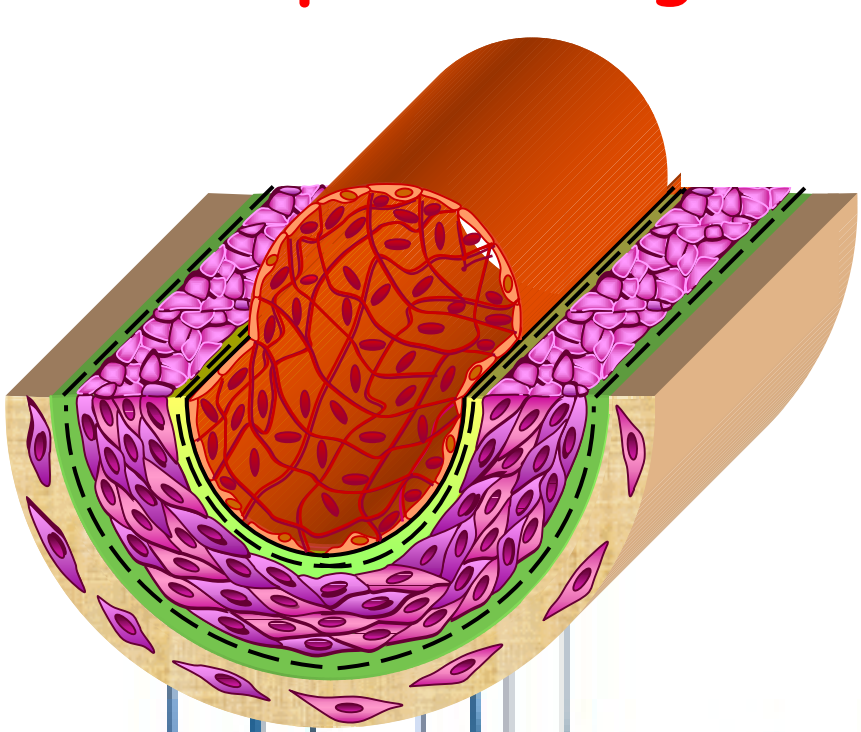
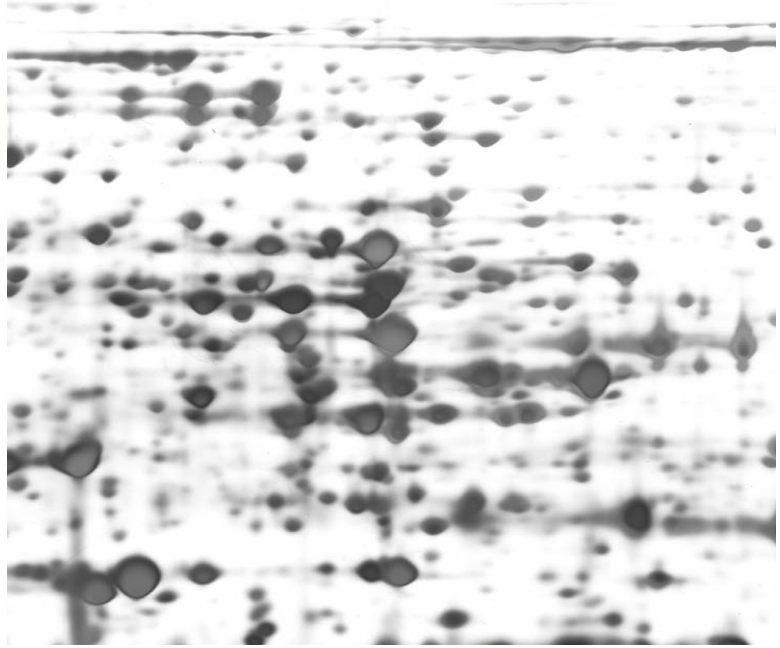
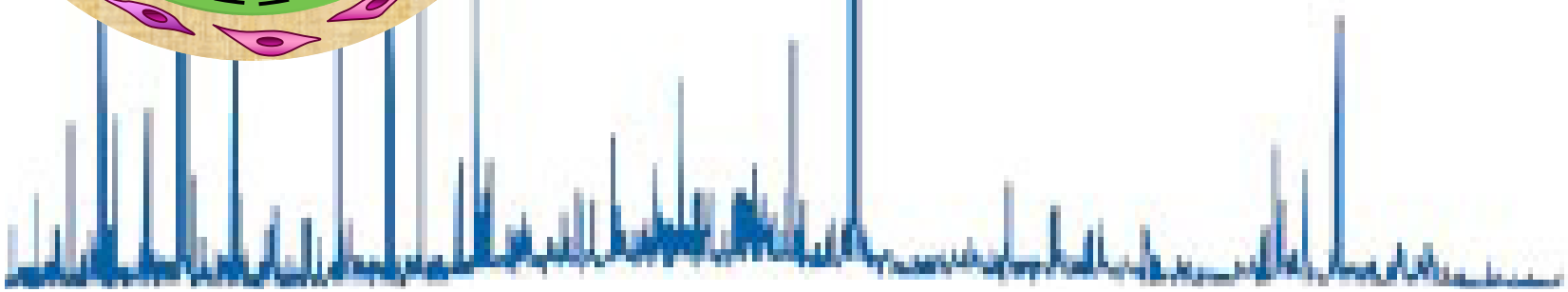
Proteomics to identify novel biomarkers and therapeutic targets in cardiovascular disease





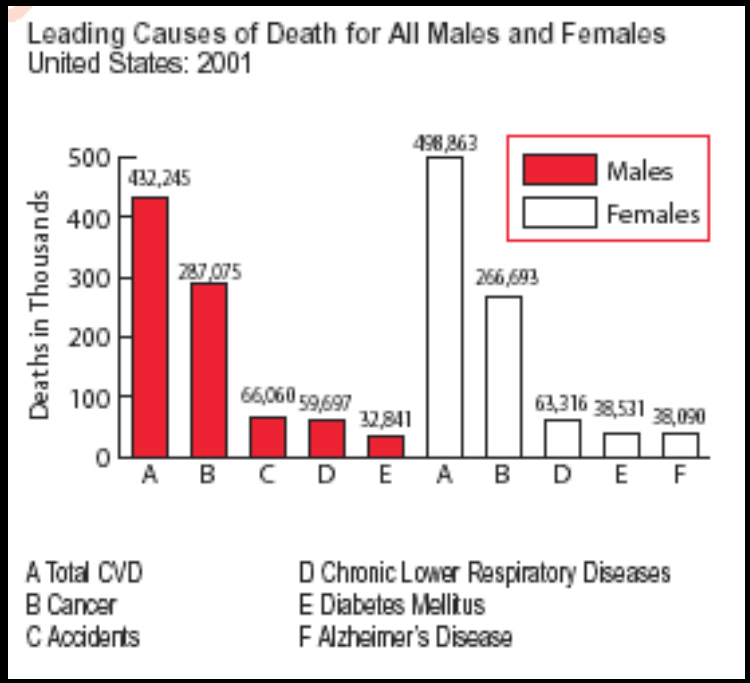


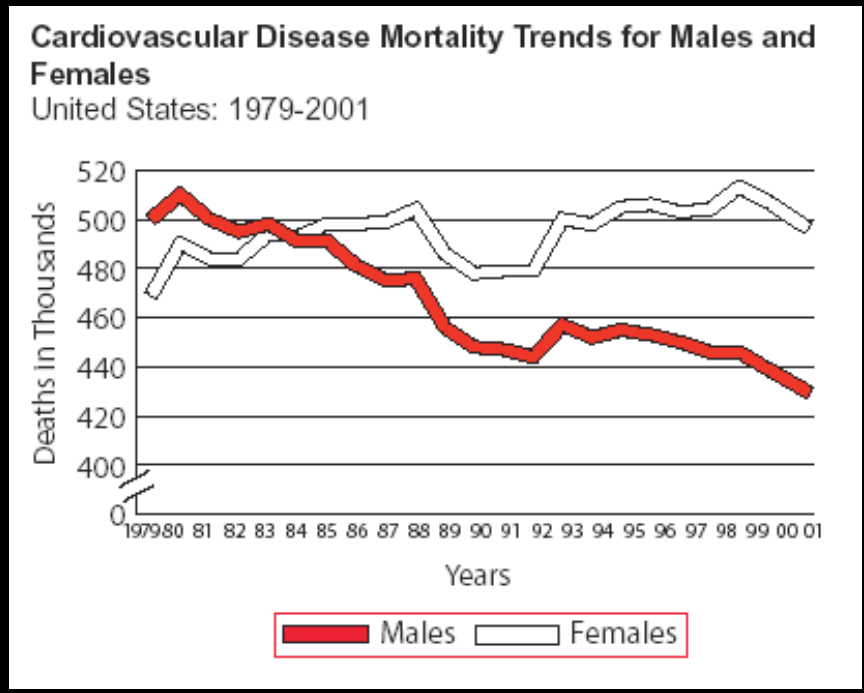
Markus Kubicek

Laboratorios de Biologia Vascular y Proteínómica Estructural, Instituto de Biomedicina de Valencia

CSIC

Cardiovascular disease - the killer number one





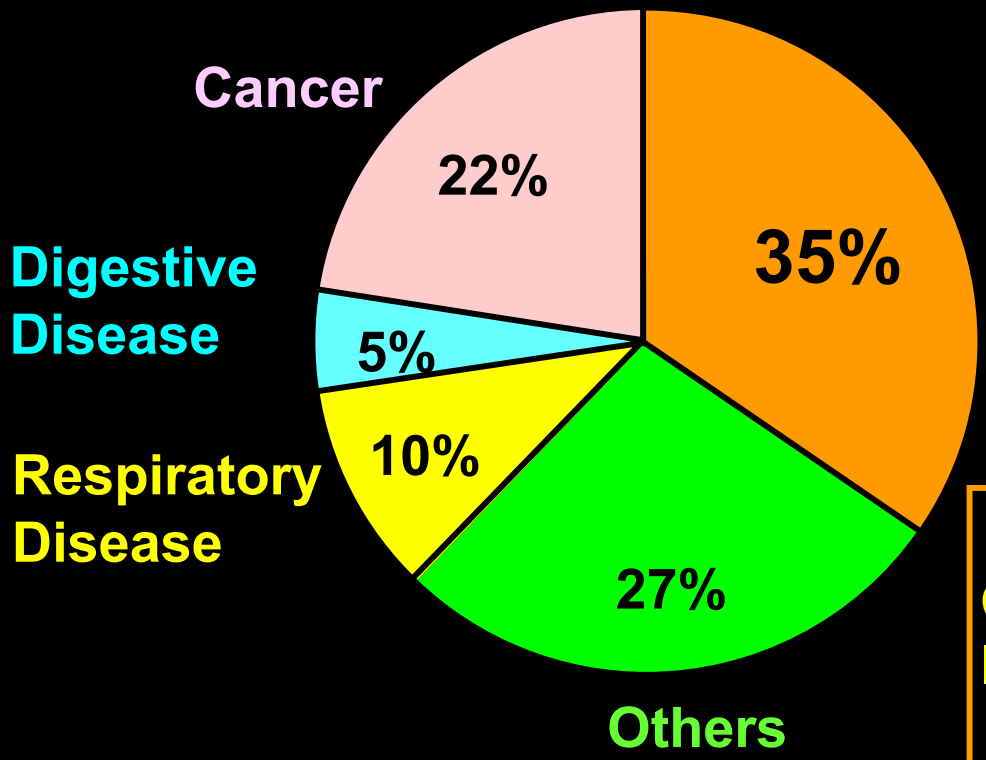
Source:CDC/NCHS

CVD accounted for 38.5 percent of all deaths or 1 of every 2.6 deaths in the United States in 2001. CVD mortality was about 60 percent of "total mortality."

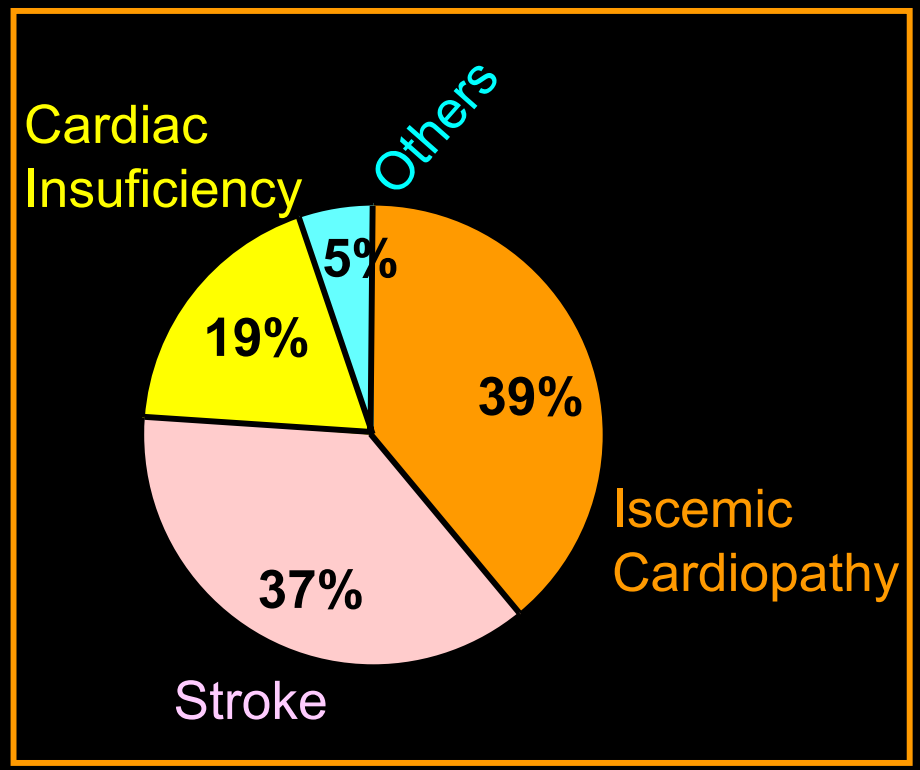
CVD cause about a death a minute among females — claiming nearly half a million female lives every year. That's more lives than the next 7 causes of death *combined*. Starting at age 75, the prevalence of CVD among women is higher than among men.

The harsh fact is, cardiovascular diseases are the No. 1 killer of women and men

MORTALITY RATES IN SPAIN

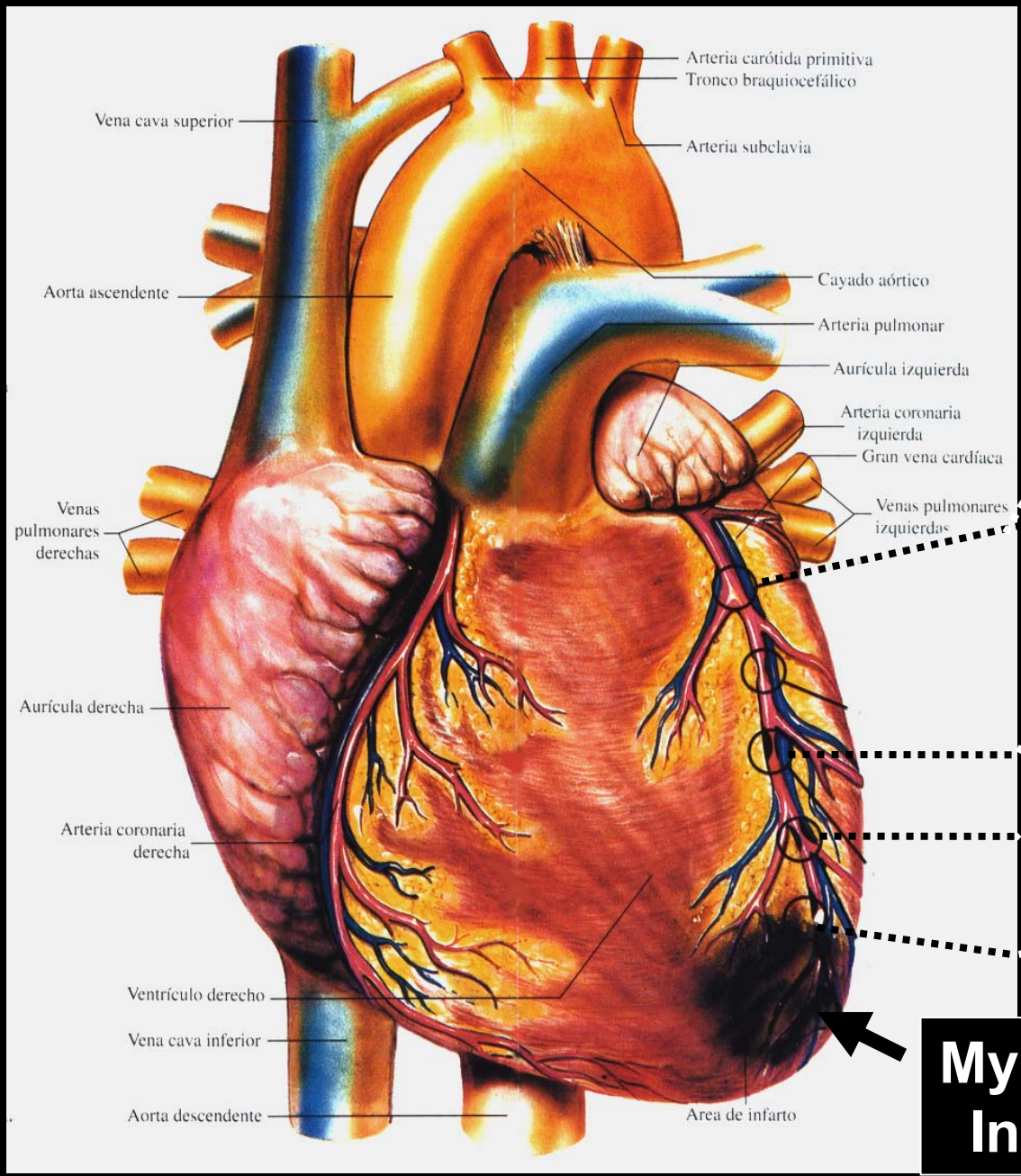


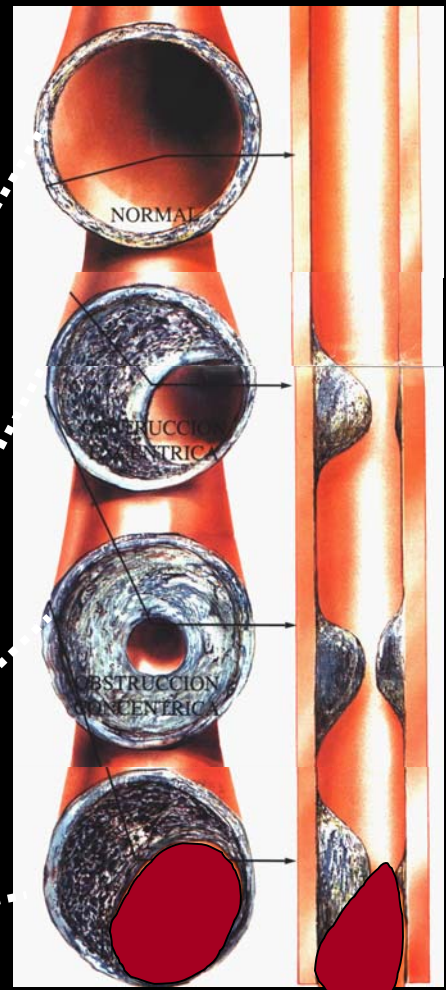
CARDIOVASCULAR DISEASES



(INE, Dic. 2003)

Coronary Atherosclerosis and Myocard Infarct

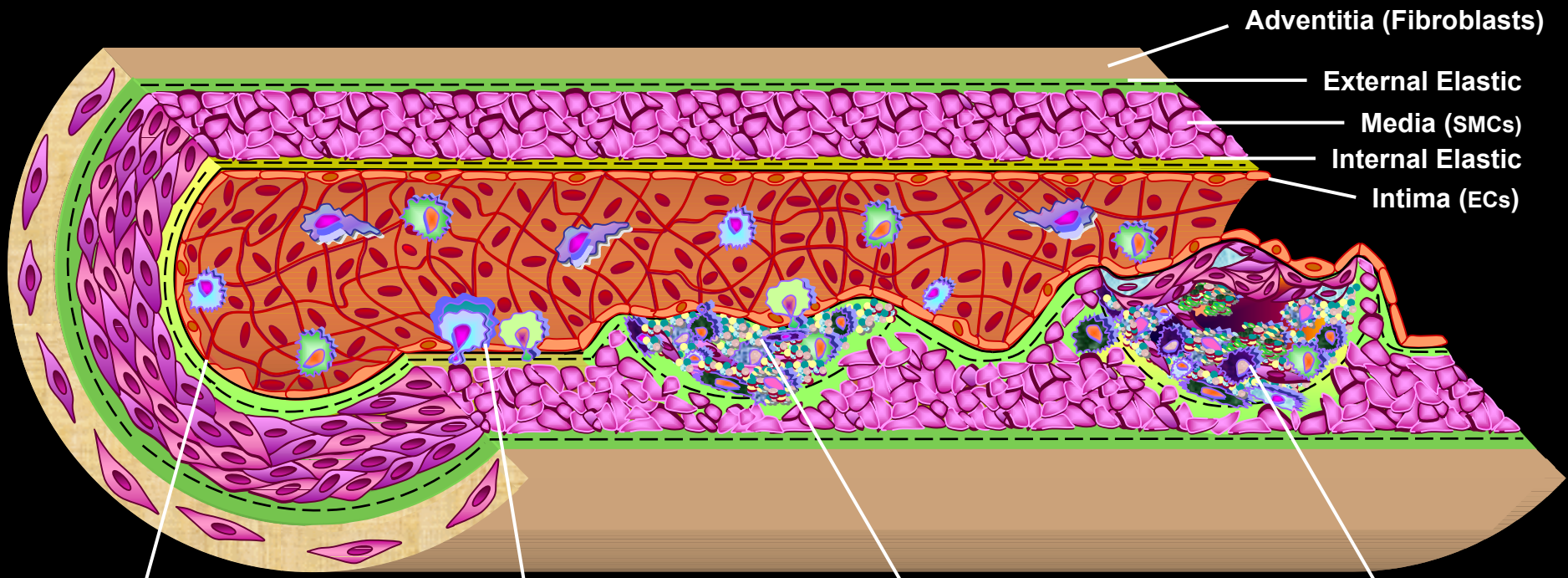




Myocard Infarct

Thrombus

Atherosclerosis:a slowly progressing disease lacking early diagnosis



"Healthy" artery

- Minor arterial cell proliferation
- Reduced leukocyte adhesion

ATHEROGENIC STIMULI →

Onset atherogenesis

- Endothelial dysfunction
- Leukocyte recruitment
- Abundant production of inflammatory cytokines/chemokines

→ **Fatty streak (early lesion)**

- Foam cell / lipid core formation
- Leukocyte proliferation
- SMC activation (proliferation/migration, extracellular matrix production)

→ **Advanced lesion**

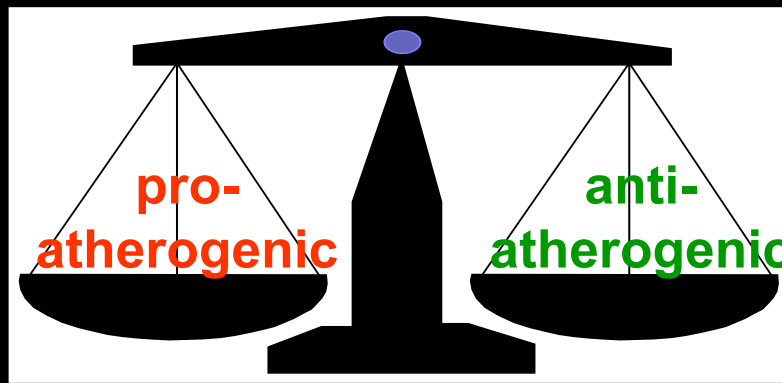
- Fibrous cap formation
- Necrotic core formation
- Plaque instability

Plaque rupture and thrombus formation ↓

ISCHEMIC EVENT (i.e, myocardial infarction, stroke)

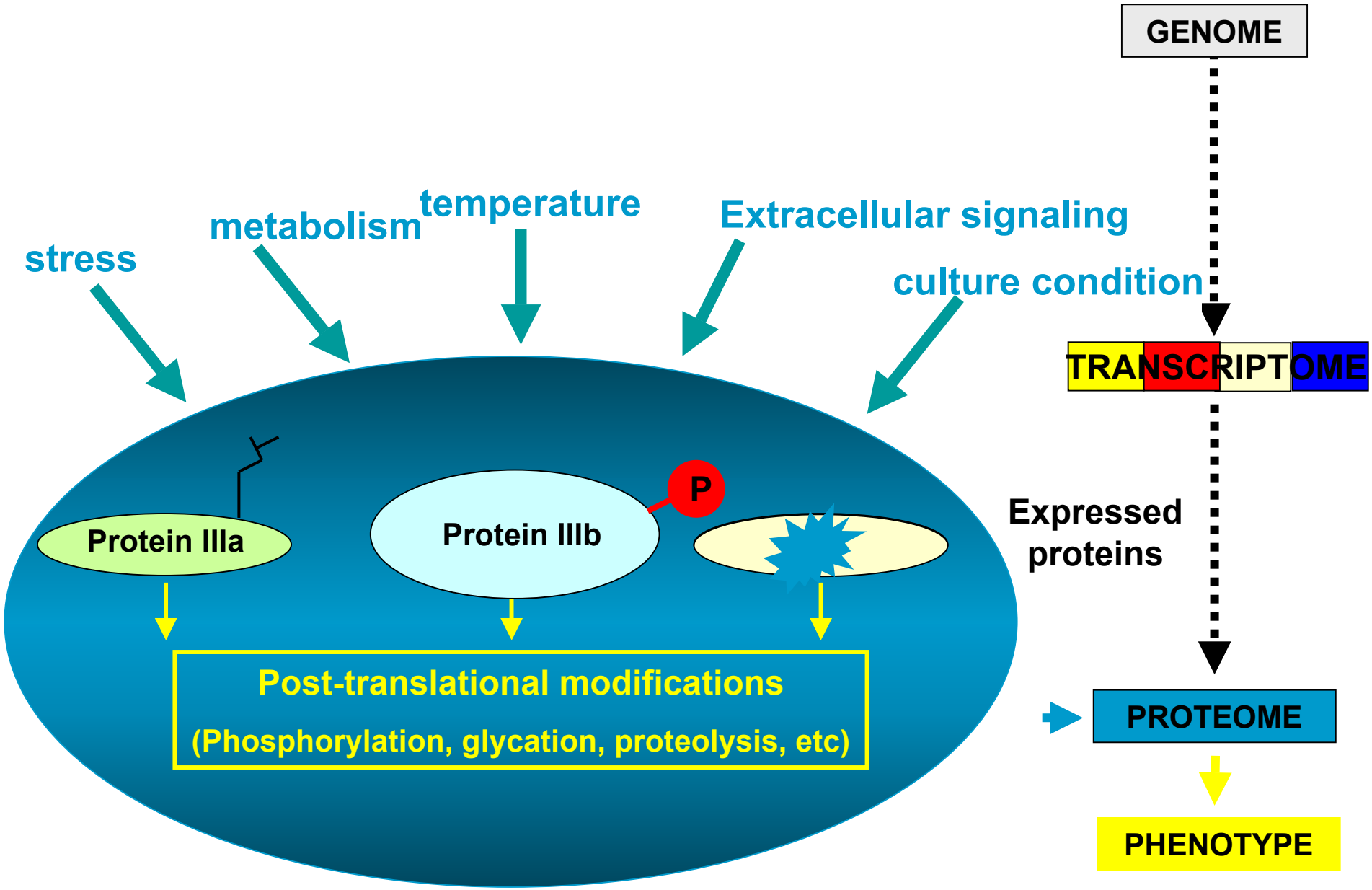
Genetic Factors

Envinromental Factors

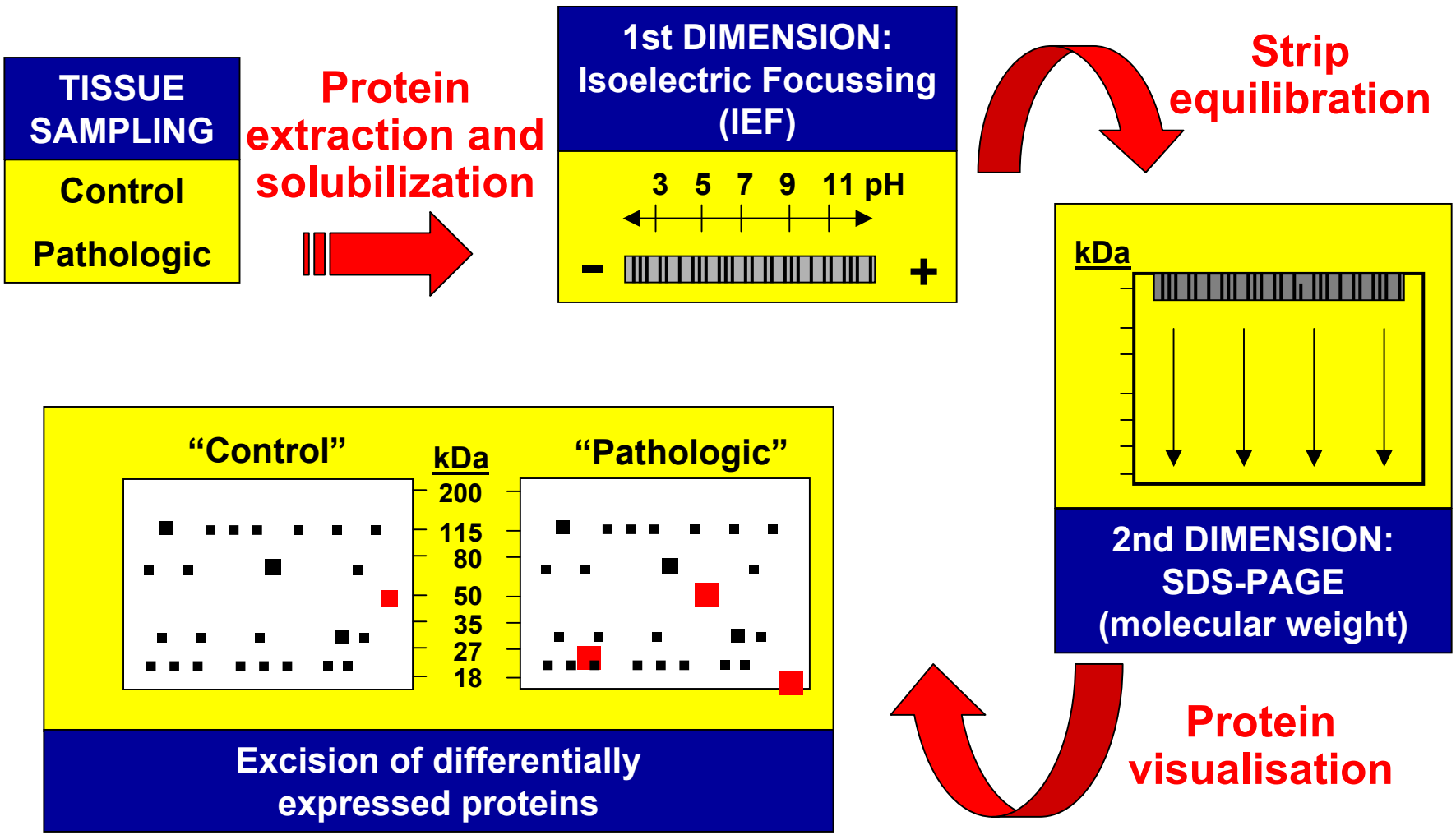


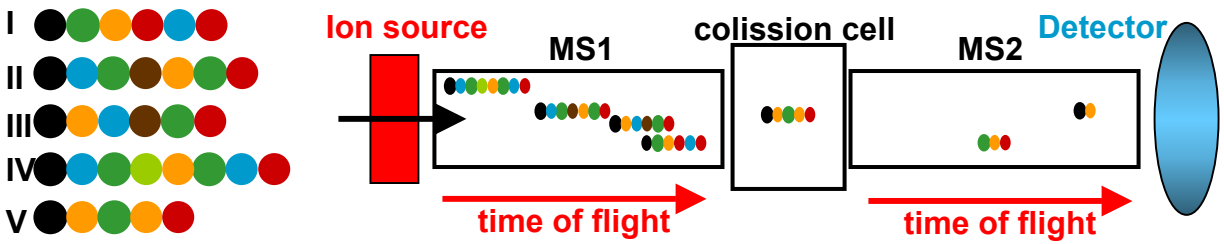
Individual risk for cardiovascular disease

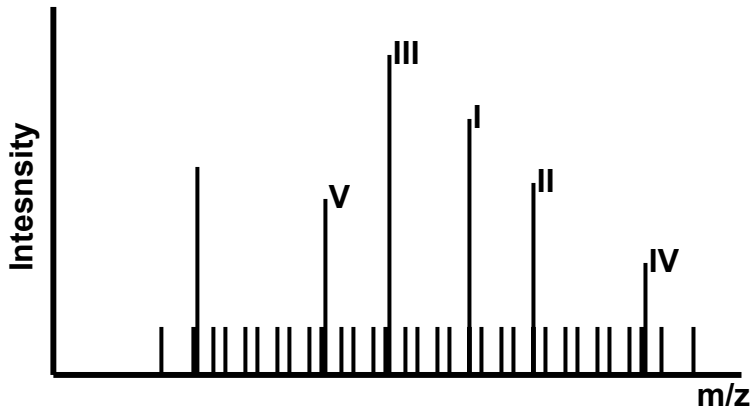
The strength and limitations of proteomics in the study of vascular disease



Separation of proteins by two-dimensional Gel-electrophoresis (2D-PAGE)







I	695.3	LGEYGL
II	720.3	LSQTFPL
III	575.3	LPCVEDYL
IV	841.3	LVQEVTDFAK
V	550.8	KQTAL

MTHSDSDILTNIEDPSSHVPEFSSSSKLSQTFPLNADFAEITKLKQT
ALAELVKLPCVEDYLTNIEDPSSHVPEFSSSSKLGEYGLFQNAILKV
QEVTKFAKKLGMRAC: Serum Albumin A05103

Proteolysis (i.e. trypsin)

↓

Peptide digestion mix

↓

MS or MS/MS

↓

Mass spectrum (fingerprits)

↓

Data base comparison

↓

Protein Identification

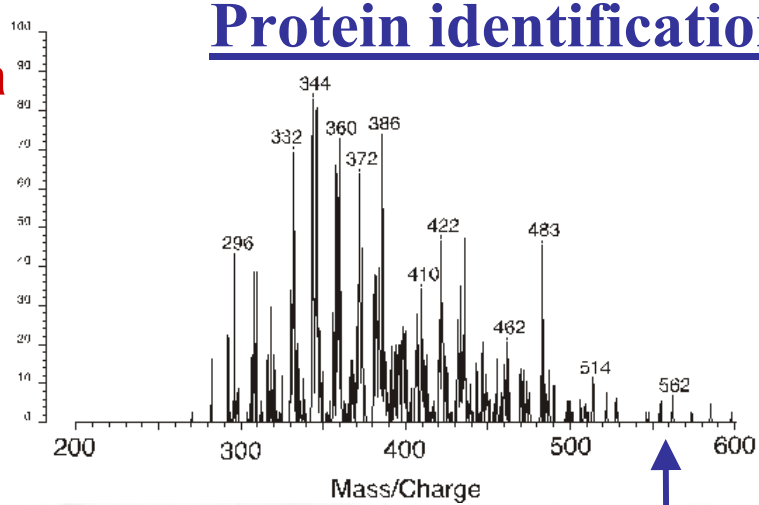
PROTEOMIC APPROACH FOR THE IDENTIFICATION OF ATHEROSCLEROSIS-BIOMARKERS FROM HUMAN PATIENT SAMPLES

Hypercholesteremia

Angina pectoris

Hypertension

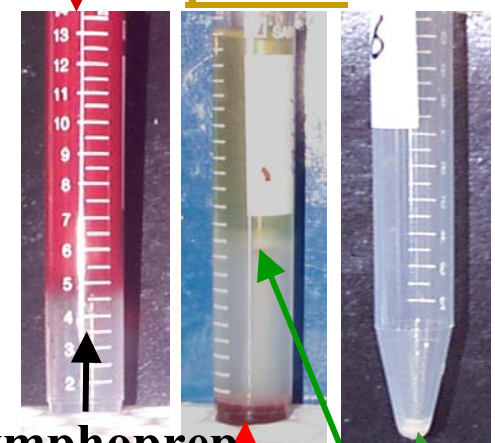
Protein identification

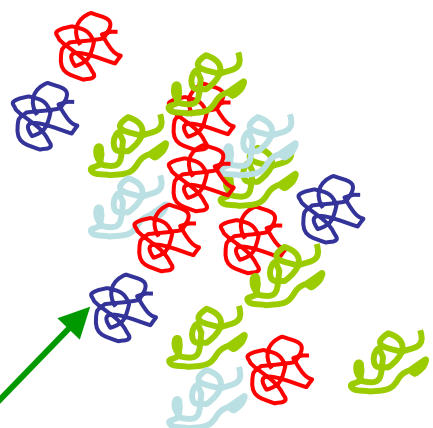


total blood

plasma

Protein extraction



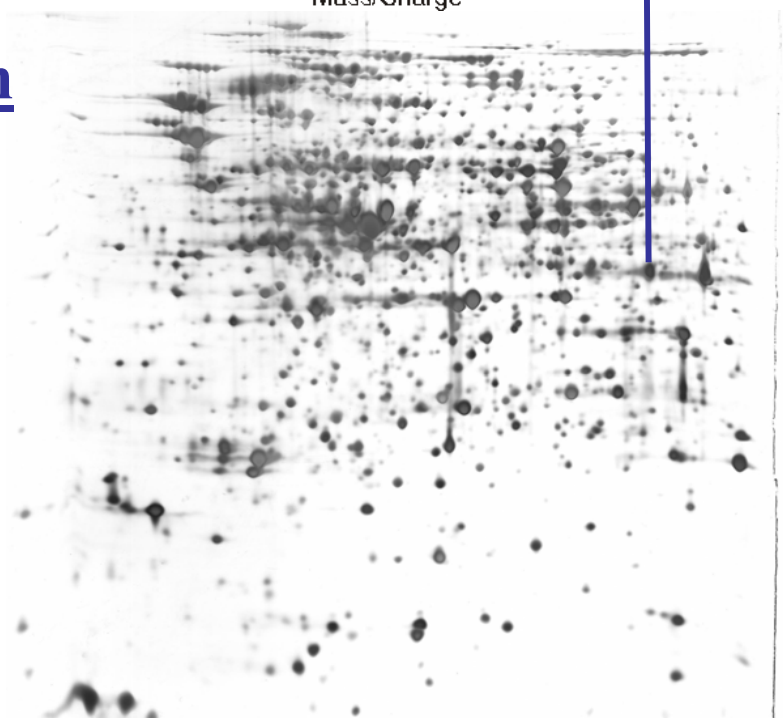


lymphoprep

mononuclear cells

erythrocytes and platelets

2D-PAGE



Proteiomic alterations in human angina pectoris patients

I) Angina pectoris

In collaboration with Dr. Panayotis Fantidis

Hospital Clinico San Carlos, Madrid

Proteiomic alterations in human hypertension patients

II) Hypertension

In collaboration with Dr. Javier Chaves and Dr. Josep Redón

Hospital Clinico Universitario de Valencia

Proteomic alteration of protein A4c is reversed after medical treatment

II) Hypertension

In collaboration with Dr. Javier Chaves and Dr. Josep Redón

Hospital Clinico Universitario de Valencia

Among proteomic alterations found in MNCs from 25 patients 5 differentially expressed proteins have been found so far to be reversed after treatment.

Proteiomic alterations associated to hypercholestermia

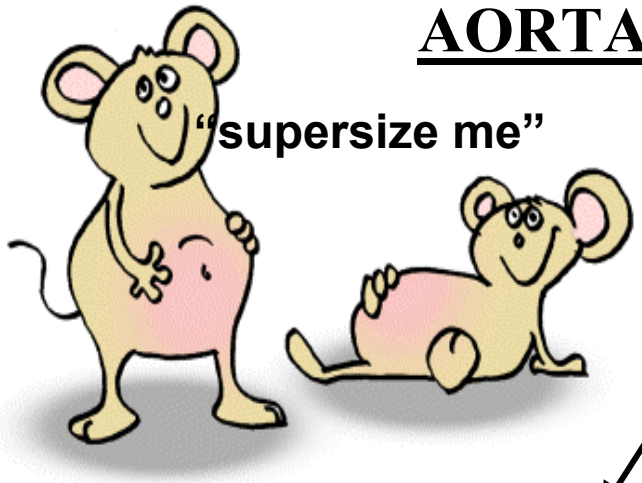
III) Hypercholesteremia

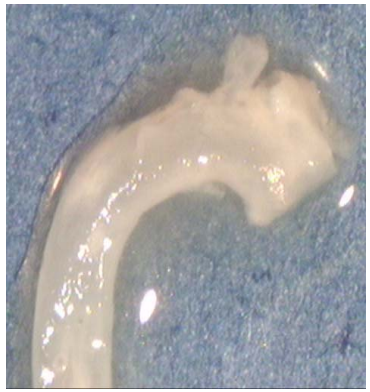
In collaboration with Dr. M. Pocoví *Universidad de Zaragoza*
human plasma bank of hereditary hypercholesteremia

MK and Kiko Verdeguer *Instituto de Biomedicina Valencia*
ApoE deficient mouse model of atherosclerotic disease

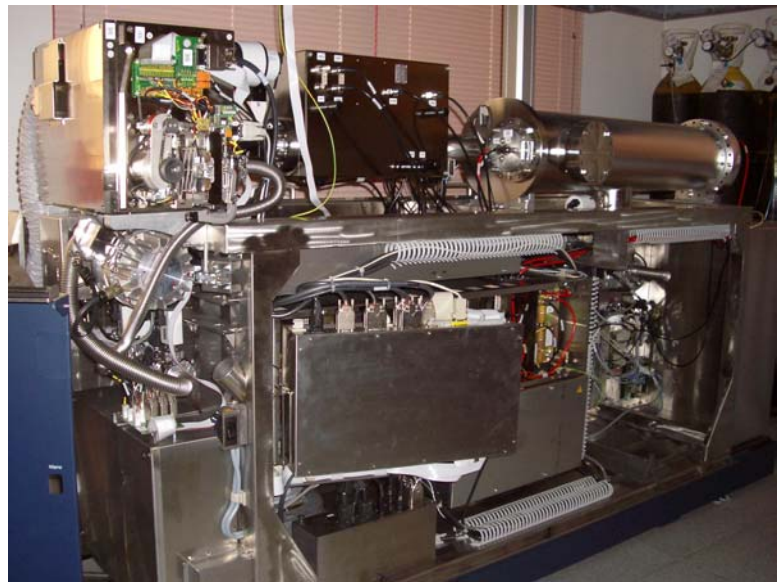
PROTEOMIC APPROACH FOR THE IDENTIFICATION OF ATHEROSCLEROSIS-BIOMARKERS FROM APOE-/- MICE

AORTA



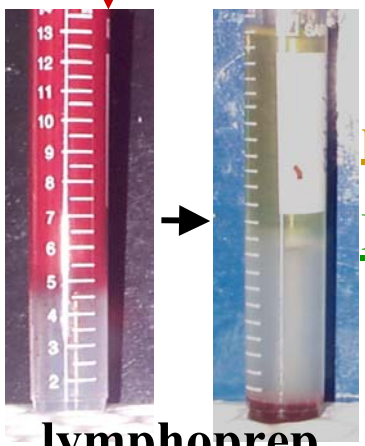


Mass Spectrometry



Protein extraction

BLOOD

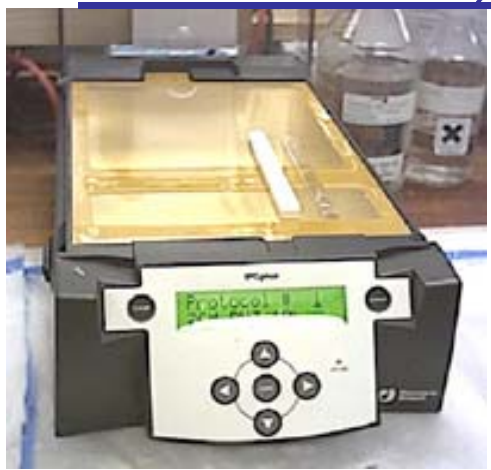


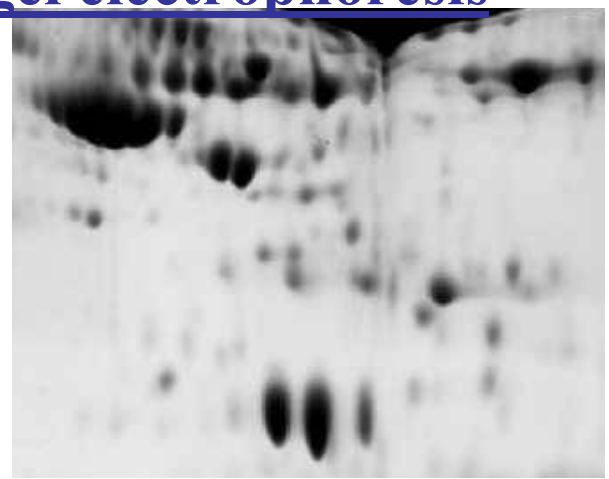
lymphoprep

plasma

MNC

2 dimensional gel electrophoresis

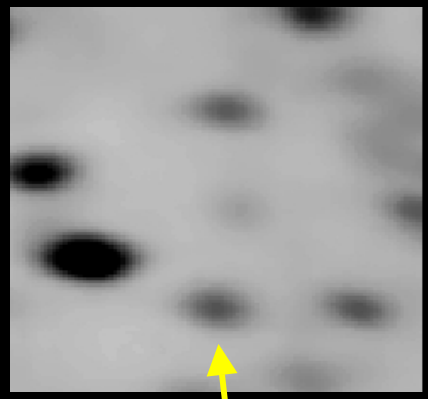




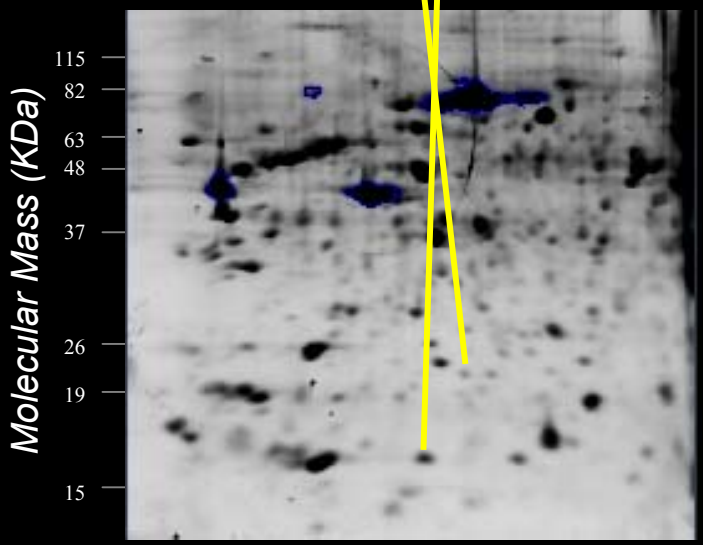
2D-ELECTROPHORESIS COMPARISON FOR DISCOVERY OF BIOMARKERS

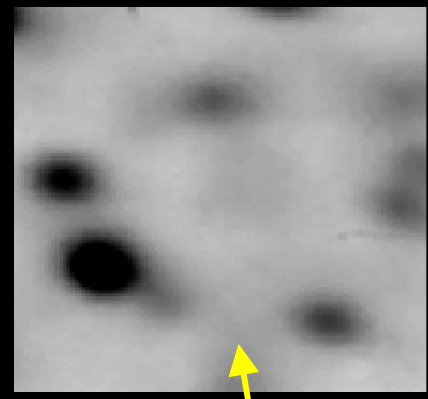
CONTROL DIET apoE KO MICE

ATHEROGENIC DIET apoE KO MICE



CONTROL DIET





ATHEROGENIC DIET

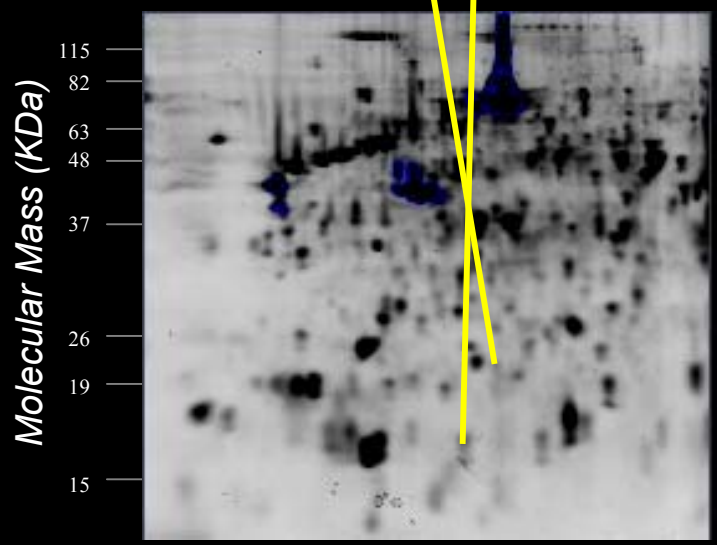
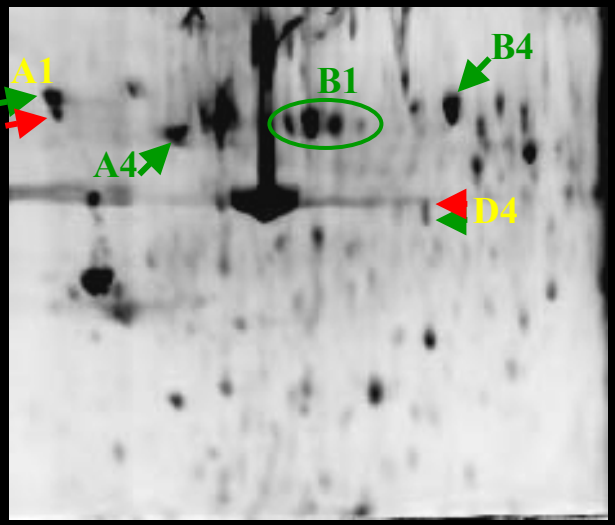


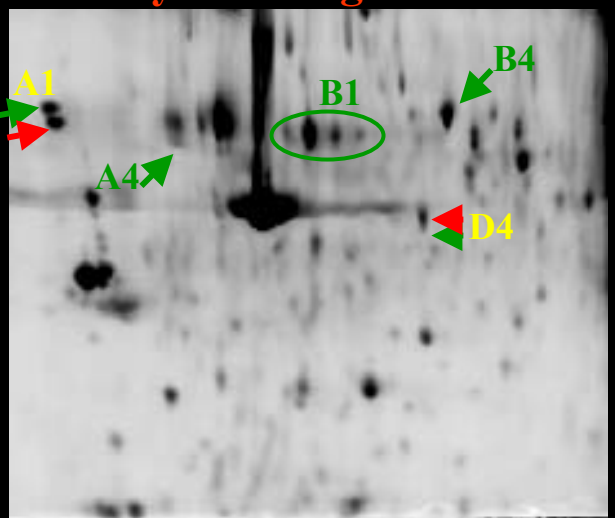
IMAGE COMPARISON WITH BIOINFORMATIC ANALYSIS

Proteiomic alterations of mononucleated cells derived from control and ATG diet fed mice

Control diet



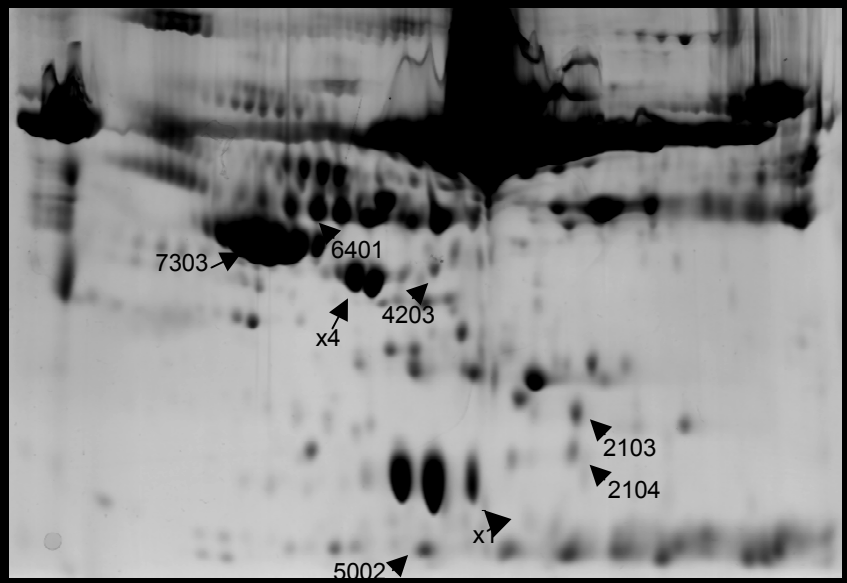
30 days atherogenic diet



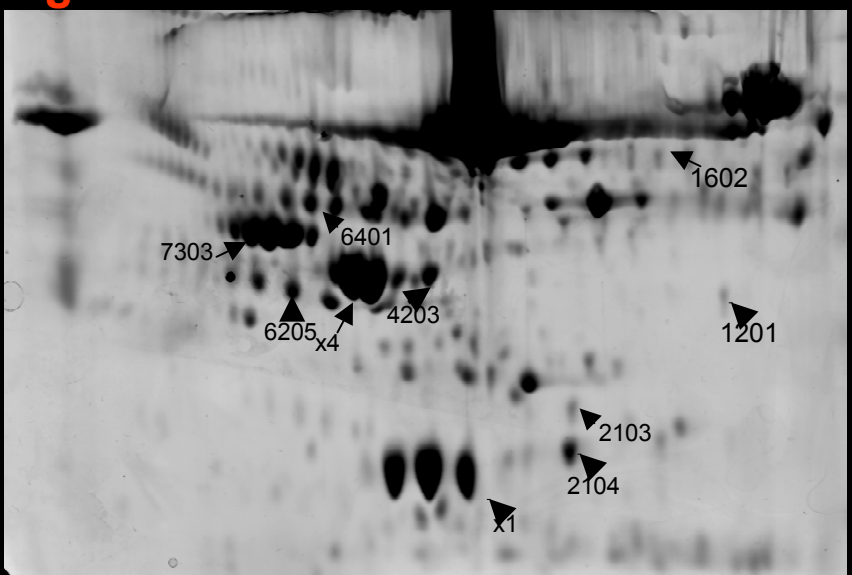
abbrv	SSP	kDA	pI	ATG vs CTR
A1a	0501	55	4,2	down 1,3
A1b	0502	53	4,2	up 1,5
A2	1501	55	4,8	down x 2,5
A3	1503	50	5	spec. Atg
A4	1401	48	5,1	down x 7
B1a	4401	49	5,7	down 1,5
B1b	4402	49	5,8	down 1,6
B1c	5401	49	5,9	down 2
B1d	6401	49	6	down 1,8
B2	7501	60	6,1	down 2
B3	7502	50	6,15	down 2
B4	7504	50	6,2	down 3
B5	8301	45	6,5	down 2,3
B6	8501	50	6,6	down 2,9
B7	8502	50	6.7	down 1,9
D1	4103	21	5,7	up 1,9
D2	5201	35	5,6	down 1,8
D3	6001/TLN	20	6	up 1,6
D4a	7301	35	6,4	down 1,8
D4b	7302	36	6,4	up 1,7
D5	9201	30	6,8	down 2,3
Z1	1	16	5	up 2,2

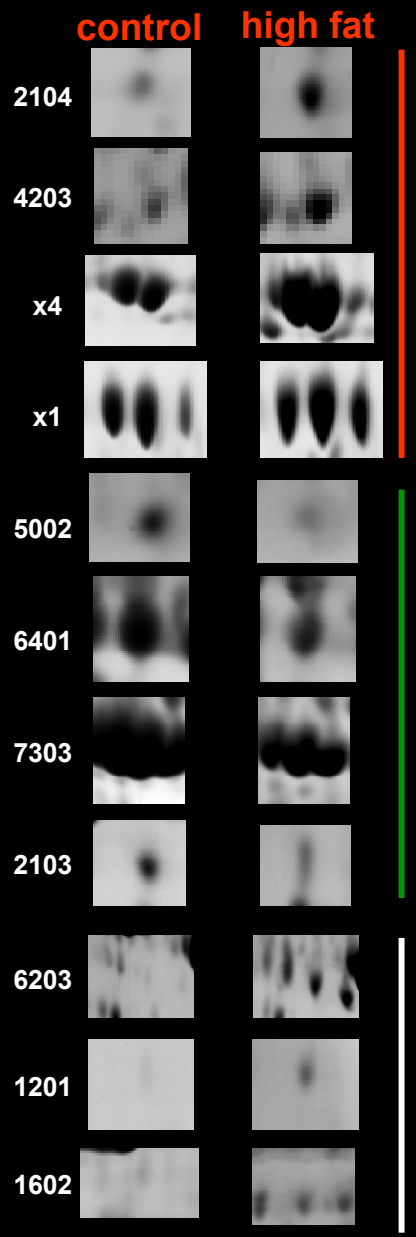
Proteiomic alterations in mouse plasma from control versus high-fat diet fed mice

Control diet



high-fat diet





Elevated in high fat

Nombre	SSP1	ATG vs CT	ATG vs CT	ATG vs CT
B1	2104	**3,83**	**2,76**	**2,49**
A8(C1)	4203	**3,66**	**10,62**	**2,71**
x1	5007	**2,65**	**2,33**	**2,28**
x4	6201	**4,79**	**2,45**	**5,83**

Reduced in high fat

Nombre	SSP1	ATG vs CT	ATG vs CT	ATG vs CT
C3	5002	**-2,94**	**-5,00**	**-4,00**
A4	6401	**-1,67**	**-2,04**	**-1,79**
A1	7303	**-3,23**	**-4,17**	**-2,56**
C4	2103	**-1,49**	**-1,75**	**-1,10**

Absent in control

Nombre	SSP1
A7	6203-8202
B5	1201
B9	1602

Proteiomic alterations associated to hypercholestermia

III) Hypercholesteremia

In collaboration with Dr. M. Pocoví *Universidad de Zaragoza*

human plasma bank of hereditary hypercholesteremia

project in progress!

MK and Kiko Verdeguer *Instituto de Biomedicina Valencia*

ApoE deficient mouse model of atherosclerotic disease

Reproducible proteomic alteration of 14 proteins in mouse serum from which interestingly 3 have been shown to be also differentially expressed in mouse aorta.

ACKNOWLEDGEMENTS

Collaborations:

Dr. Javier Chaves — **Dr. Josep Redón**

Hospital Clinico Universitario de Valencia

Dr. Panayotis Fantidis

Hospital Clinico San Carlos, Madrid

Dr. M. Pocoví

Universidad de Zaragoza