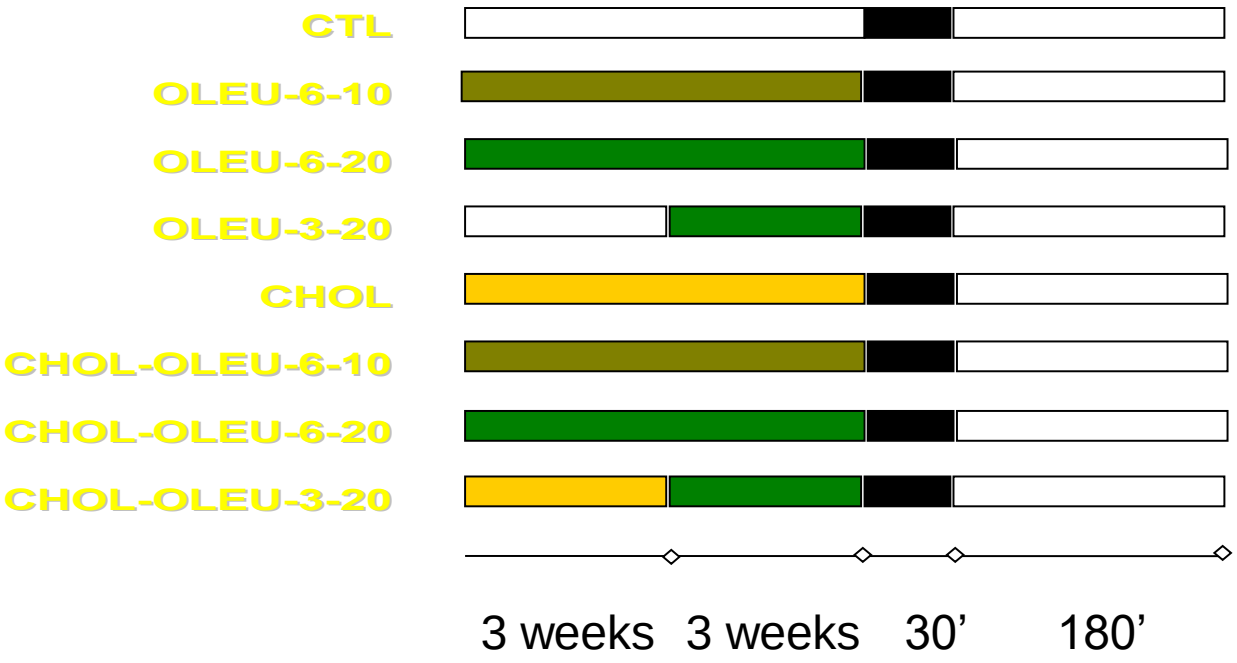


Cohen MV, Downey JM: Br J Pharmacol 2007

The Journal of Nutrition
Nutrition and Disease

The Olive Constituent Oleuropein Exhibits Anti-Ischemic, Antioxidative, and Hypolipidemic Effects in Anesthetized Rabbits[1,2]

Ioanna Andreadou,[3,4]* Efstathios K. Iliodromitis,[3] Emmanuel Mikros,[4] Maria Constantinou,[4] Apostolos Agalias,[5] Prokopios Magiatis,[5] Alexios Leandros Skaltsounis,[5] Elli Kamber,[6] Anna Tsantili-Kakoulidou,[4] and Dimitrios Th Kremastinos[3]



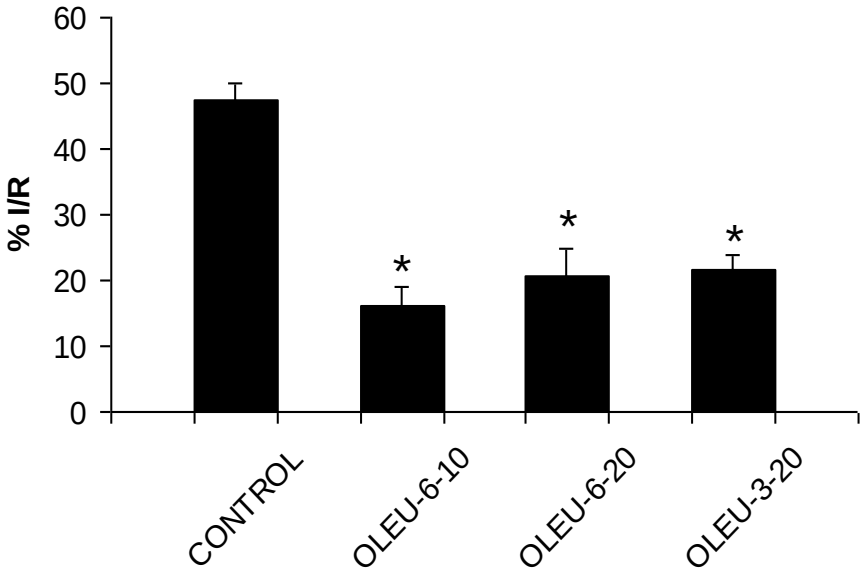
Dosage protocol

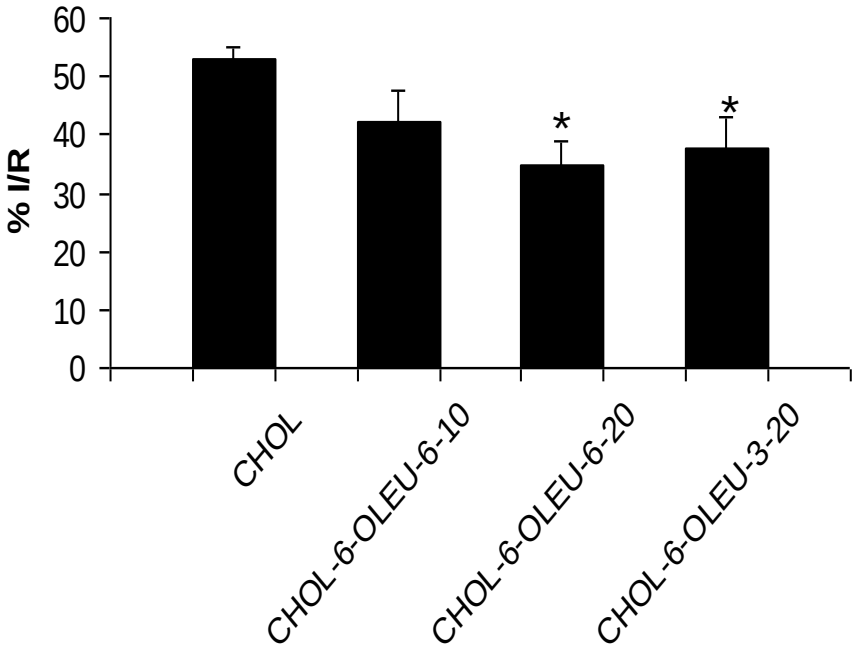
Dosage protocol. The dose used in the in vivo experiments was based on the average consumption of olive drupes and olive oil (17) in the Mediterranean area. Oleuropein is the major polyphenol of olive tree leaves, unprocessed drupes, and bitter table olives. The majority of polyphenolic metabolites found in olives or in olive oil (hydroxytyrosol, oleuropein aglycone, demethyloleuropein, elenolic acid) originate from the hydrolysis of oleuropein (18). Therefore, we expressed total polyphenol consumption from olive drupes or olive oil as the molecular equivalent of oleuropein, calculated to be ~100 mg/d.

For the extrapolation of the dosage from humans to rabbits, we used the metabolic body size or food intake rather than body weight as a criterion (19). The estimated quantity of oleuropein expressed per unit of human diet was 0.2 mg/g of dry food. Considering an energy density of ~16.4 kJ/g of dry food, the dose was calculated to be 0.012 mg/kJ. For rabbits, this consumption corresponded to a dose of 10 mg/(kg body weight·d). A higher dose of 20 mg/(kg·d) was also given to test for a dose response.

Andreadou et al., J Nutr. 2006 Aug;136(8):2213-9

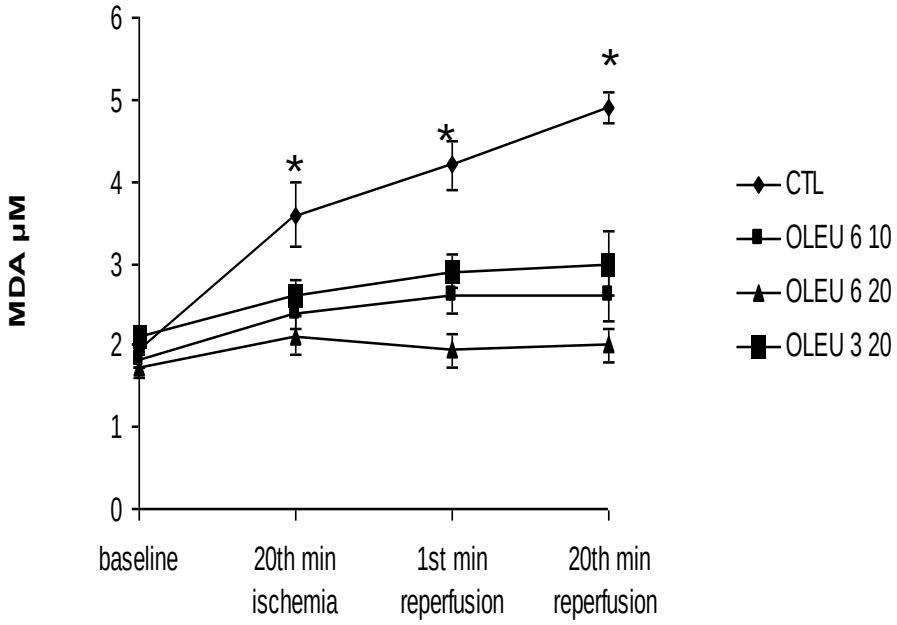
Chronic treatment of oleuropein reduces myocardial infarct size in normal fed rabbits and in higher doses in cholesterol fed rabbits

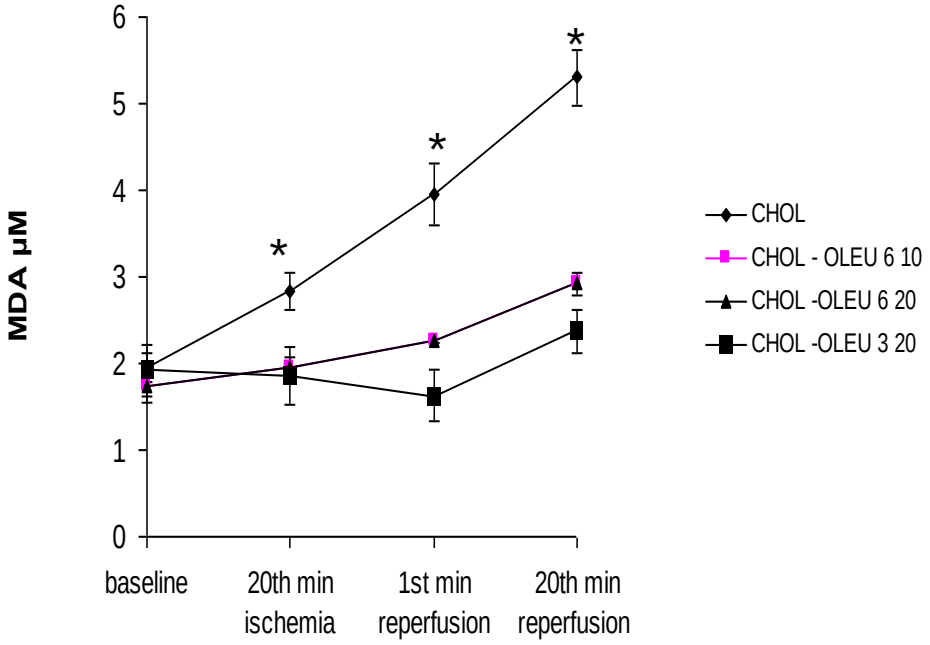




Andreadou I, et al., J Nutr. 2006 Aug;136(8):2213-9

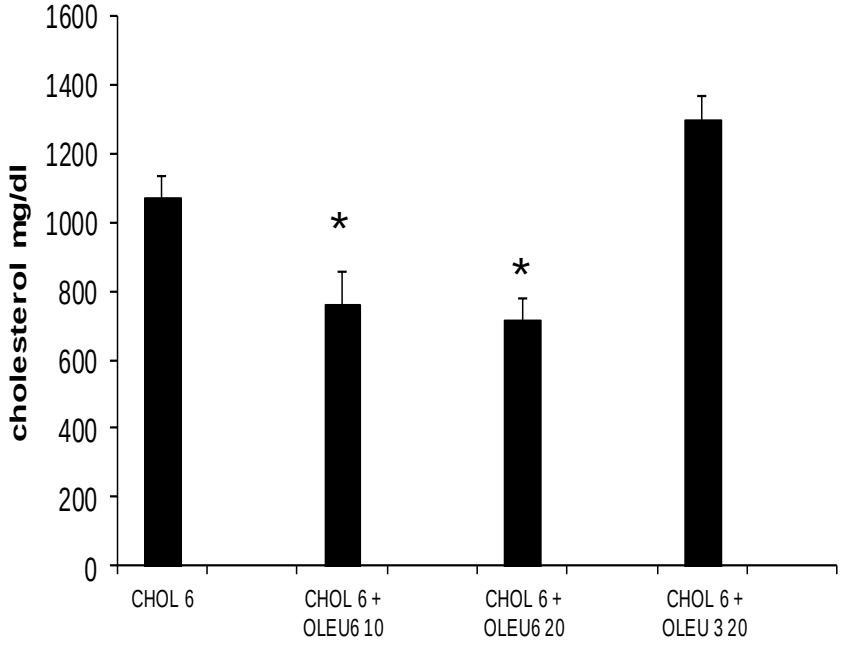
Oleuropein reduces circulatory lipid peroxidation products during myocardial ischemia/reperfusion

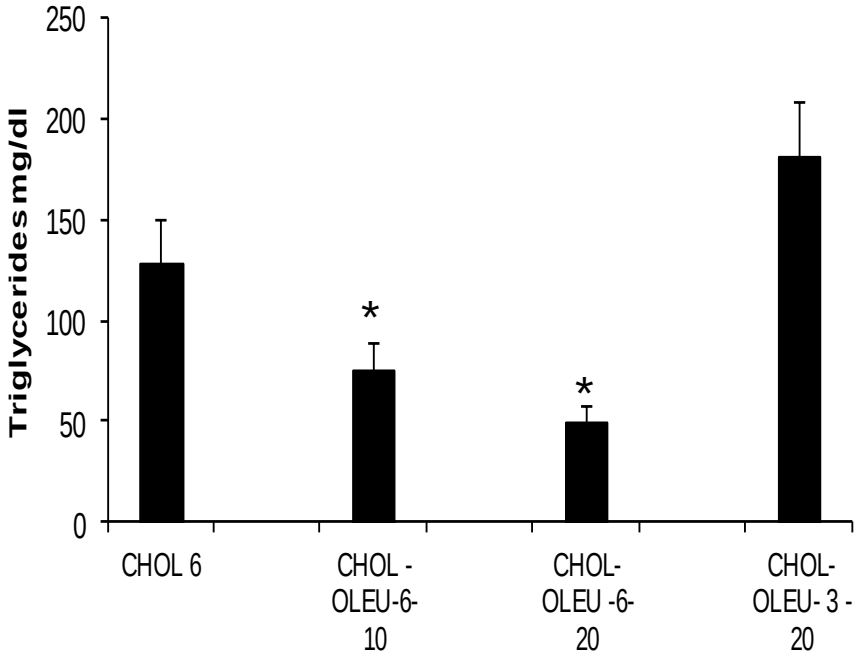




Andreadou et al., J Nutr. 2006 Aug;136(8):2213-9

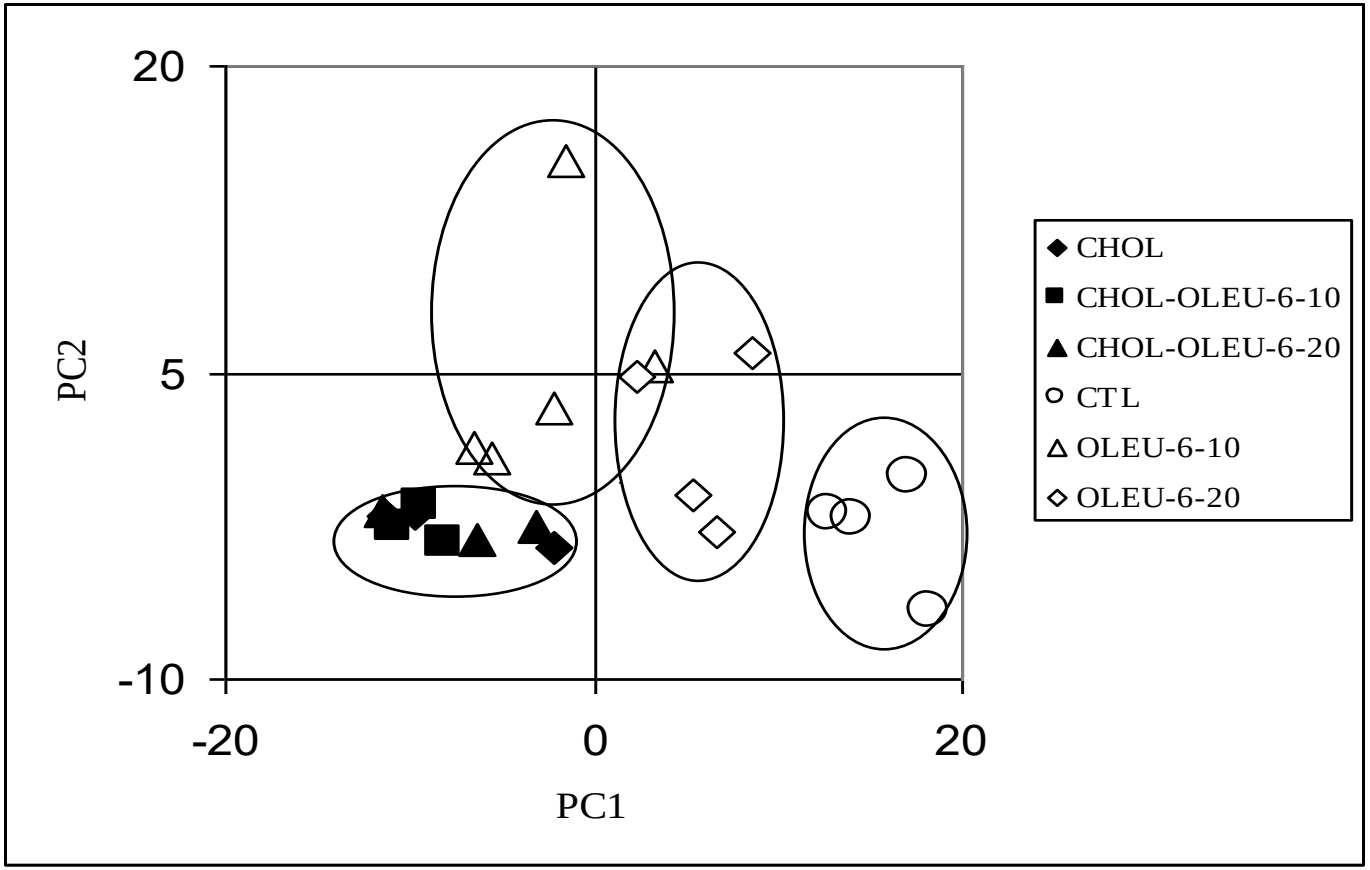
6 weeks treatment with oleuropein reduced total cholesterol and triglyceride levels





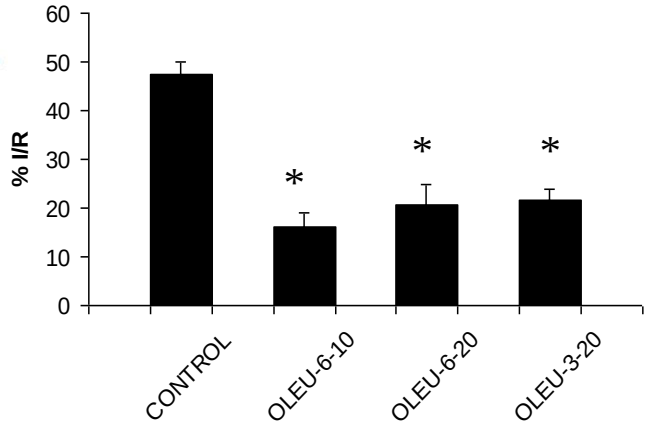
Andreadou et al., J Nutr. 2006 Aug;136(8):2213-9

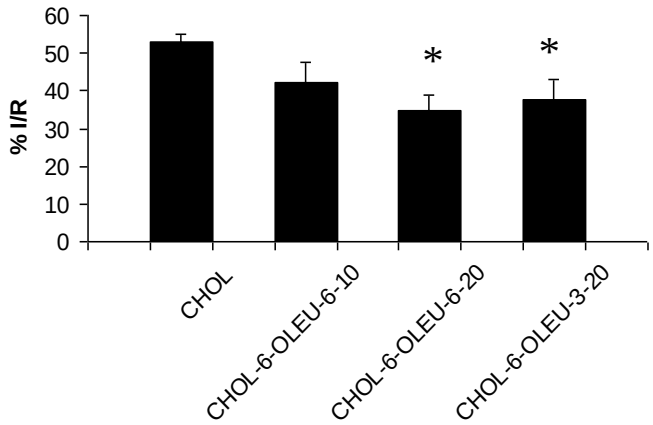
Treatment with oleuropein influences the metabolic profile of normal subjects, restoring the mechanism of aerobic glycolysis and providing cardioprotection even before the onset of ischemia.



PCA scores plot based on ^{1}H NMR CPMG plasma spectra of samples treated with different doses of OLEU and CHOL at 20 min of reperfusion.

Andreadou et al., J Nutr. 2006 Aug;136(8):2213-9





Comorbidities

Diabetes

Hypertrophy

Ageing

Hypercholesterolemia

PC Increased Threshold

The effectiveness of postconditioning and preconditioning on infarct size in hypercholesterolemic and normal anesthetized rabbits

Efstathios K. Iliodromitis [a,*], Anastasia Zoga [a], Agathi Vrettou [a], Ioanna Andreadou [a,*], Ioannis A. Paraskevaidis [b], Loukas Kaklamanis [b], Dimitrios Th. Kremastinos [a]

[a] *Second University Department of Cardiology, Medical School, Attikon General Hospital, University of Athens, Rimini 1, 124 62 Athens, Greece*
[b] *Onassis Cardiac Surgery Center, 356 Sygrou Avenue, 176 74 Athens, Greece*

Received 8 July 2005; received in revised form 10 November 2005; accepted 14 November 2005

Basic Res Cardiol (2010) 105:193–203
DOI 10.1007/s00395-009-0078-3

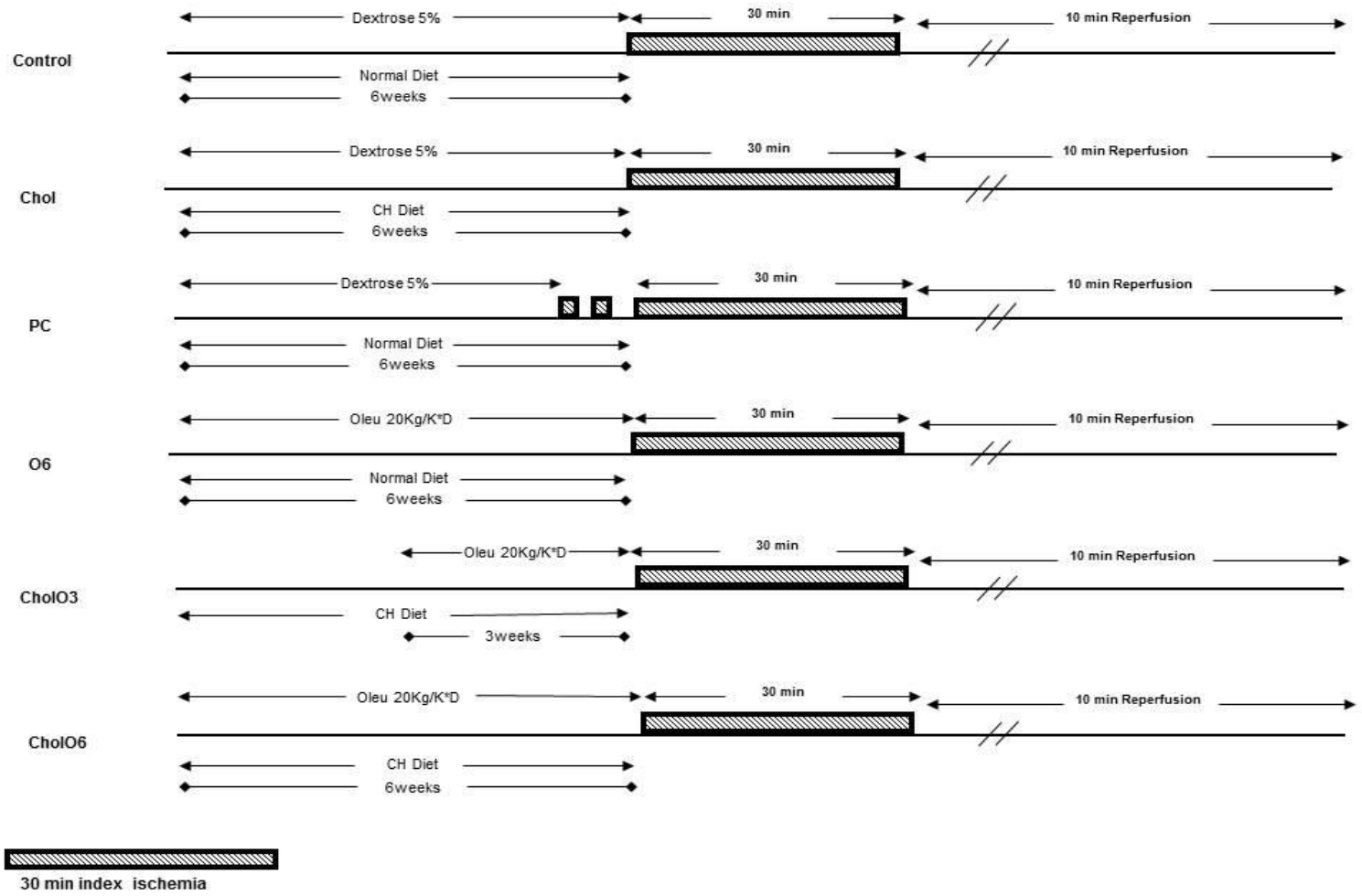
ORIGINAL CONTRIBUTION

Simvastatin in contrast to postconditioning reduces infarct size in hyperlipidemic rabbits: possible role of oxidative/nitrosative stress attenuation

Efstathios K. Iliodromitis · Ioanna Andreadou · Eftihios Prokovas · Anastasia Zoga · Dimitrios Farmakis · Theano Fotopoulou · Konstantinos Ioannidis · Ioannis A. Paraskevaidis · Dimitrios Th. Kremastinos

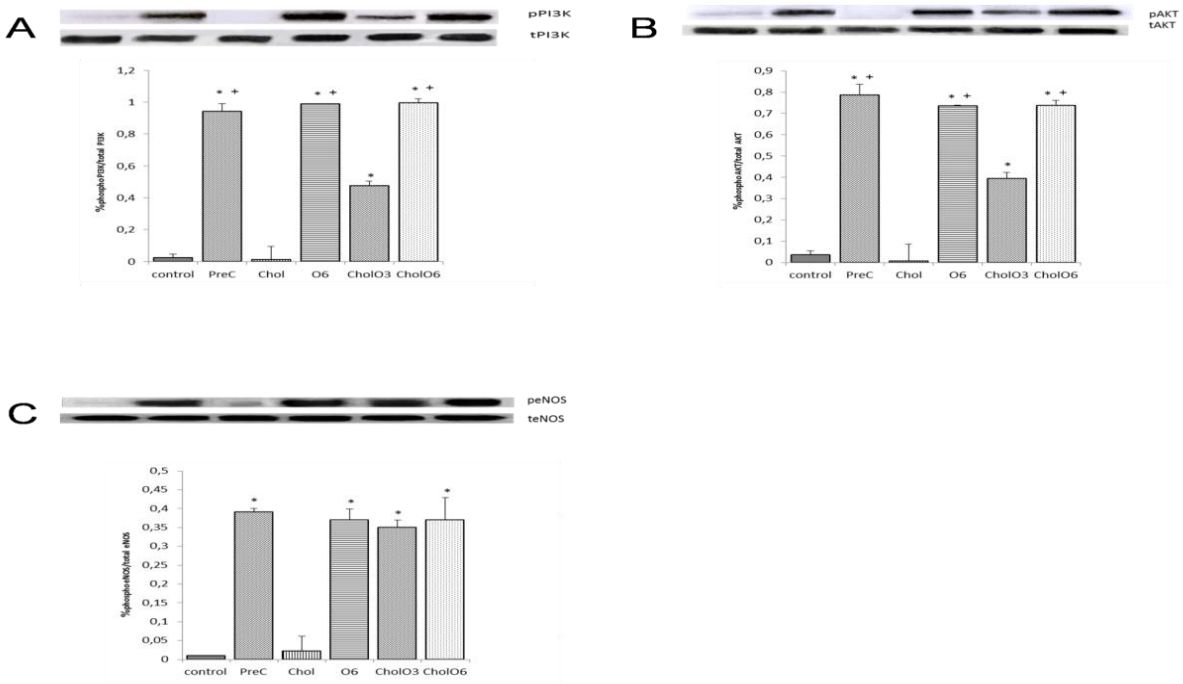
Cardiovasc Res.2012 Jun 1;94(3):501-9.
Short-term statin administration in hypercholesterolaemic rabbits resistant to postconditioning: effects on infarct size, endothelial nitric oxide synthase, and nitro-oxidative stress.
Andreadou I, Farmakis D, Prokovas E, Sigala F, Zoga A, Spyridaki K, Papalois A, Papapetropoulos A, Anastasiou-Nana M, Kremastinos DT, Iliodromitis EK

The natural olive oil constituent oleuropein induces nutritional preconditioning in normal and cholesterol fed rabbits.



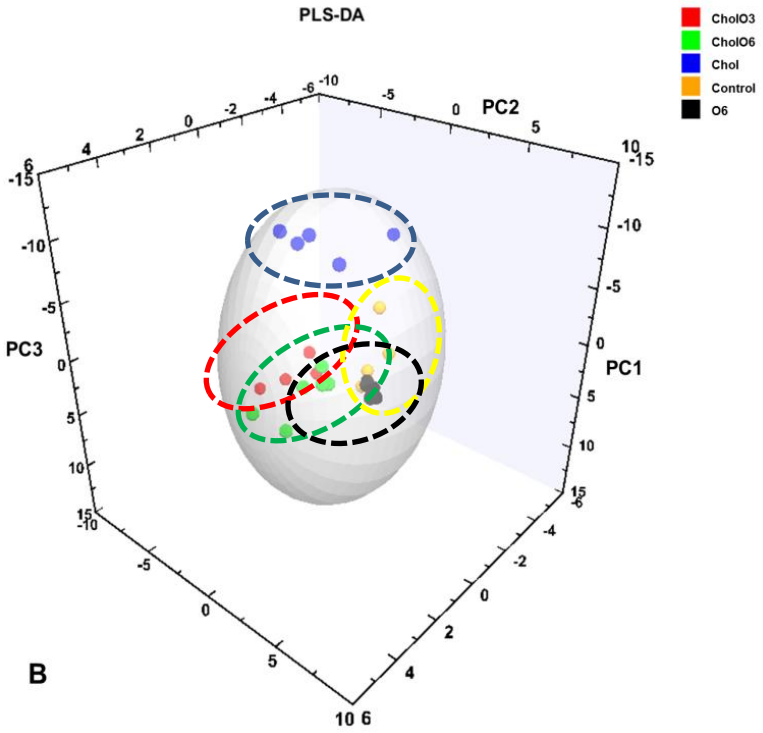
Andreadou I, et al, Planta Medica, 81:655-63, 2015

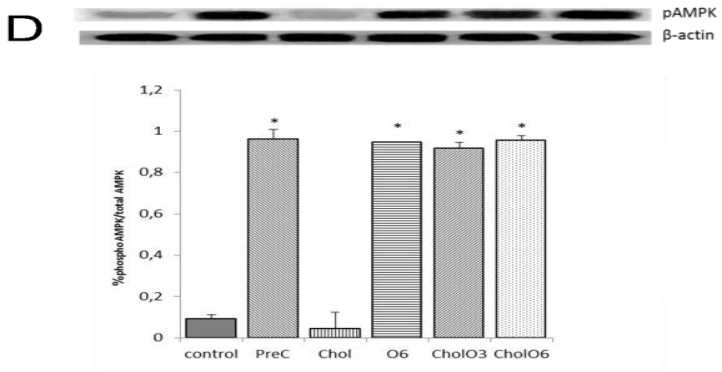
Oleuropein activates intracellular signaling cascades similar to those of preconditioning



Andreadou I, et al, Planta Medica, 81:655-63, 2015

The holistic NMR-based metabonomic analysis showed a clear metabolic profile distinction between the study groups, and the main metabolites responsible for this distinction can be related to the energy metabolism.





Andreadou I, et al, Planta Medica, 81:655-63, 2015

SUMMARY 1

OLEUROPEIN AS PROPHYLACTIC TREATMENT IN AMI

- Oleuropein when administered at a nutritional dose reduces infarct size in normal fed rabbits and in hypercholesterolemic rabbits and induces nutritional PC.

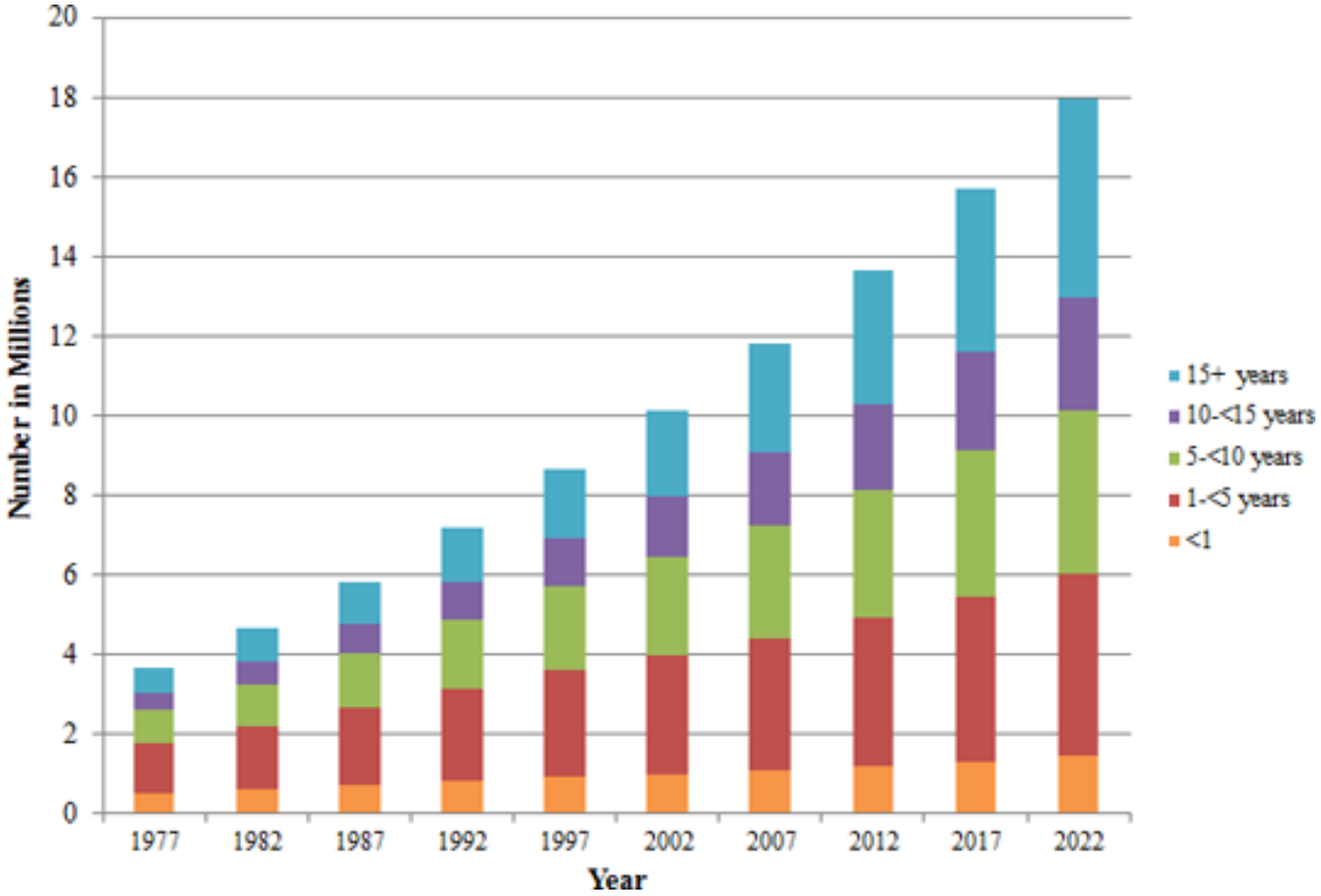
- Oleuropein significantly reduces nitro-oxidative stress, activates intracellular signaling cascades similar to those of preconditioning

- Reduces the levels of total cholesterol 28.7% and 33.1% and triglyceriles 41.1% and 61.4% (10 and 20 $mg.kg^{-1}.day^{-1}$ respectively)

- The activation of AMPK by oleuropein at the first min of reperfusion may be a mediator of cardioprotection and might contribute to bioenergetic restoration of the myocardium.

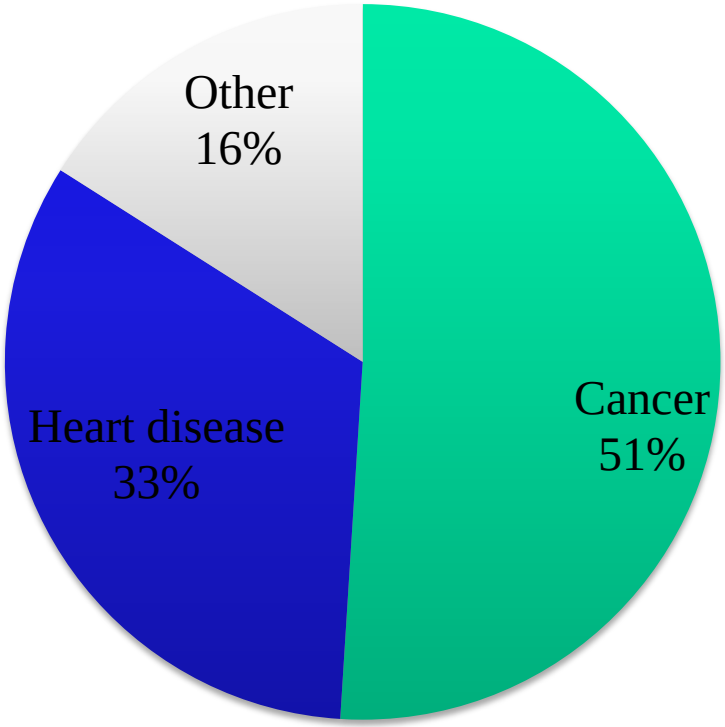
Investigating the cardioprotective effects of oleuropein on acute and chronic cardiomyopathy induced by Doxorubicin

Estimated and projected cancer survivors in USA



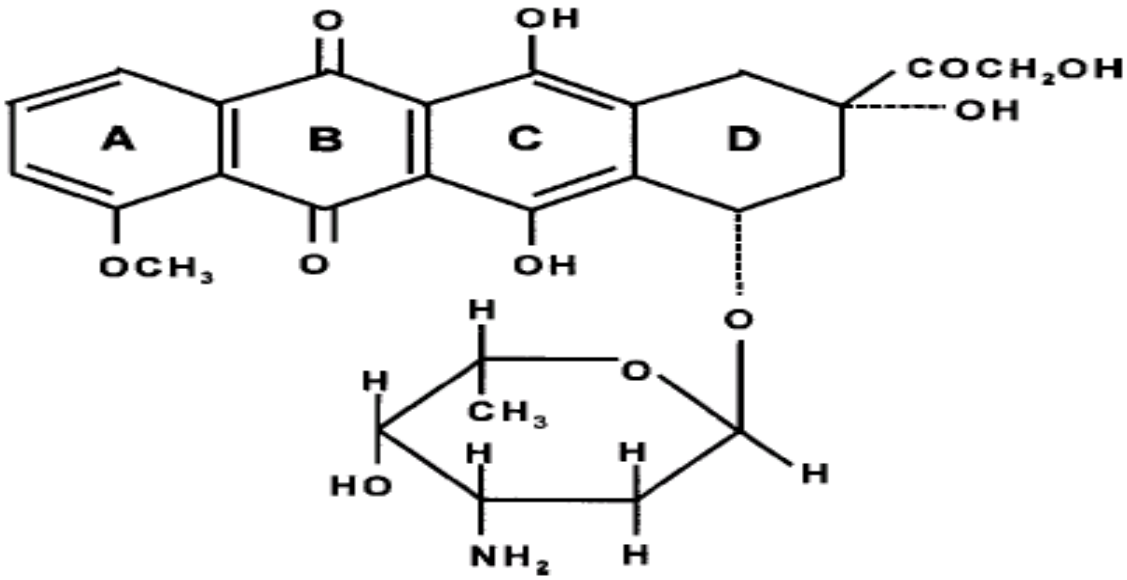
de Moor JS et al. Cancer Epidemiol Biomarkers Prev 2013

Causes of death in cancer survivors



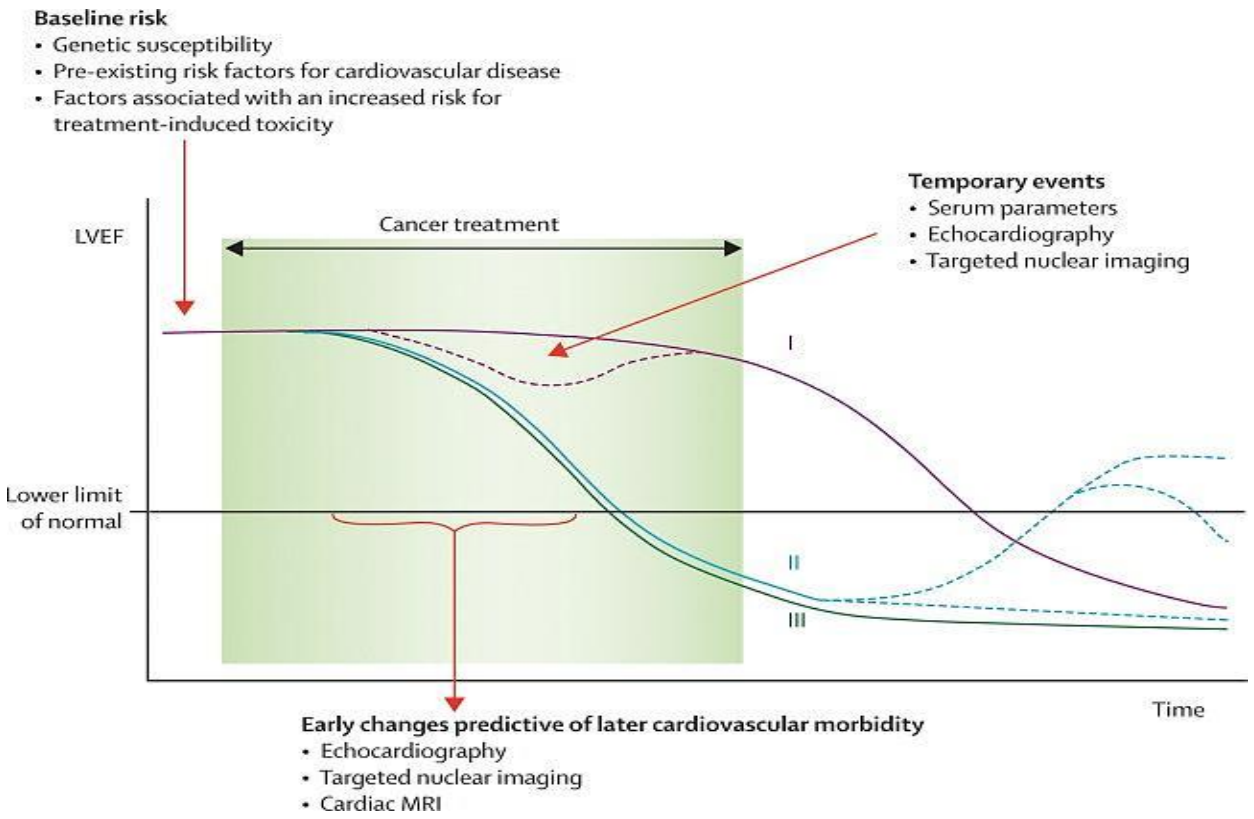
Ning et al. Cancer Res 2012

ADRIAMYCIN-DOXORUBYCIN(DXR)



- Antibiotic agent, chemotherapy drug
- Anthracyclin
- **Side effect**: Dose dependent cardiomyopathy and heart failure

DOXORUBICIN INDUCES ACUTE AND CHRONIC CARDIOTOXICITY



(I) Late onset cardiotoxicity (radiotherapy, anthracyclines) (II) reversible cardiotoxicity (trastuzumab: dotted line indicates long-term consequences are still unknown); (III) irreversible cardiotoxicity during treatment (anthracyclines)

Altena R., et al. Lancet Oncol., 10(4):391-9, 2009.

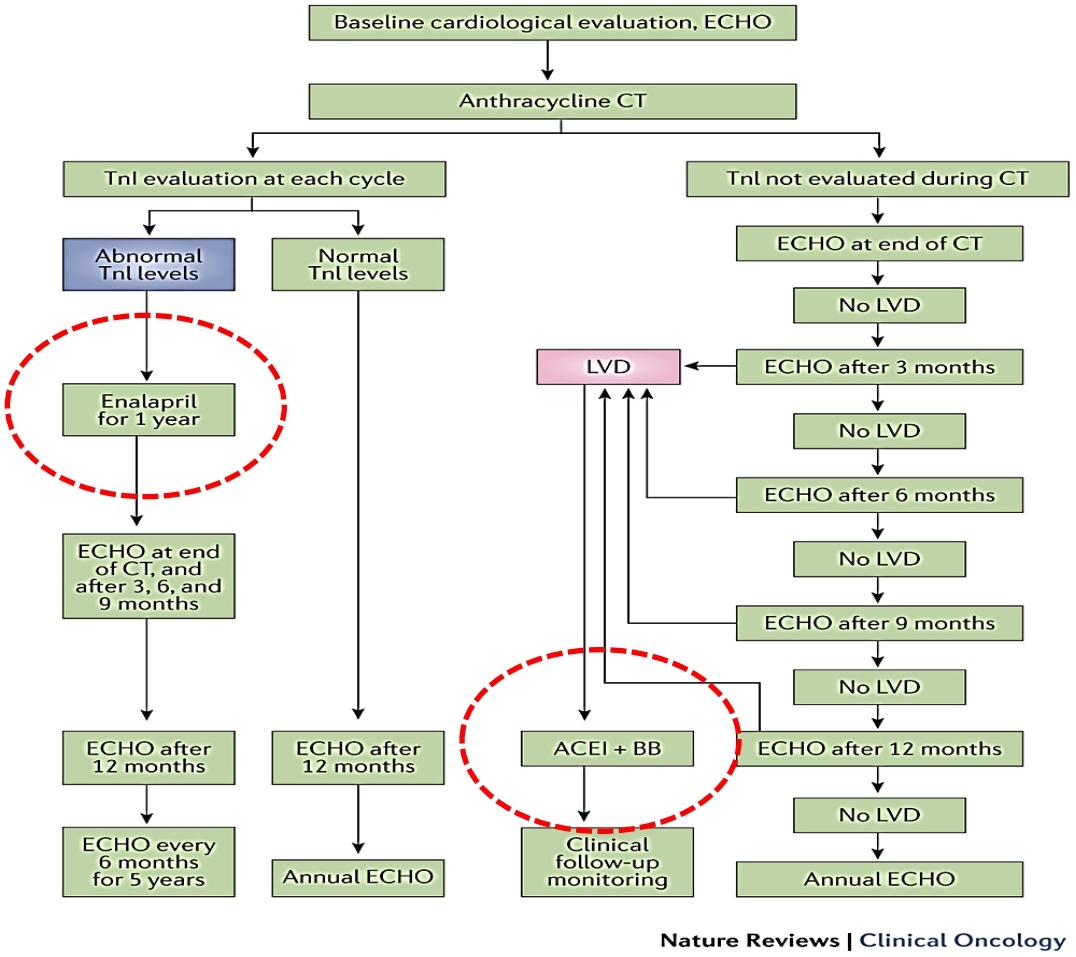
Clinical Management of Doxorubicin Cardiotoxicity

Guidelines for stopping treatment in the event of reduced function based on Herceptin Adjuvant (HERA) trial protocol is as follows:

Asymptomatic patients

LVEF	Absolute decrease of <10%	Absolute decrease of 10–15%	Absolute decrease of ≥16%
Within normal limits	Continue	Continue	Hold*
1–5% below normal limits	Continue	Hold*	Hold*
>6% below normal limits	Continue*	Hold*	Hold*

Pfeffer B., Br J Cardiol., 16(2), 85-89, 2009.

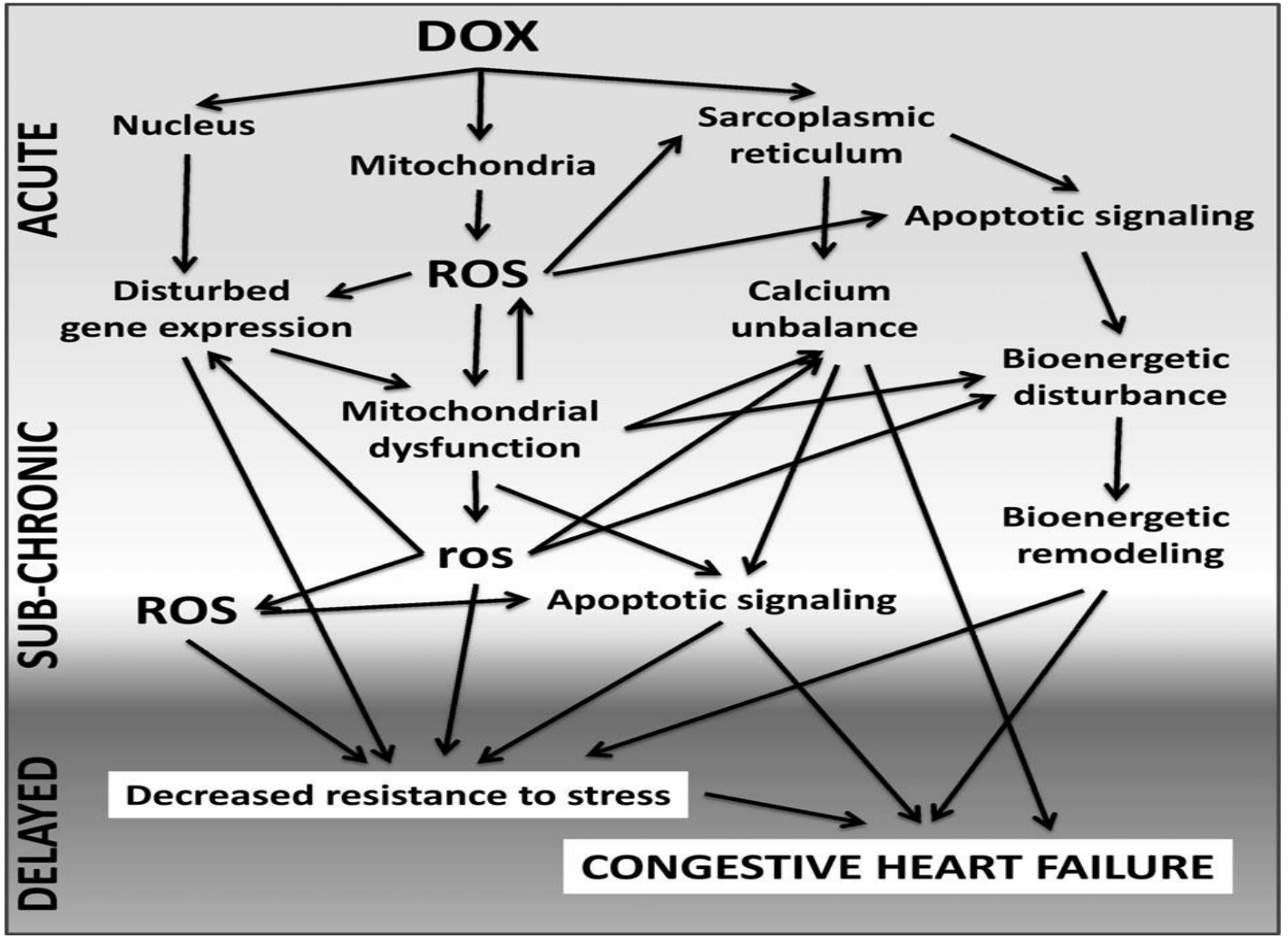


Zagar TM., Nat Rev Clin Oncol. Nov 24., 2015

Although extensive research has also been done to find effective treatment of doxorubicin cardiomyopathy, no such treatment has been discovered. Similarly, extensive research has been done and is being done to prevent doxorubicin cardiotoxicity. However, an effective preventive treatment is yet to be discovered.

Chatterjee K. et al Cardiology; 115(2): 155–162, 2010

UNDERLYING MECHANISM OF CARDIOTOXICITY



Carvalho FS., Med Res Rev.;34(1):106-35, 2014.

Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular and Cellular Cardiology 42 (2007) 549–558
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Original article

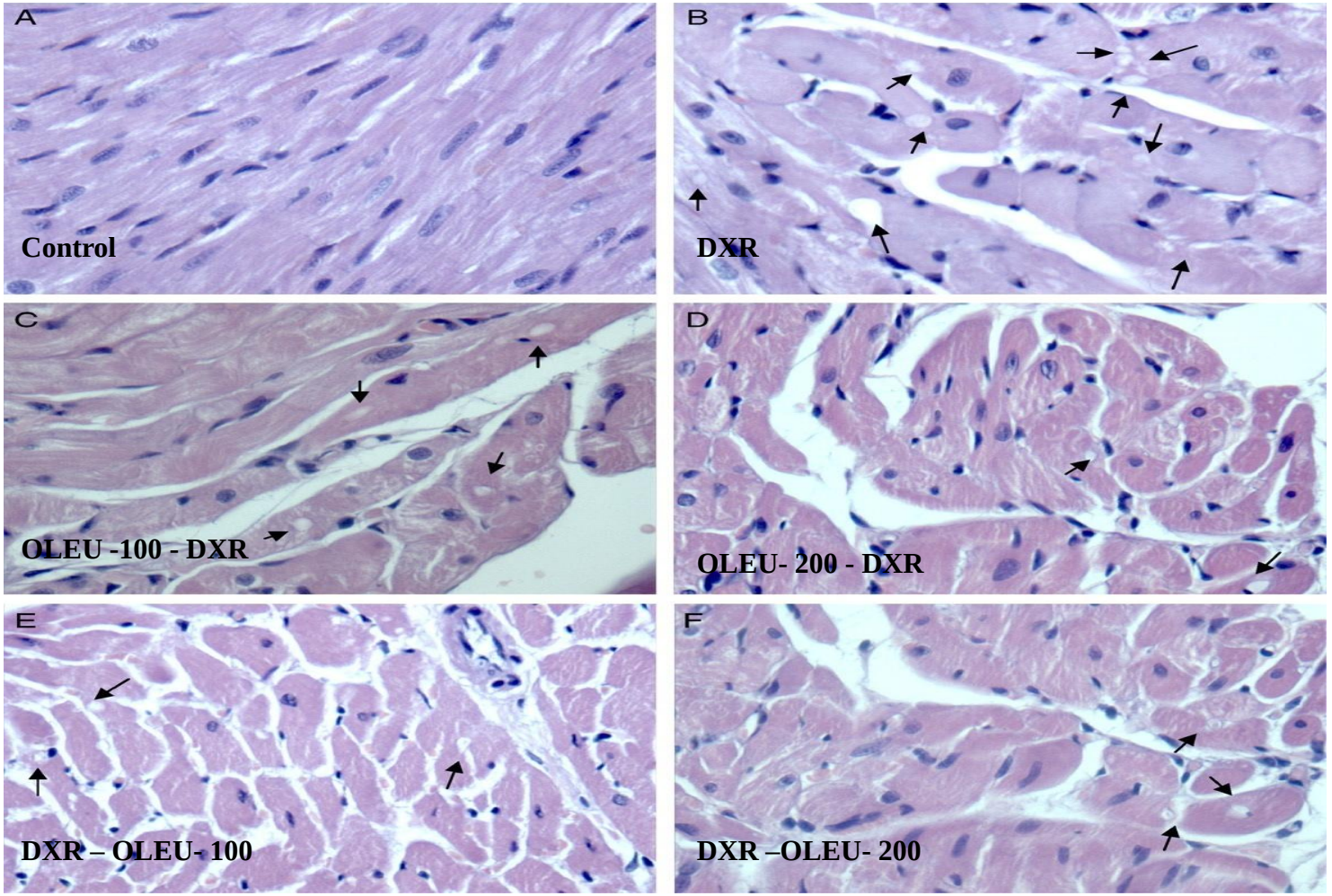
Acute doxorubicin cardiotoxicity is successfully treated with the phytochemical oleuropein through suppression of oxidative and nitrosative stress

Ioanna Andreadou [a,b,*], Fragiska Sigala [c], Efstathios K. Iliodromitis [a], Maria Papaefthimiou [b], Constantinos Sigalas [c], Nektarios Aligiannis [d], Paraskevi Savvari [e], Vassilis Gorgoulis [e], Efstathios Papalabros [c], Dimitrios. Th. Kremastinos [a]

50 Wistar rats divided in 6 groups:

1) Control
2) DXR: i.p. DXR 20 mg . kg^{-1}
3) Oleu 100-DXR: 5 days i.p. oleuropein 100 mg. kg^{-1} . day^{-1}
Oleuropein was administrated 2 days before DXR administration and for 2 days following DXR administration
4) Oleu 200-DXR: 5 days i.p. oleuropein 200 mg. kg^{-1} . day^{-1}
Oleuropein was administrated 2 days before DXR administration and for 2 days following DXR administration
5) DXR-Oleu 100: DXR administration and then i.p. oleuropein administration 100 mg. kg^{-1} . day^{-1} for 2 days
6) DXR-Oleu 200: DXR administration and then i.p. oleuropein administration 200 mg. kg^{-1} . day^{-1} for 2 days

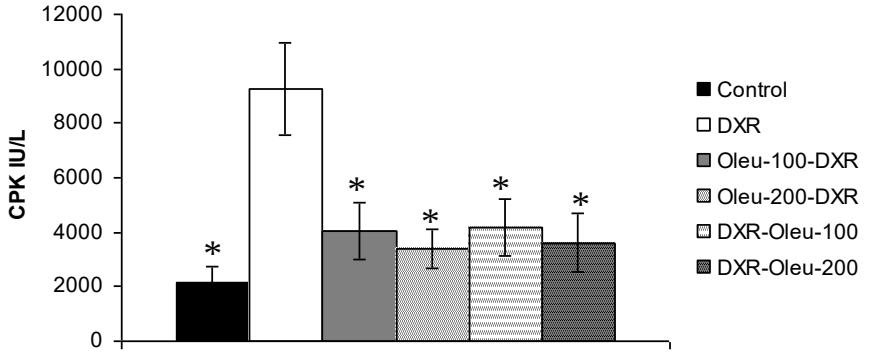
Oleuropein groups had very low cytoplasmic vacuolisation in cardiomyocytes compared to the DXR group

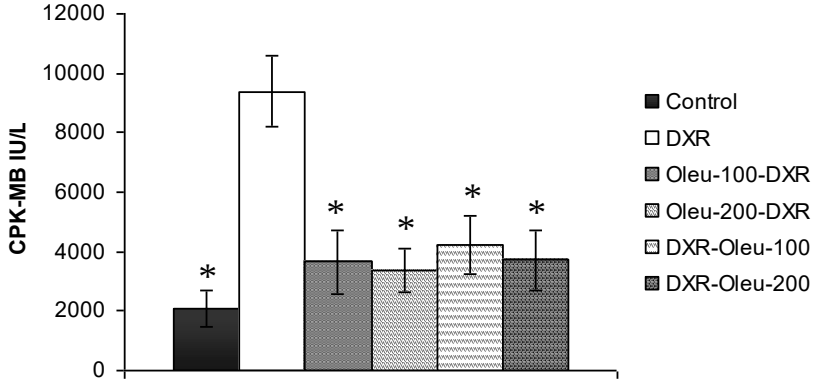


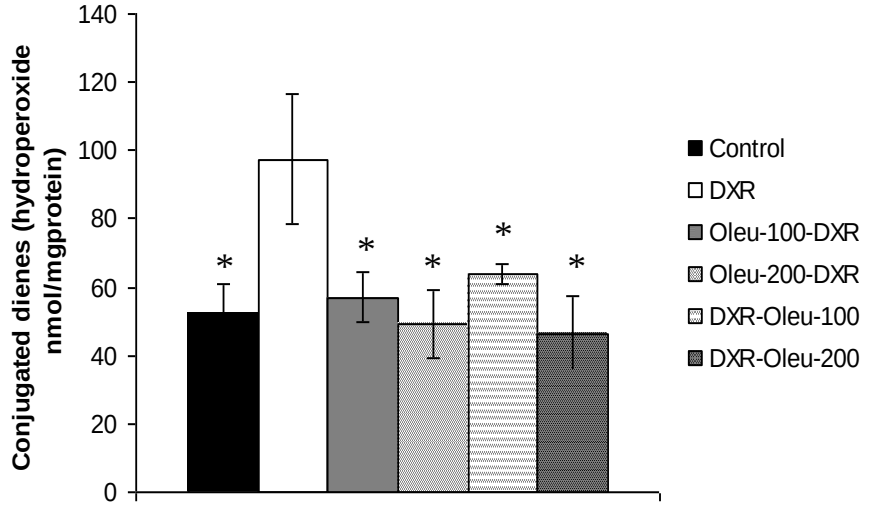
Representative histopathological findings from study groups. Arrows indicate cytoplasmic vacuolisation (magnification x 400).

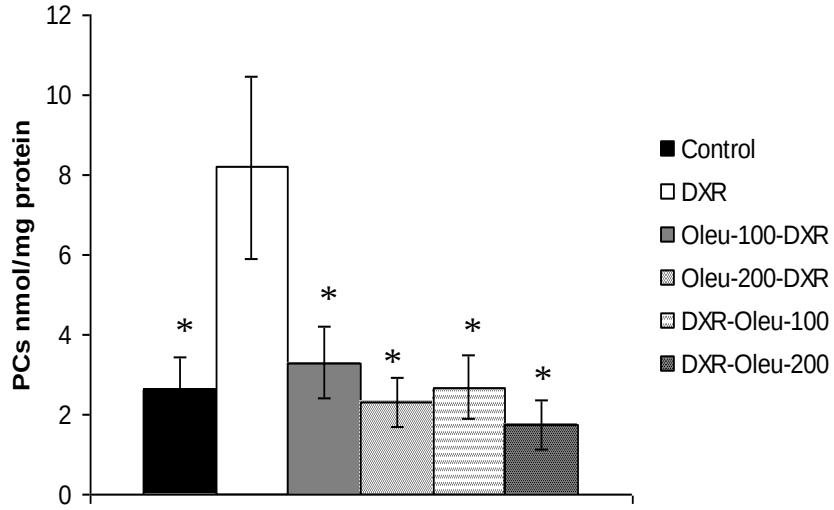
Andreadou et al., J Mol Cell Cardiol. 2007 Mar;42(3):549-58.

Two different doses and treatment regimes of oleuropein prevented the increase of cardiac necrosis and oxidative stress biomarkers



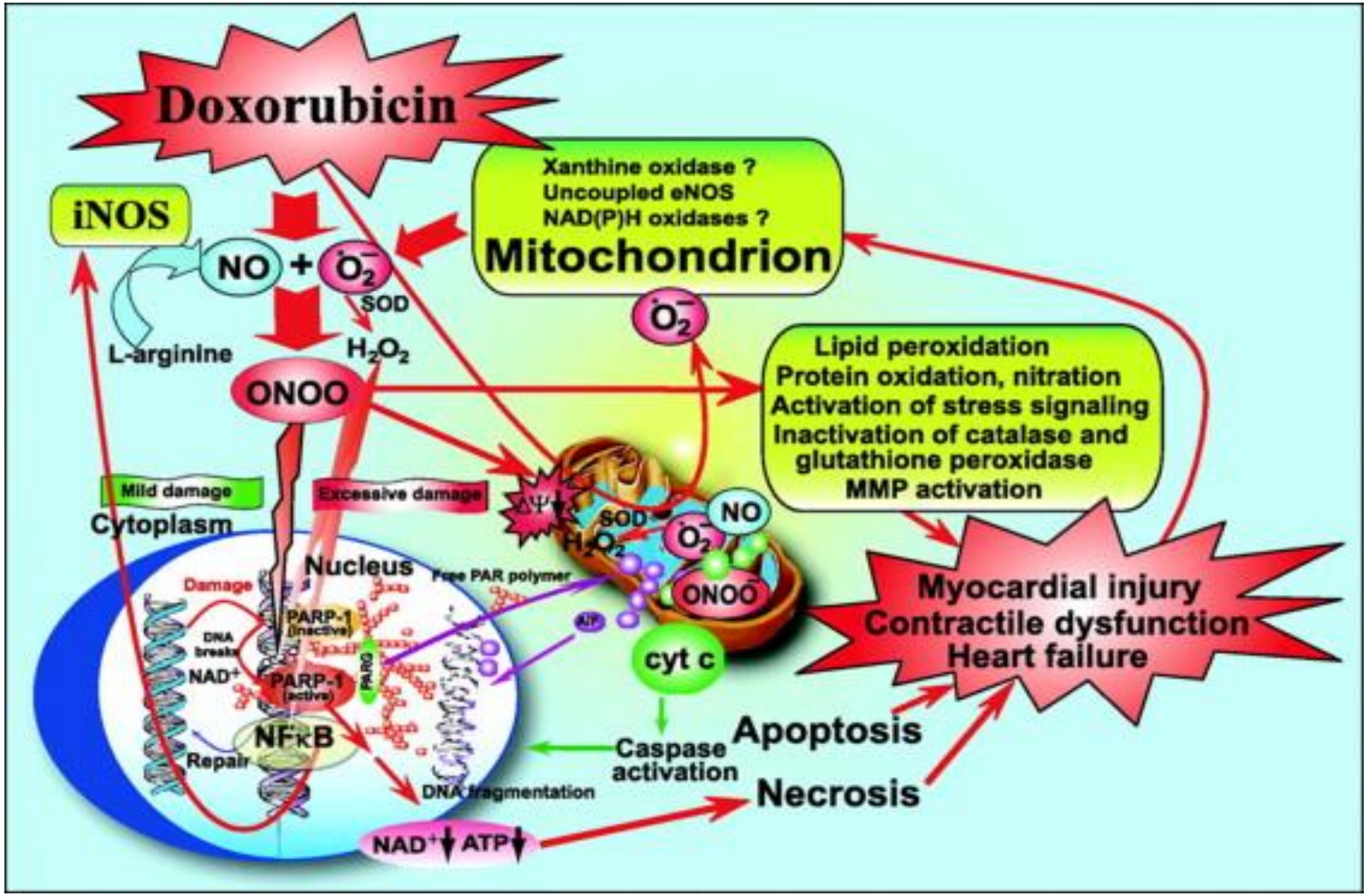






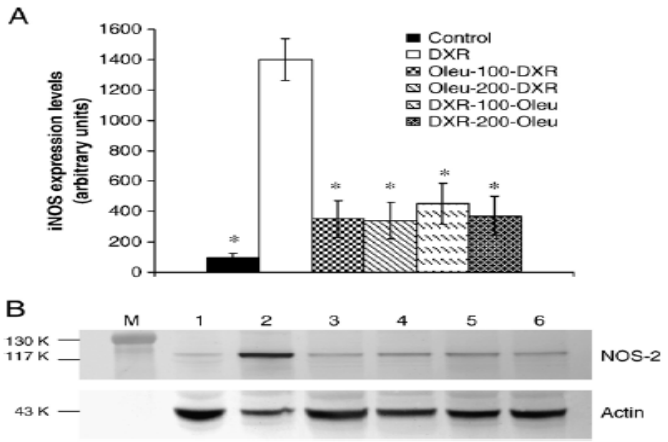
Andreadou et al., J Mol Cell Cardiol. 2007 Mar;42(3):549-58.

DXR induces iNOS expression and nitrooxidative stress

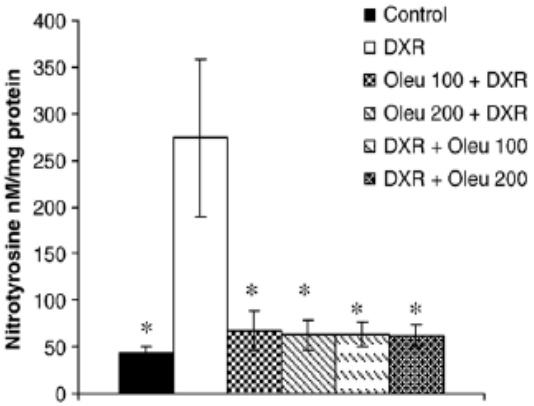


Roberts R., et al. Toxicological and pathophysiological roles of reactive oxygen and nitrogen species 2010, 276:85–94

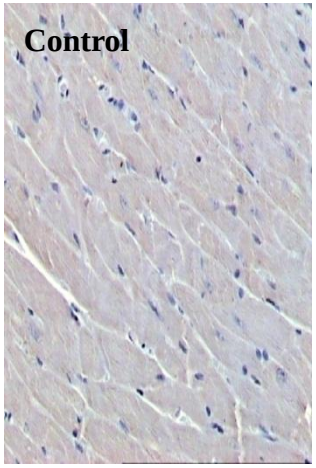
Oleuropein administration significantly reduced nitrotyrosine formation and iNOS expression in myocardium

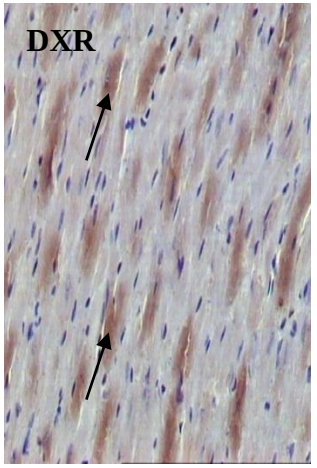


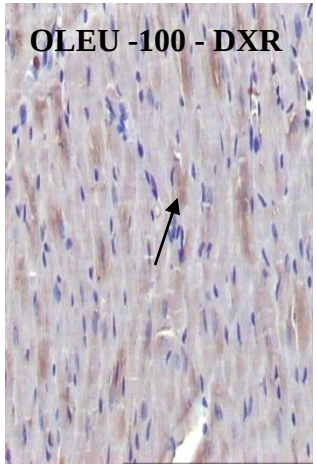
Representative wetsern blot of iNOS. *p<0.05 vs DXR

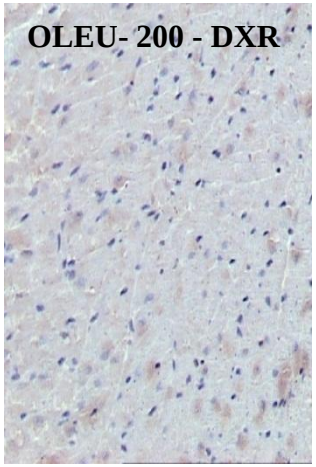


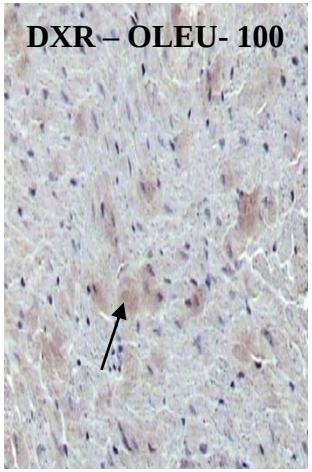
Nitrotyrosine measurment . *p<0.05 vs DXR

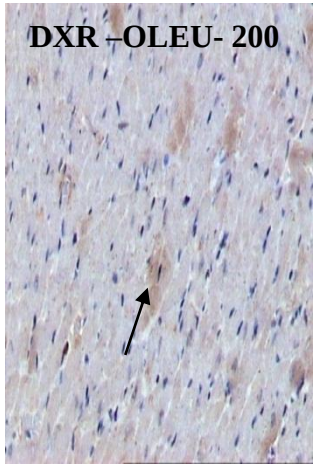












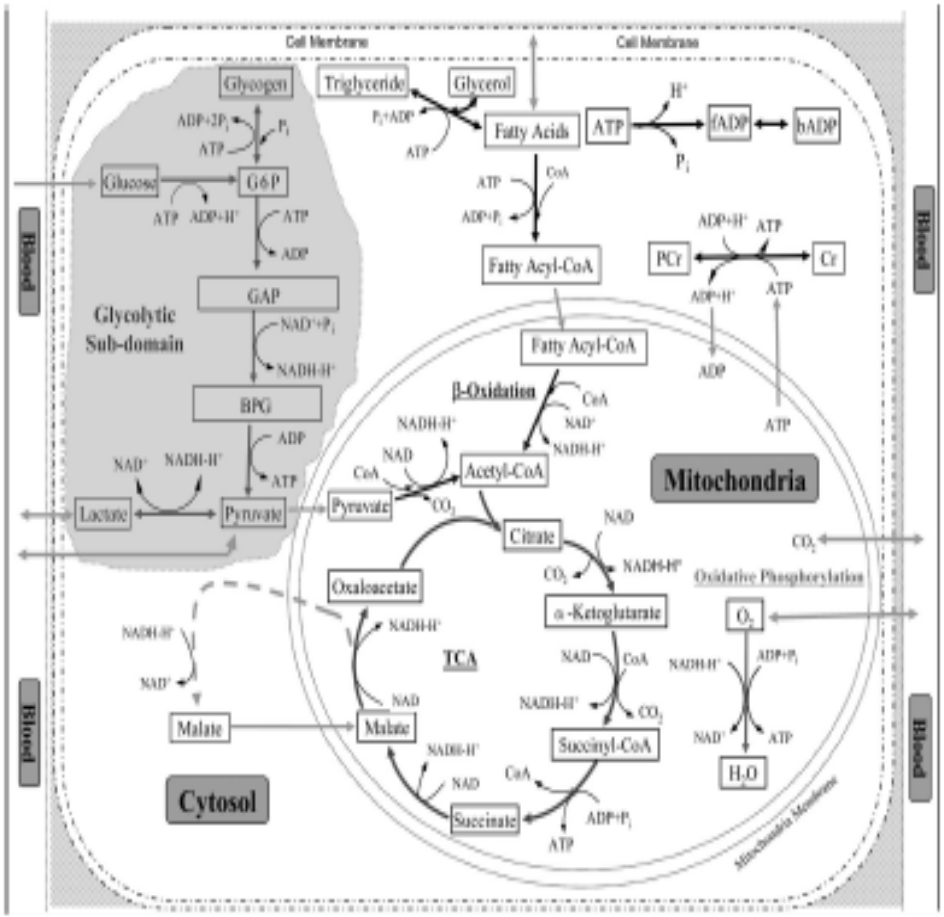
Representative immunohistochemistry analysis of iNOS in the experimental study groups. Arrows indicate iNOS positive cells (magnification x 200).

Andreadou et al., J Mol Cell Cardiol. 2007 Mar;42(3):549-58.

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



Research Article

NMR IN BIOMEDICINE

Received: 23 April 2008, Revised: 8 December 2008, Accepted: 10 January 2009, Published online in Wiley InterScience: 23 March 2009

(www.interscience.wiley.com) DOI:10.1002/nbm.1370

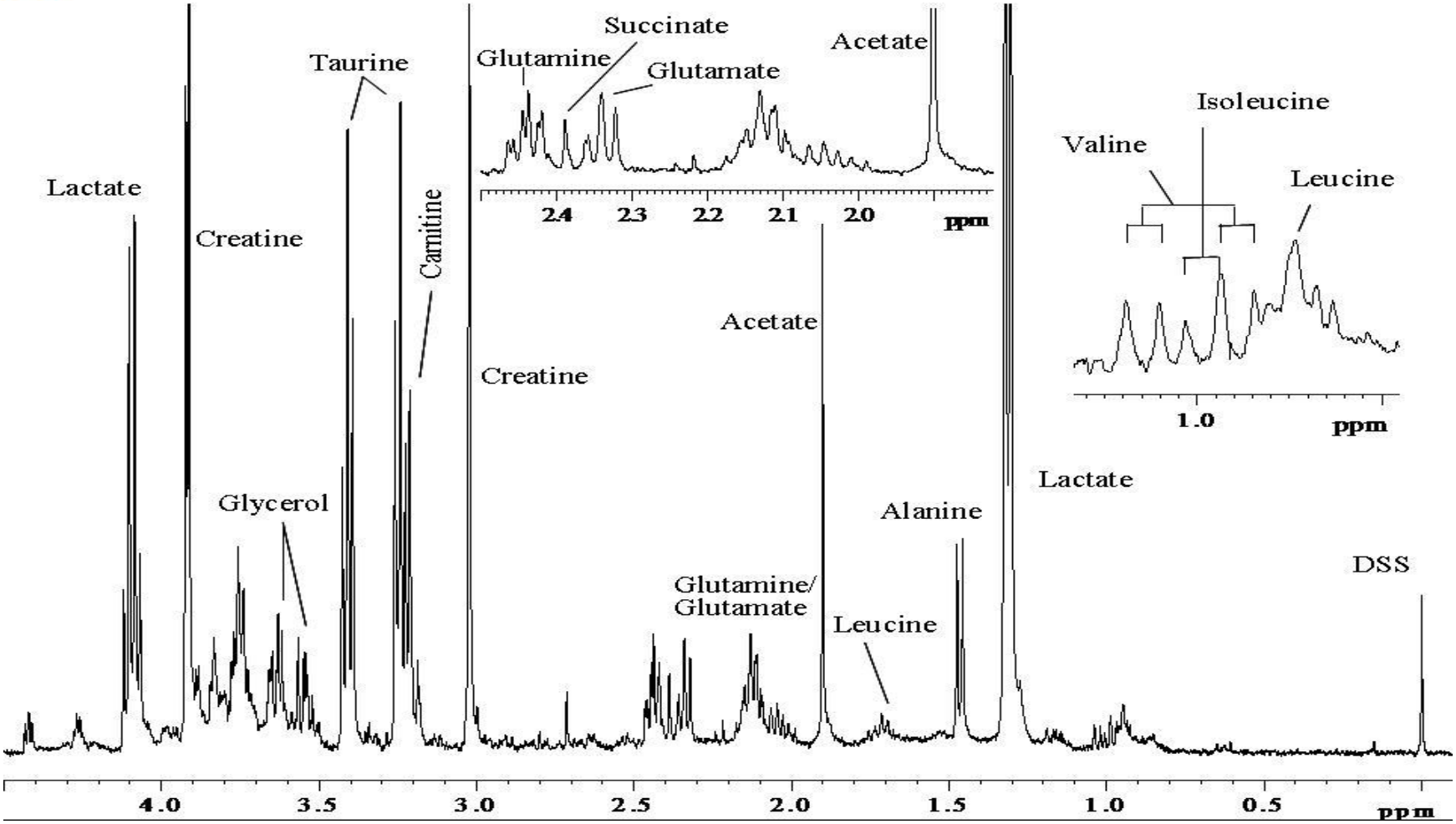
Metabonomic identification of novel biomarkers in doxorubicin cardiotoxicity and protective effect of the natural antioxidant oleuropein

Ioanna Andreadou[a,b], Maria Papaefthimiou[a], Athina Zira[a], Maria Constantinou[a], Fragiska Sigala[c], Alexios-Leandros Skaltsounis[d], Anna Tsantili-Kakoulidou[a], Efstathios K. Iliodromitis[b], Dimitrios T. Kremastinos[b] and Emmanuel Mikros[a]*

Effects of oleuropein in cardiac energy metabolism

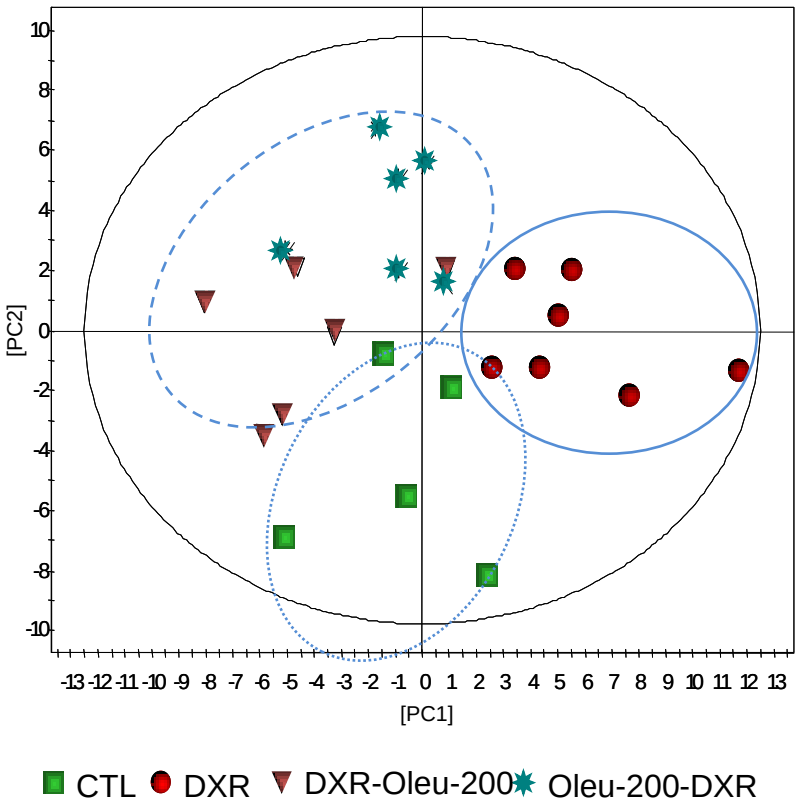
Metabonomic analysis with NMR

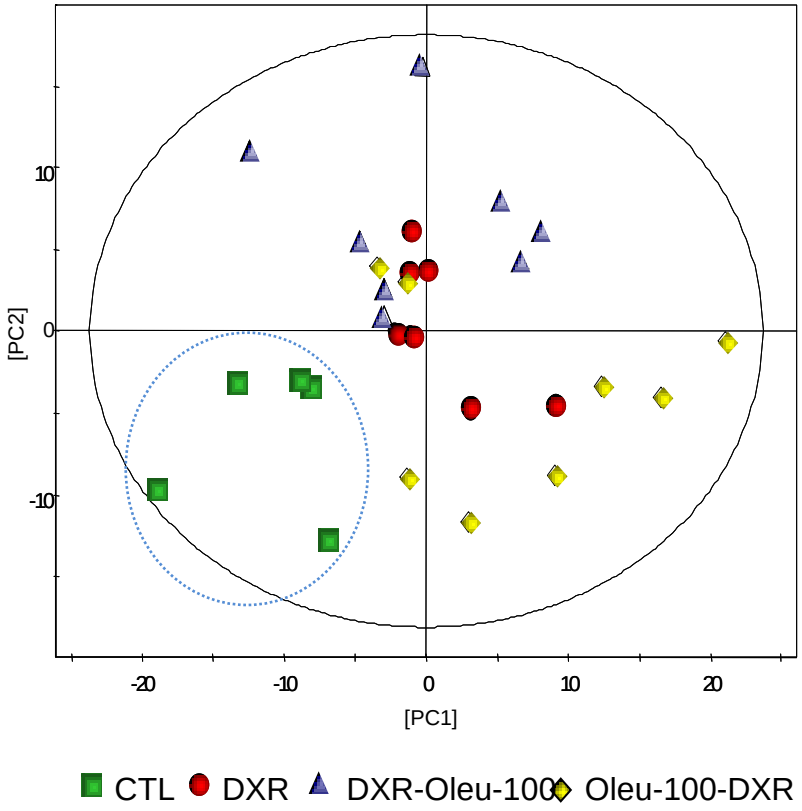
Representative 400 MHz 1H-NMR spectra (d 4.5–0.5) of aqueous myocardial extract collected from control group



Andreadou et al., NMR Biomed. 2009 Jul;22(6):585-92..

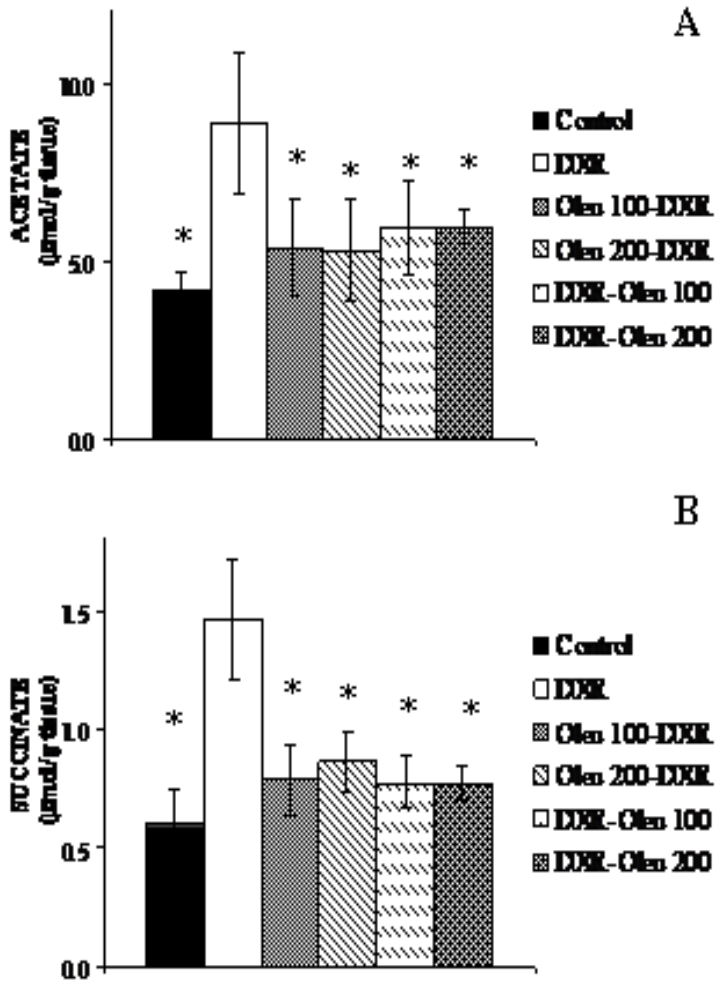
Dose-dependent discrimination





Andreadou et al., NMR Biomed. 2009, 22(6):585-92..

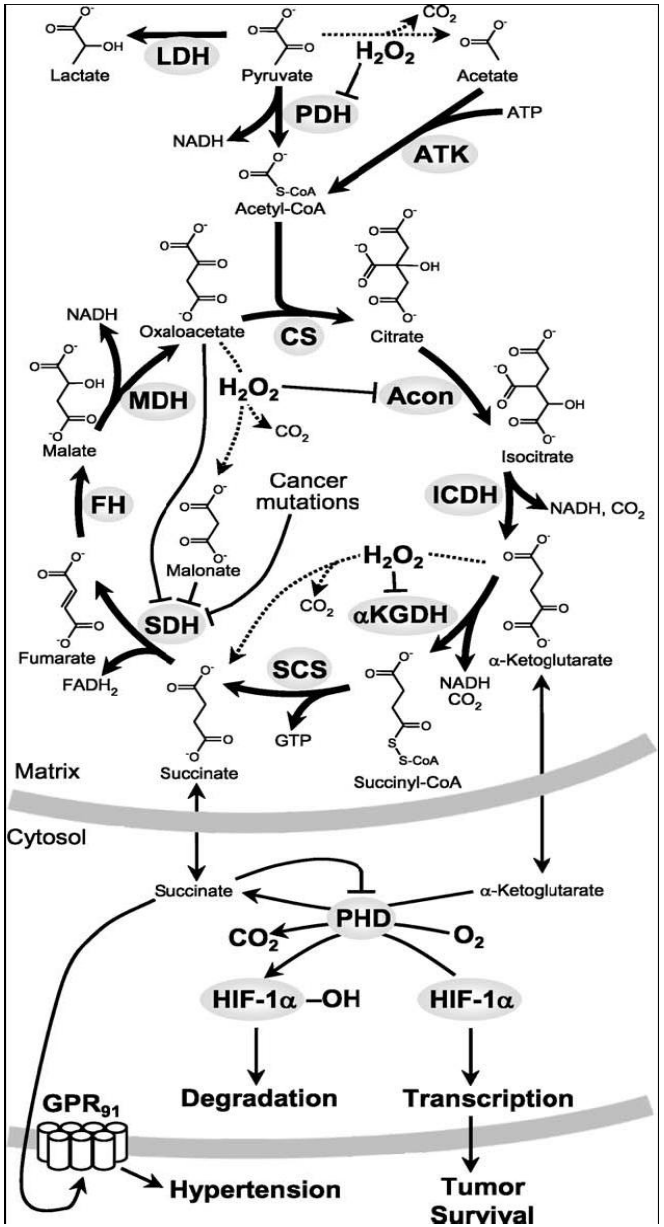
Oleuropein repairs cardiac energy metabolism as protective agent



Oleuropein eliminates succinate and acetate accumulation in myocardium

Andreadou et al., NMR Biomed. 2009 Jul;22(6):585-92..

Acetate and succinate constitute novel biomarkers related to DXR acute cardiotoxicity



Nonezymatic formation of succinate in mitochondria under oxidative stress

Fedotcheva, N. I. et al., Free Radic.Biol. Med.; 2006.

Oxidation of pyruvate by peroxynitrite produces acetate in much higher yields than by hydrogen peroxide.

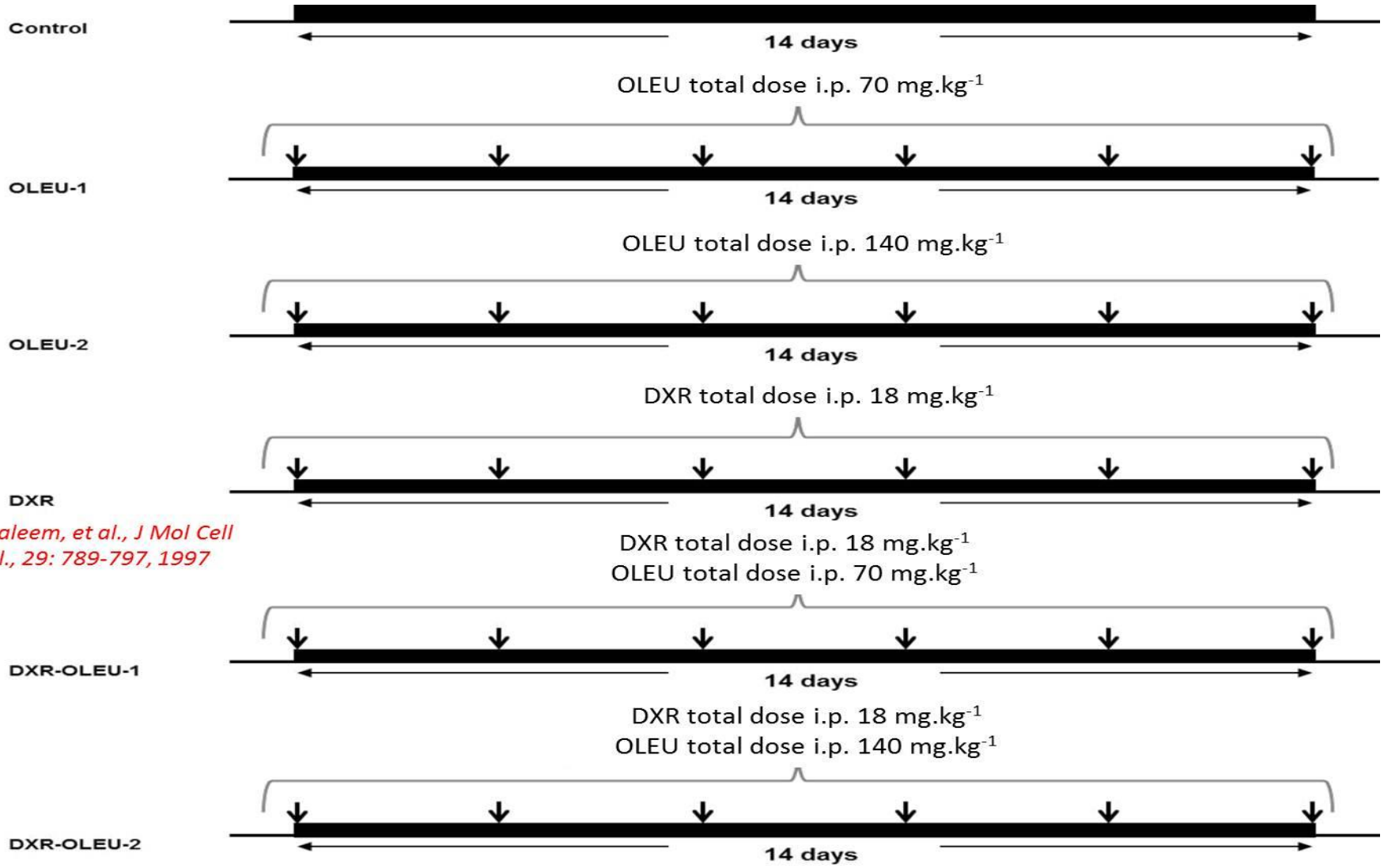
Vasquez-Vivar J, et al., Chem Res Tox, 1997

SUMMARY 3

1. Oleuropein prevents and treats the acute cardiotoxicity induced by DXR.

2. Oleuropein reduces significantly peroxynitrite and iNOS expression in cardiomyocytes reducing simultaneously acetate and succinate levels leading to improvement of the impaired energy metabolism in myocardium.

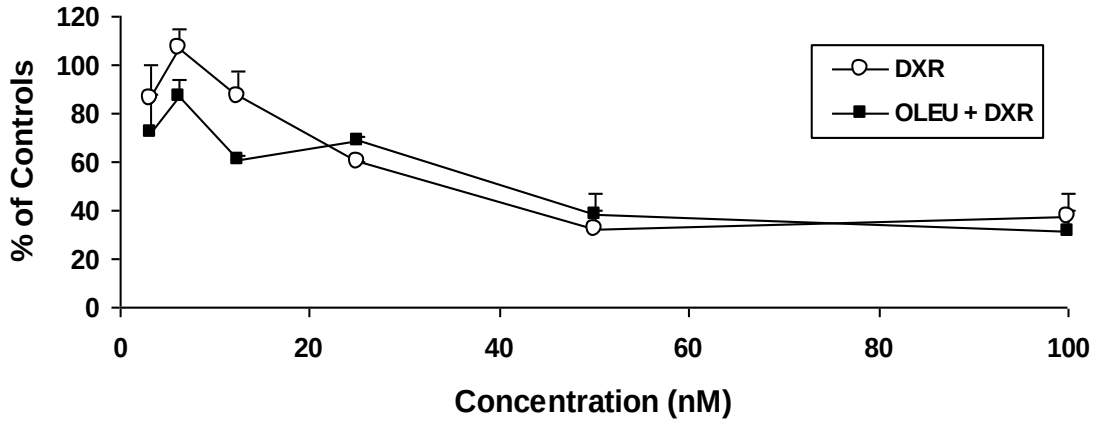
The role of Oleuropein in chronic DXR-induced cardiomyopathy



Abdel-aleem, et al., J Mol Cell Cardiol., 29: 789-797, 1997

Andreadou I, et al., J Mol Cell Cardiol, 69:4-16, 2014

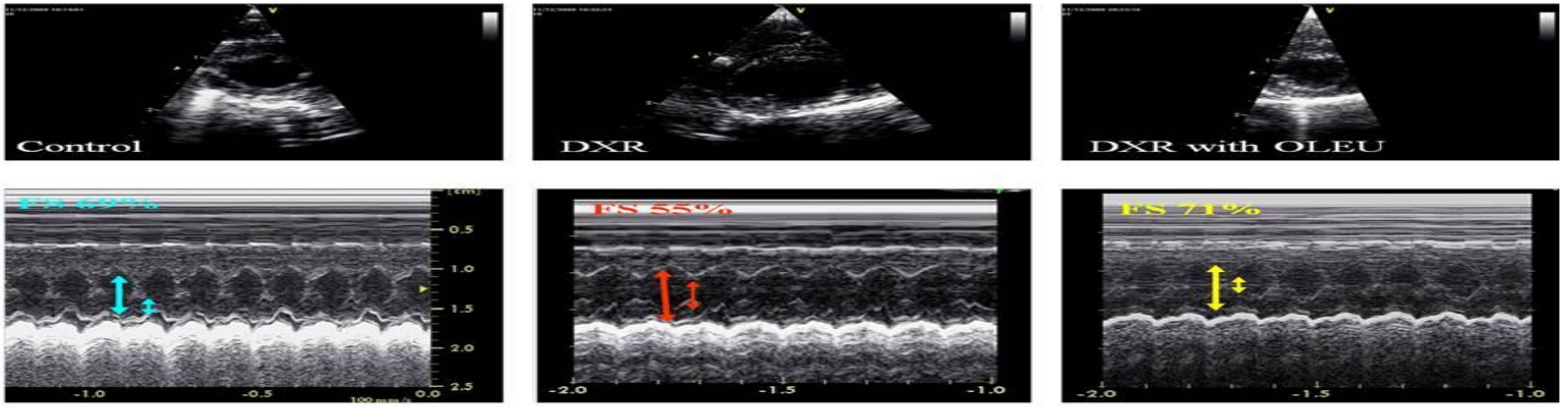
The cotreatment of PC-3 cells with DXR (3–100 nM) and OLEU (100 μg/ml) did not alter the cell proliferation inhibition obtained by DXR alone



Andreadou I, et al., J Mol Cell Cardiol, 69:4-16, 2014

Oleuropein increases significantly FS% compared to the DXR group

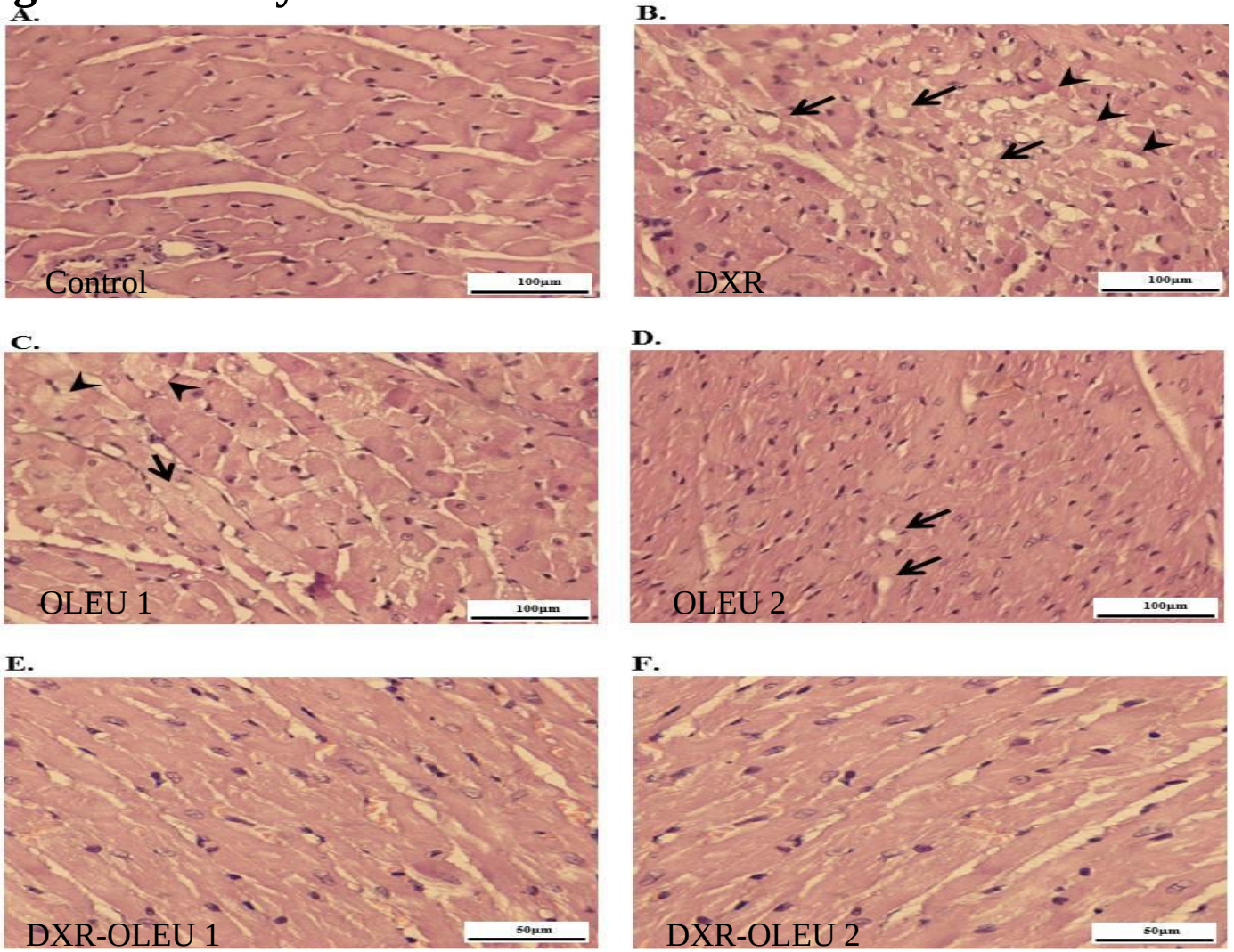
	Control (n=5)	DXR (n=8)	DXR-OLEU 1 (n=5)	DXR-OLEU 2 (n=5)	OLEU 1 (n=5)	OLEU 2 (n=5)
Heart rate, bpm	472(34)	337(65)*	345(15)*	386(31)*	438(43)	395(72)
AWTd, mm	2.1(0.2)	1.7(0.4)	2.0(0.2)	2.0(0.1)	2.0(0.1)	2.0(0.4)
PWTd, mm	2.7(0.5)	1.6(0.3)*	2.1(0.6)	1.9(0.2)	2.6(0.5)	2.3(0.5)
LVDP, mm	4.9(0.5)	5.0(1.4)	5.0(0.7)	5.2(0.2)	4.4(0.5)	4.2(0.3)
LVDs, mm	2.0(0.7)	2.9(1.0)	2.0(0.5)	1.9(0.6)	1.9(0.5)	1.7(0.4)
FS, %	65.0(8.2)	43.5(7.0)*	61.1(7.1)	71.0(3.5)	68.9(5.2)	71.67(7.8)
LVmass, g	0.85 (0.13)	0.50 (0.22)*	0.65 (0.10)	0.67 (0.12)	0.72 (0.11)	0.69 (0.22)
R/H ratio	4.0(1.5)	7.4(2.9)*	5.2(1.7)	5.8(1.1)	3.5(0.8)	3.5(0.3)



2D echo (Vivid-i, GE Healthcare με 12MHz probe).

Andreadou I, et al., J Mol Cell Cardiol, 69:4-16, 2014

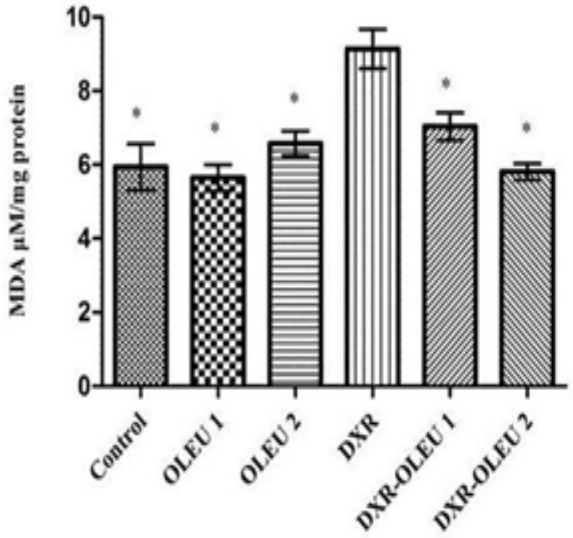
Oleuropein attenuated the development of inflammatory and degenerative myocardial lesions

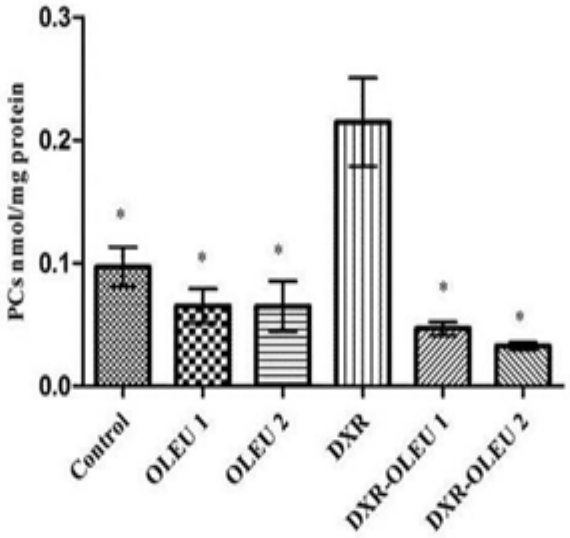


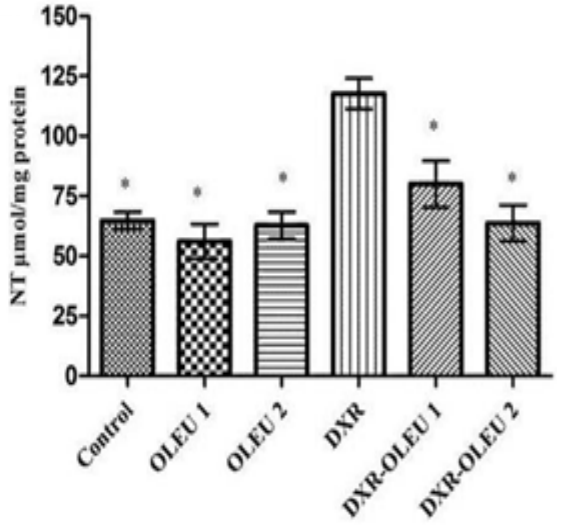
Histological evaluation of cardiac morphology showing profound focal edema, vacuolarization and degeneration of myocardiac fibers in the DXR group

Andreadou I, et al., J Mol Cell Cardiol, 69:4-16, 2014

Oleuropein treatment significantly reduced the elevated nitro-oxidative stress biomarkers in the DXR treated rats.







Andreadou I, et al., J Mol Cell Cardiol, 69:4-16, 2014

The combined reduction in nitro-oxidative stress, and endothelin may caused the early improvement in aortic function

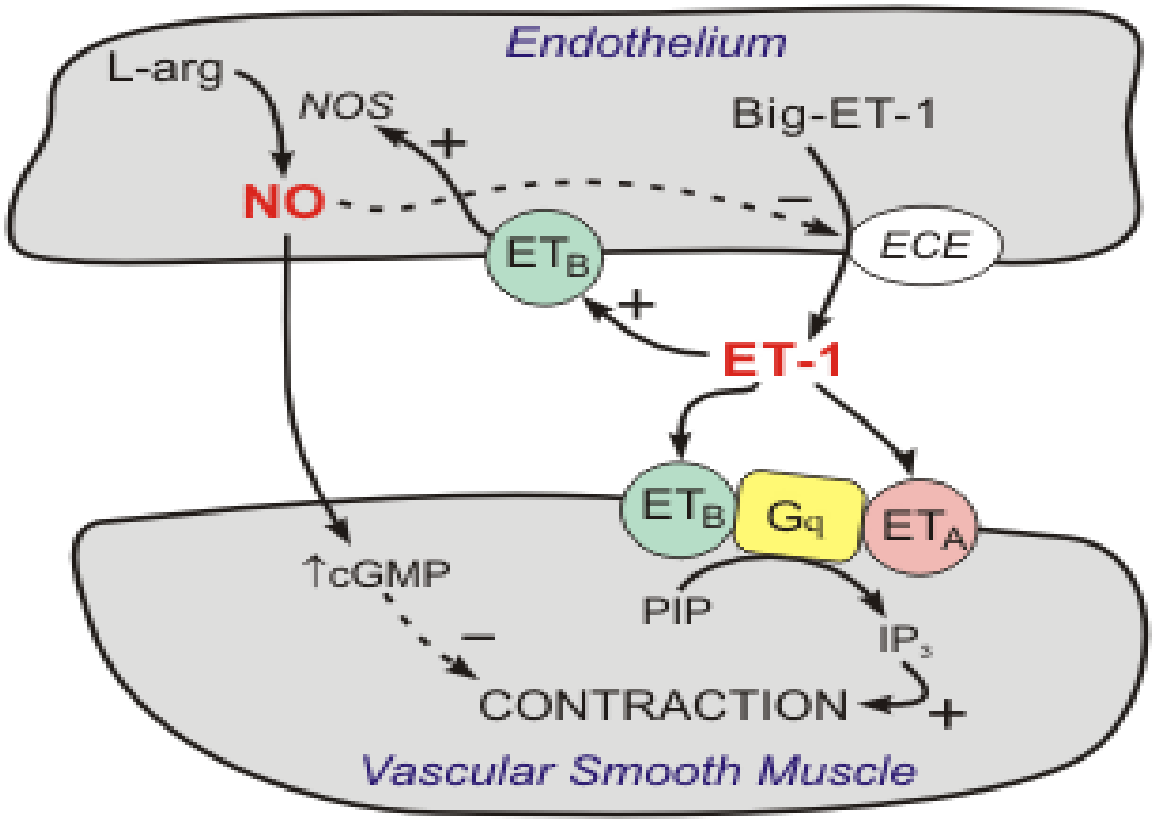
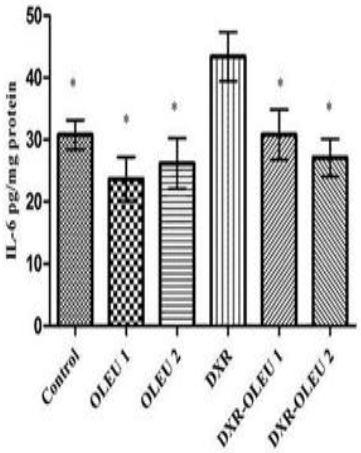
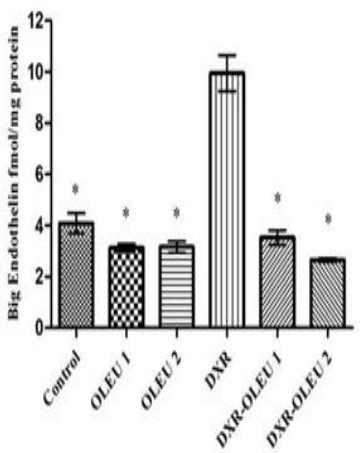


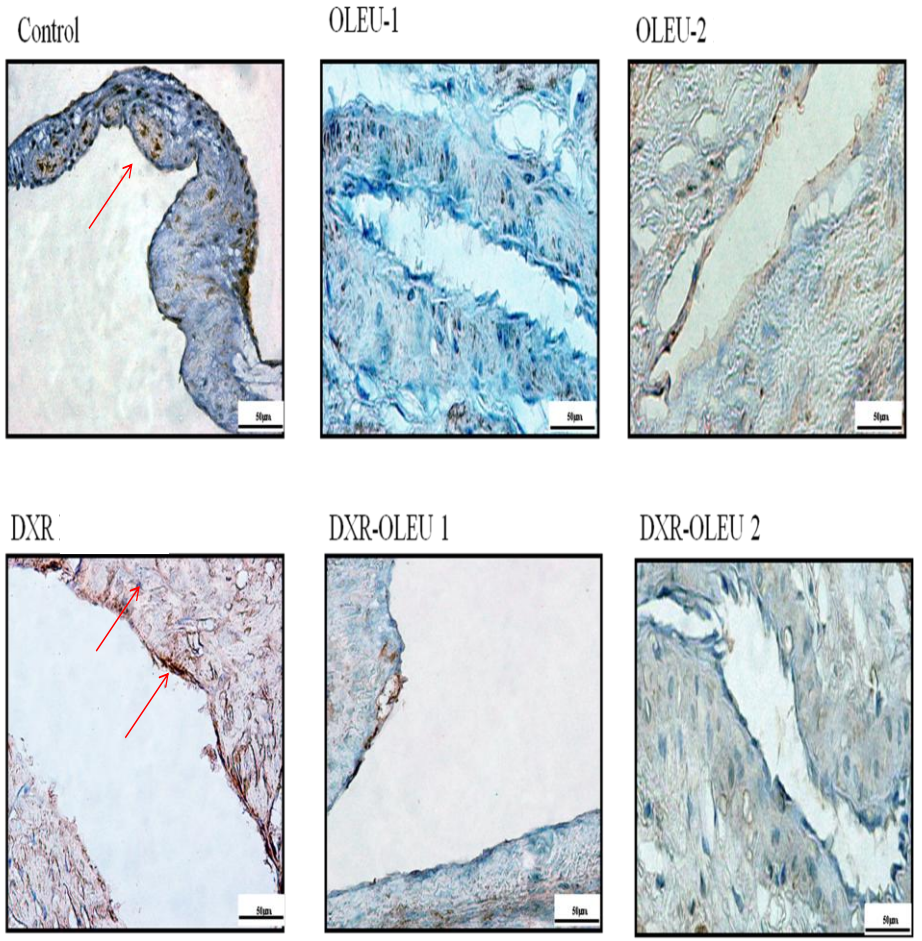
Figure 2. Endothelin receptors and interactions with nitric oxide.

Ikonomidis I, Lekakis I, Andreadou I, Circulation, 117: 2662-2669, 2008

Oleuropein treatment significantly reduced the elevated IL-6 and ET-1 levels in the DXR treated rats.



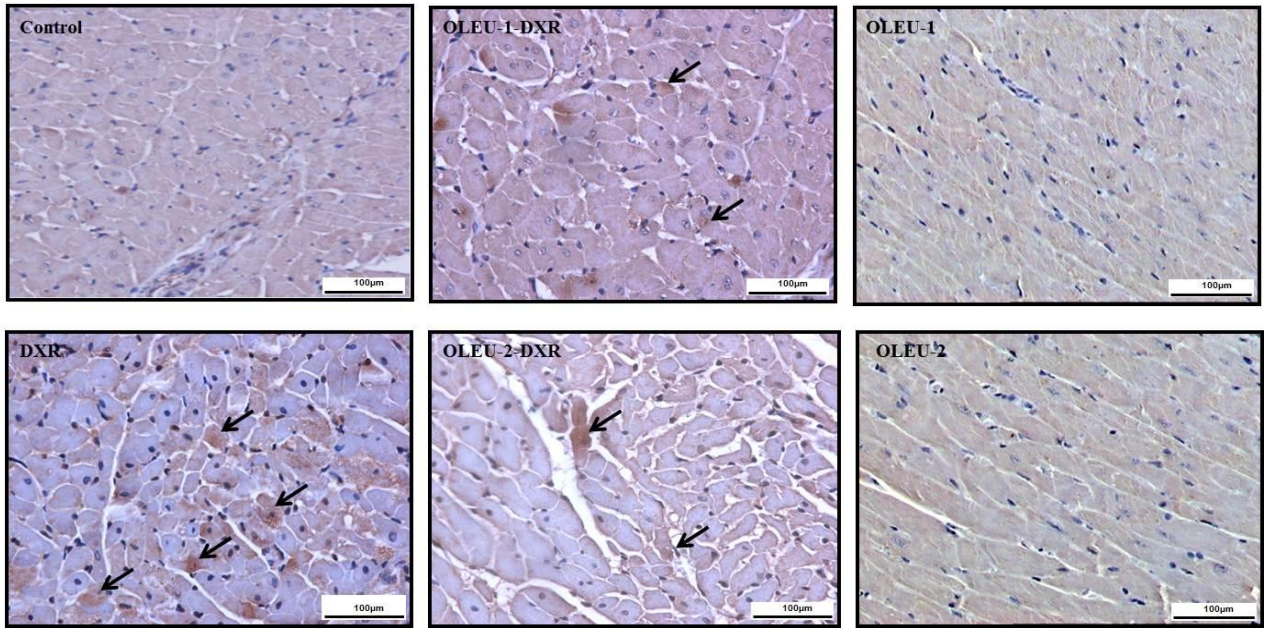


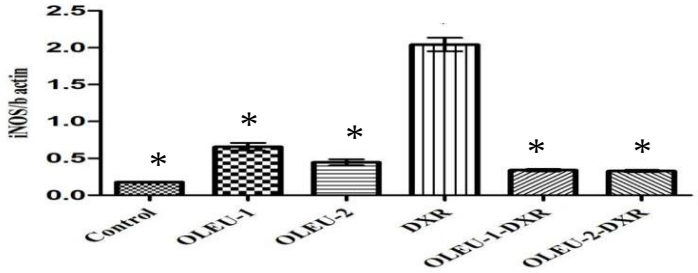


Immunohistochemistry of ET-1 (400x)
Insets indicate ET-1 positive cells.

Andreadou I, et al., J Mol Cell Cardiol, 69:4-16, 2014

Oleuropein treatment significantly reduces the iNOS expression in cardiomyocytes





Andreadou I, et al., J Mol Cell Cardiol, 69:4-16, 2014

The higher eNOS expression in the vascular endothelium and the lower iNOS expression in the cardiomyocytes of oleuropein-treated groups indicate the protective role of oleuropein.

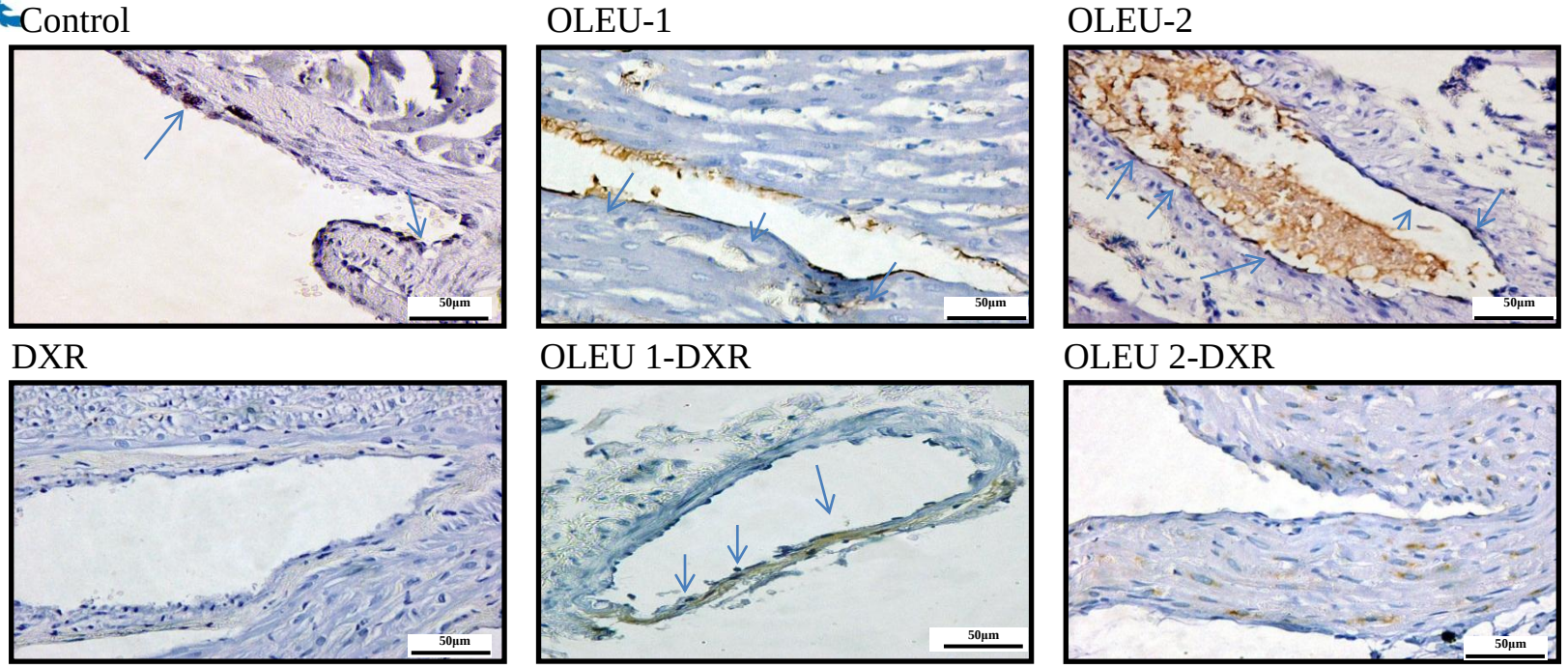
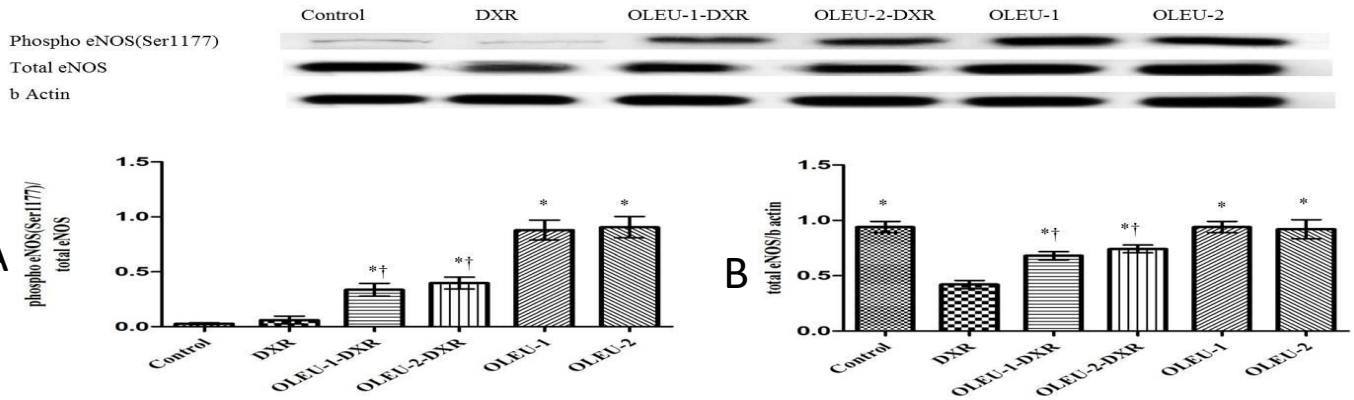


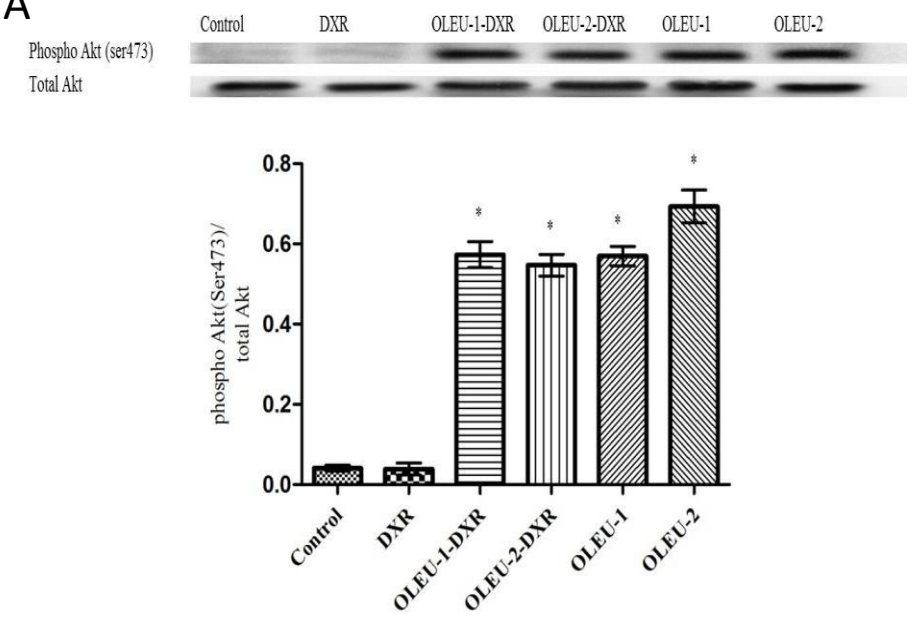
Figure 5D



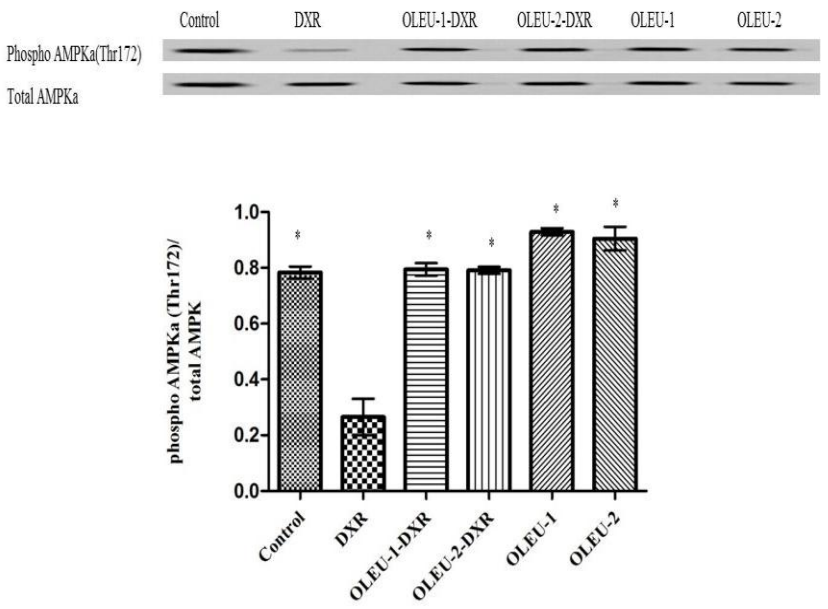
Andreadou I, et al., J Mol Cell Cardiol, 69:4-16, 2014

Oleuropein attenuated the impaired pro-survival mechanisms (Akt), the cardiac energy metabolism (AMPK) and the homeostasis of NO (eNOS) induced by DXR

A

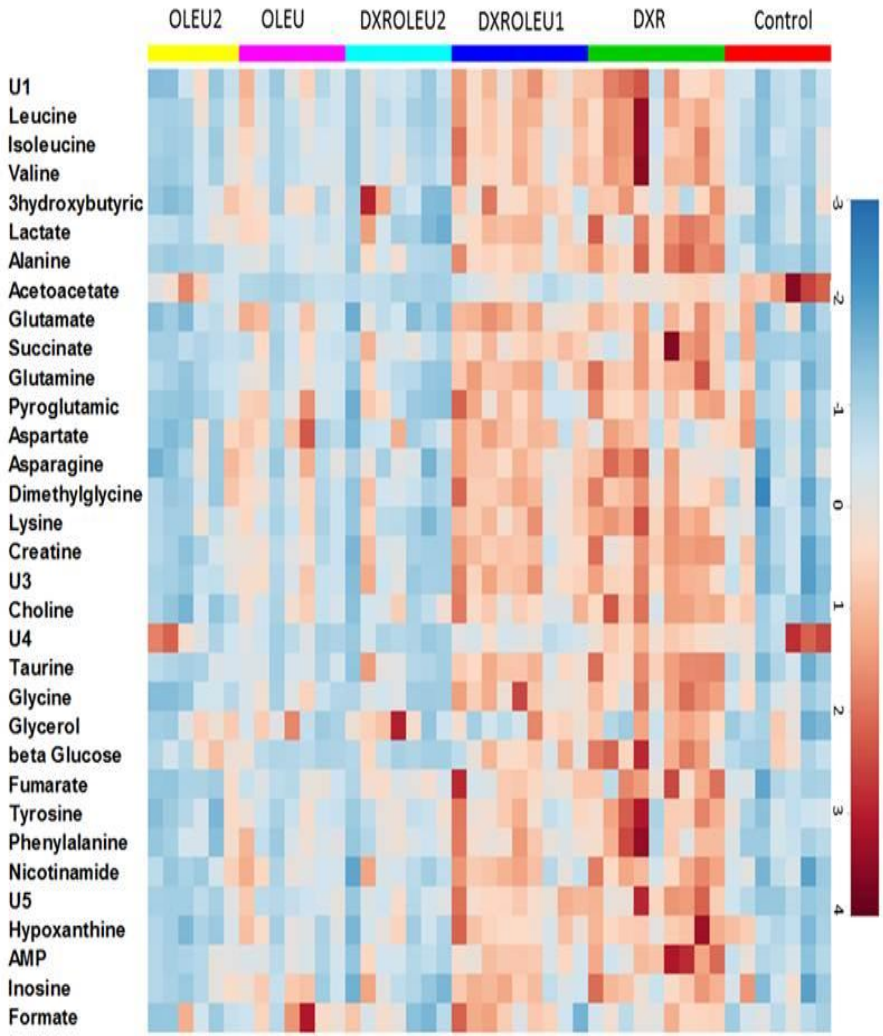


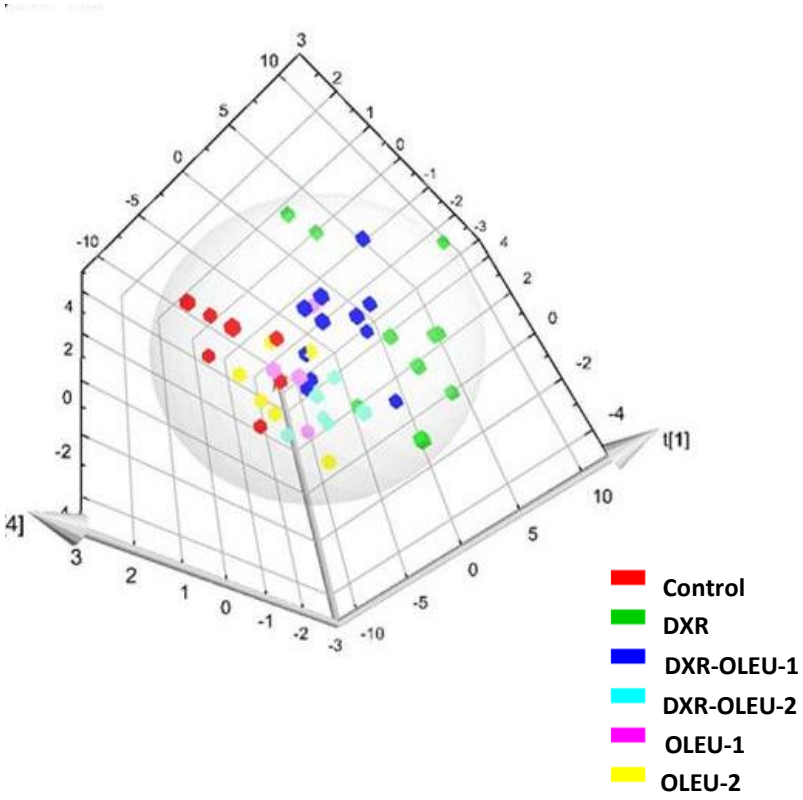
B



Andreadou I, et al., J Mol Cell Cardiol, 69:4-16, 2014

NMR Metabonomics showed a clear distinction between *the study groups*





Andreadou I, et al., J Mol Cell Cardiol, 69:4-16, 2014

The metabolites responsible for this distinction are related to the energy metabolism and the remodeling events in the failing heart

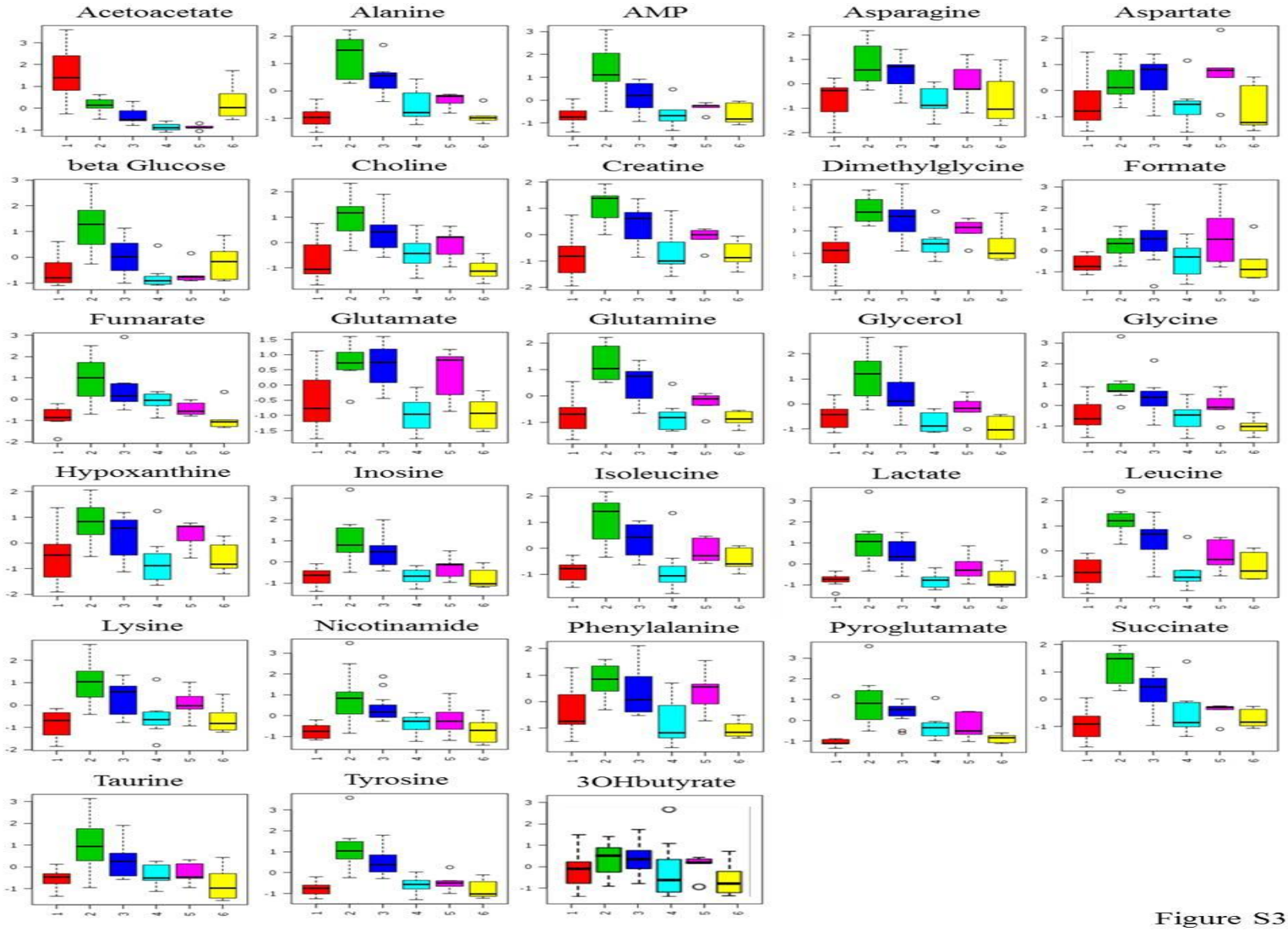


Figure S3

The activation of AMPK by oleuropein seems to be the key point of the restoration of the levels of amino acids and protein biosynthesis

Increase of all aminoacids suggests that protein degradation is increased in the failing heart

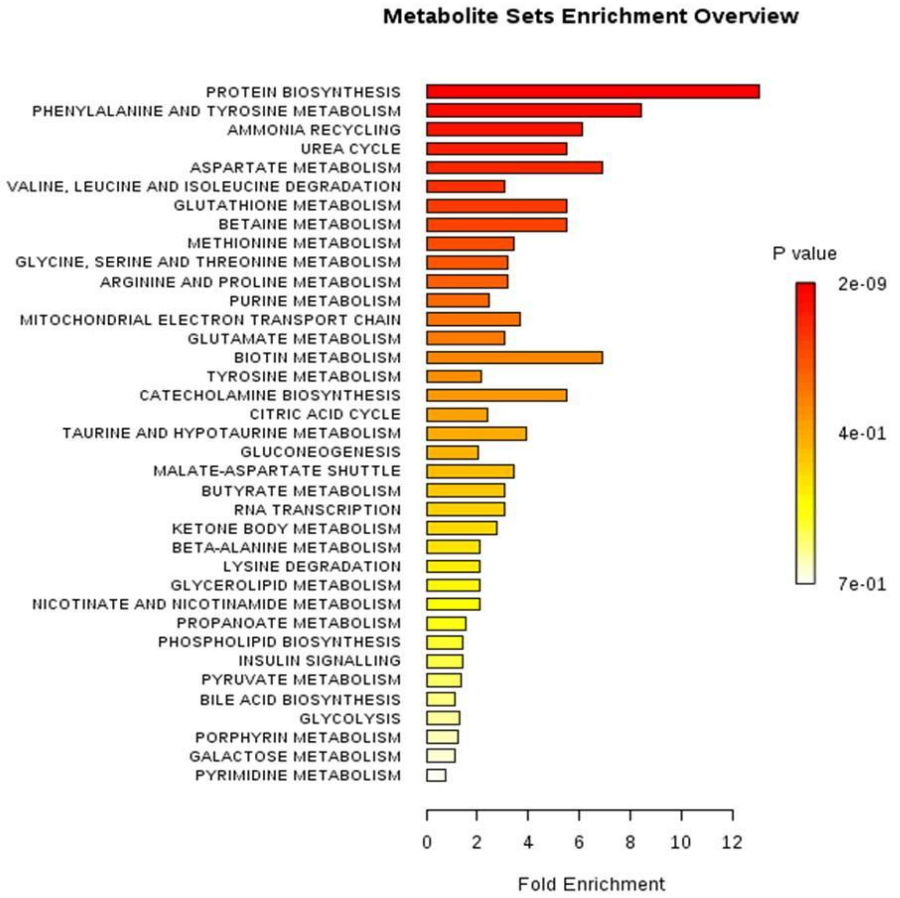
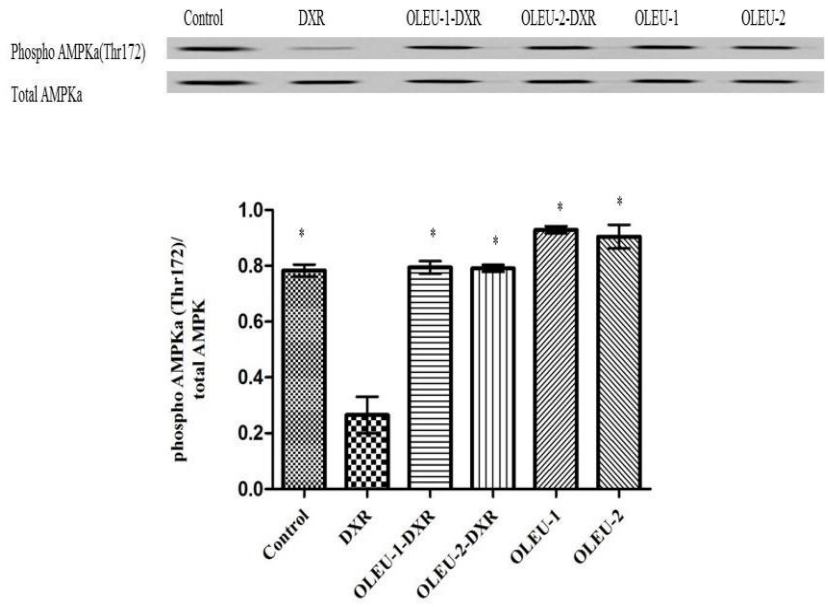


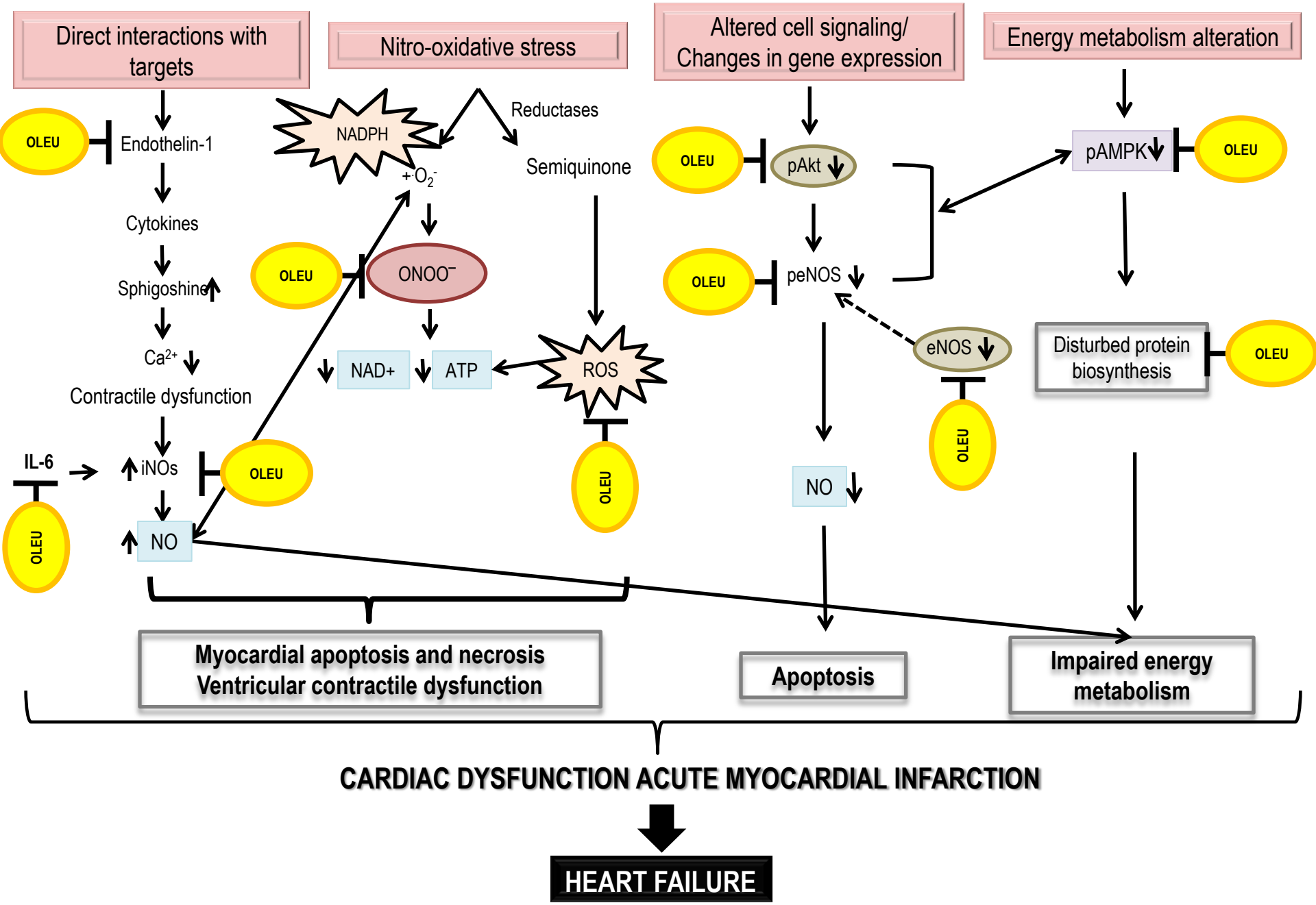
Figure S4

B



Andreadou I, et al., J Mol Cell Cardiol, 69:4-16, 2014

CONCLUSION


Direct interactions with targets
Nitro-oxidative stress
Altered cell signaling/ Changes in gene expression
Energy metabolism alteration
OLEU
Endothelin-1
Cytokines
Sphigoshine
Ca2+
Contractile dysfunction
IL-6
iNOs
NO
NADPH
+·O2-
Reductases
Semiquinone
ONOO−
NAD+
ATP
ROS
pAkt
peNOS
eNOS
pAMPK
Disturbed protein biosynthesis
NO
Myocardial apoptosis and necrosis
Ventricular contractile dysfunction
Apoptosis
Impaired energy metabolism
CARDIAC DYSFUNCTION ACUTE MYOCARDIAL INFARCTION
HEART FAILURE

Acknowledgments

Financial Support: This study has partly been founded by a grant received from the Central National Health Council.

- *K. Ioannides*
- *M. Papaefthimiou*
- *S.I. Bibli*
- *S. Toumpas*
- *M. Konstantinou*
- *S. Vasiliou*
- *P. Efentakis*

Medical School, University of Athens, Vascular Surgery

- *F. Sigala*

Medical School, University of Ioannina, Cardiology

- *K. Naka*

School of Pharmacy, University of Athens

- E. Gikas
- E. Mikros
- A.L. Skaltsounis

B' Cardiology Department of Cardiology, Medical School, University of Athens

- E. Iliodromitis
- D. Th. Kremastinos