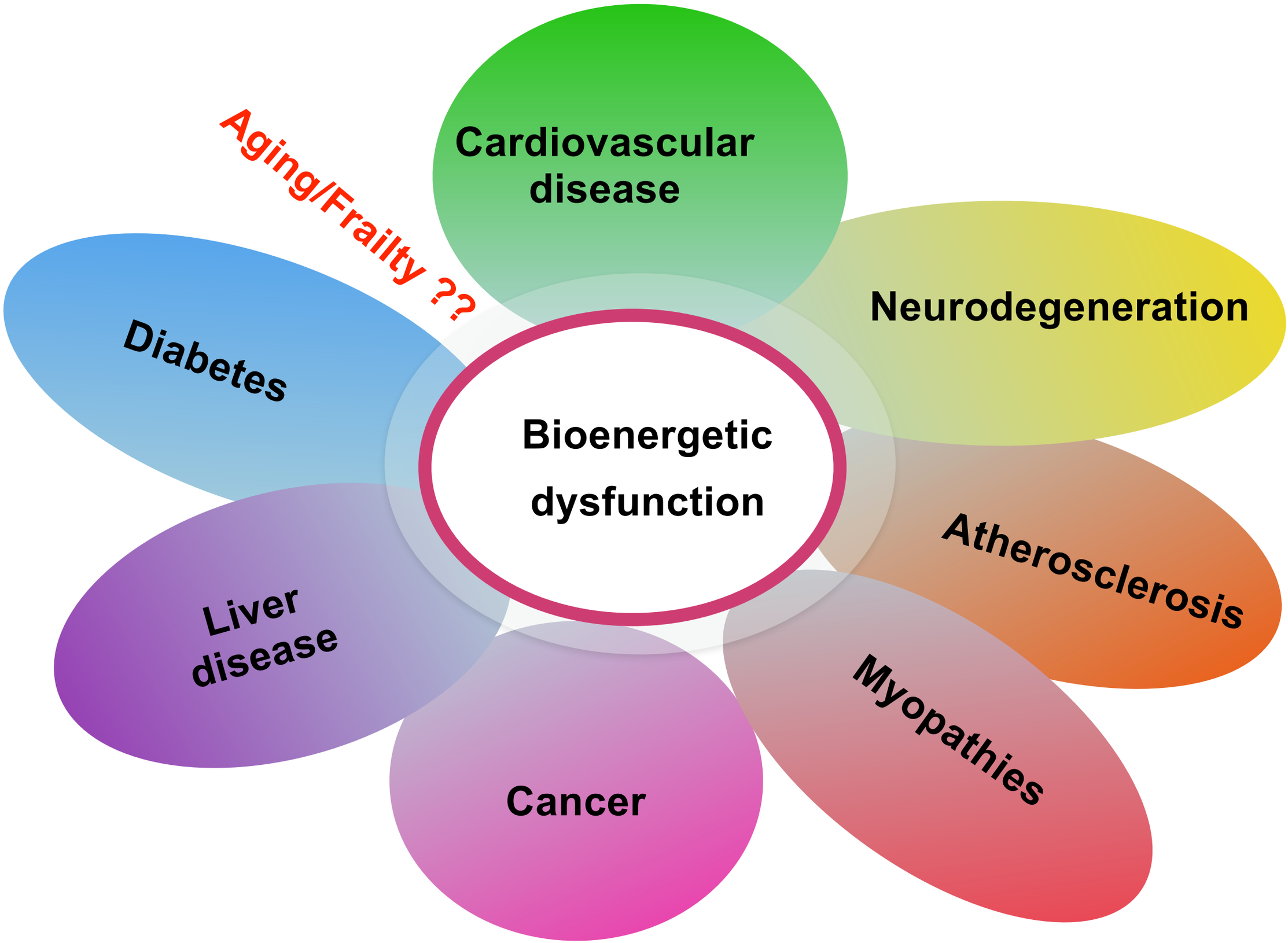
UNIVERSITÀ
di VERONA
Dipartimento
di NEUROSCIENZE,
BIOMEDICINA E MOVIMENTO

Evolution Age Gender Lifestyle Environment
MITO
Mitochondrial fitness mapping - Quality management network

BLOOD CELLS MITOCHONDRIAL ACTIVITY IN AGING AND FRAILTY

Elisa Calabria


Aging/Frailty ??
Cardiovascular disease
Neurodegeneration
Diabetes
Bioenergetic dysfunction
Atherosclerosis
Liver disease
Myopathies
Cancer

Aging is the progressive accumulation of biological and structural modifications occurring with time and leading to impairment of several physiological systems.

Limiting factors in aging:

- **sarcopenia** (loss of muscle mass, strength and regenerative capacity),
- **immunosenescence** (functional deterioration of the immune system, alteration in the profile of immune cells composition, increased inflammatory markers and reduced efficacy of vaccinations)
- **cognitive impairment.**

Measures of physical function are good predictors of morbidity and mortality.

Aging is associated to a progressive decline of physical function with associated health consequences.

The biological mechanisms underlying this decline are not yet understood.

The decline of bioenergetic processes leading to ATP production in skeletal muscle has been associated to reduced physical function and aerobic capacity.

With aging we can become

healthy elderly

frail elderly

Frailty is often associated with

- heart failure,

- chronic obstructive pulmonary diseaseas,

- sarcopenia.

Frailty is associated with functional impairment, hospitalization and with risk of mortality.

A number of conceptualizations of frailty have been proposed:….

….a clinical phenotype of slowed walking speed, low physical activity, unintentional weight loss, low energy, and low grip strength

Nonetheless **there is still a lack of consensus as to how best to assess and diagnose frailty** (Lee et al., 2015).

Mitochondrial dysfunction
(↑ ox. stress ↓ capacity of energy production)

→ human ageing

↓ secondary effects

↓ Juvenility.

Physical performance

Aging

and Frailty Index

SPPB — ? — ? Frailty ?

IRAP

?

Mitoch funchon muscle?

blood ??

Mitochondrial function

The aim:

Identify patterns of mitochondrial function/dys-function in blood cells specifically related to frailty.

Hypothesis: blood cells mitochondrial properties differ in healthy elderly relative to frail elderly people.

The **aim** to have identity a tool useful to predict higher probability of frailty and allowing interventions on the lifestyle to prevent or attenuate the decline of physical functions.

RELEVANCE OF BLOOD CELLS IN THE STUDY OF THE MITOCHONDRIAL FUNCTION

The main source of biological samples to identify mitochondrial dysfunction

are often mitochondria rich tissues —-> muscle biopsy

Biopsies implies a number of ethical issues that make them a difficult starting point for research, that is interested not only in diagnosis of the dysfunction, but also in the physiology of the healthy mitochondria, or to unveil the effects of drugs and nutrients on the core of cell metabolism.

Recent studies showed that its possible to measure of mitochondrial function in human blood cells

…and that temporary cryopreservation of blood cells allows mitochondrial measures (Karabatsiakis 2014 Transl Psychiatry)

Genes differentially expressed between adults healthy elderl

570 genes
differentially
expressed

229 genes
up-regulated in
Adults (40-50yrs)

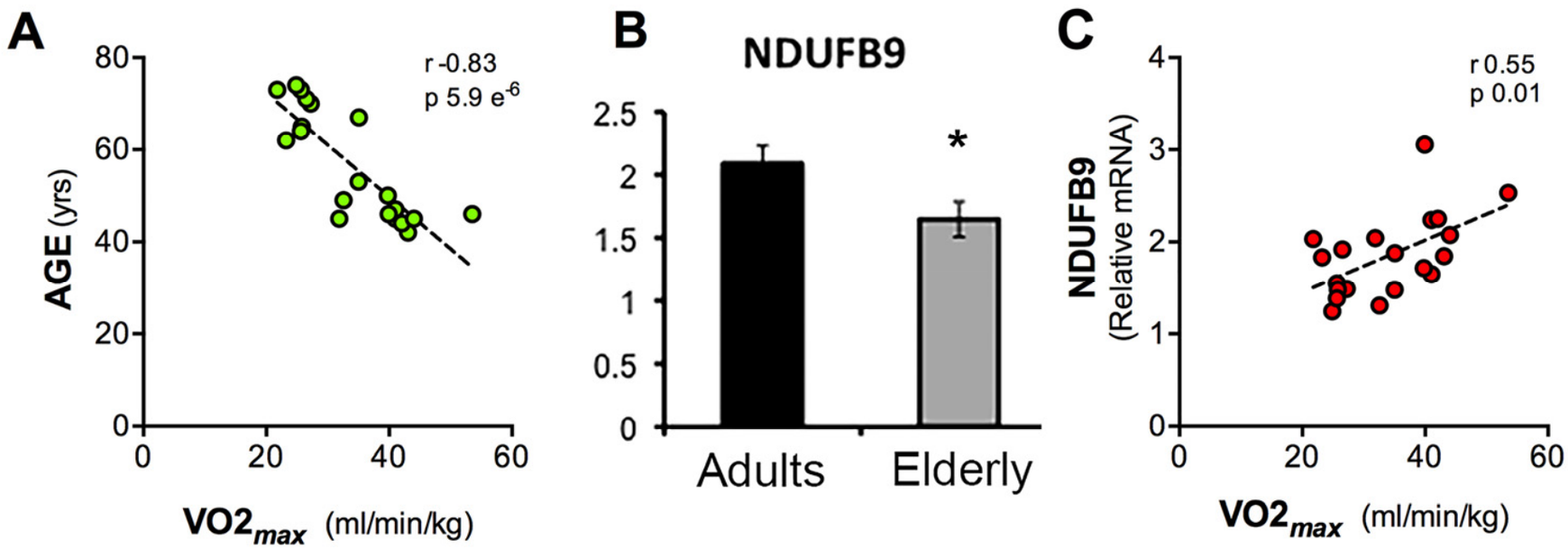
mitochondrial genes

341 genes
up-regulated in
Elderly (70yrs)

Anova p<0.05

Calabria et al., 2016

COMPLEX I TRANSCRIPTS LEVELS DIRECTLY ASSOCIATED WITH RESPIRATORY CAPACITY



Calabria et al., 2016

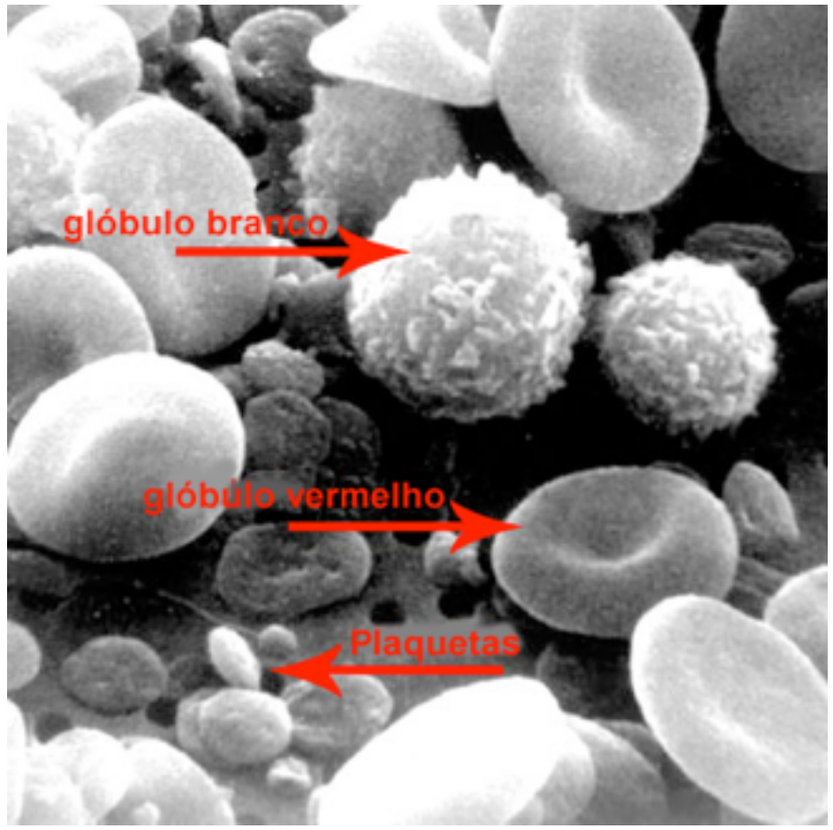
BLOOD

- Since ancient times blood exerted on men an ambiguous fascination.
- It was considered positively as the basis of life processes, was consecrated in sacrificial offerings to the gods, was drunk from the wounded body of the enemies to acquire the strength but at the same time caused and causes still a strong disturbance and an innate repulsion.
- From a medical point of view the blood is an wonderful source of information;
- through **its analysis information are obtained about the health state of the individuals, inflammatory processes and disease**
- but only in the early 1900s blood groups have been distinguished and Rh factor was only discovered in 1941.

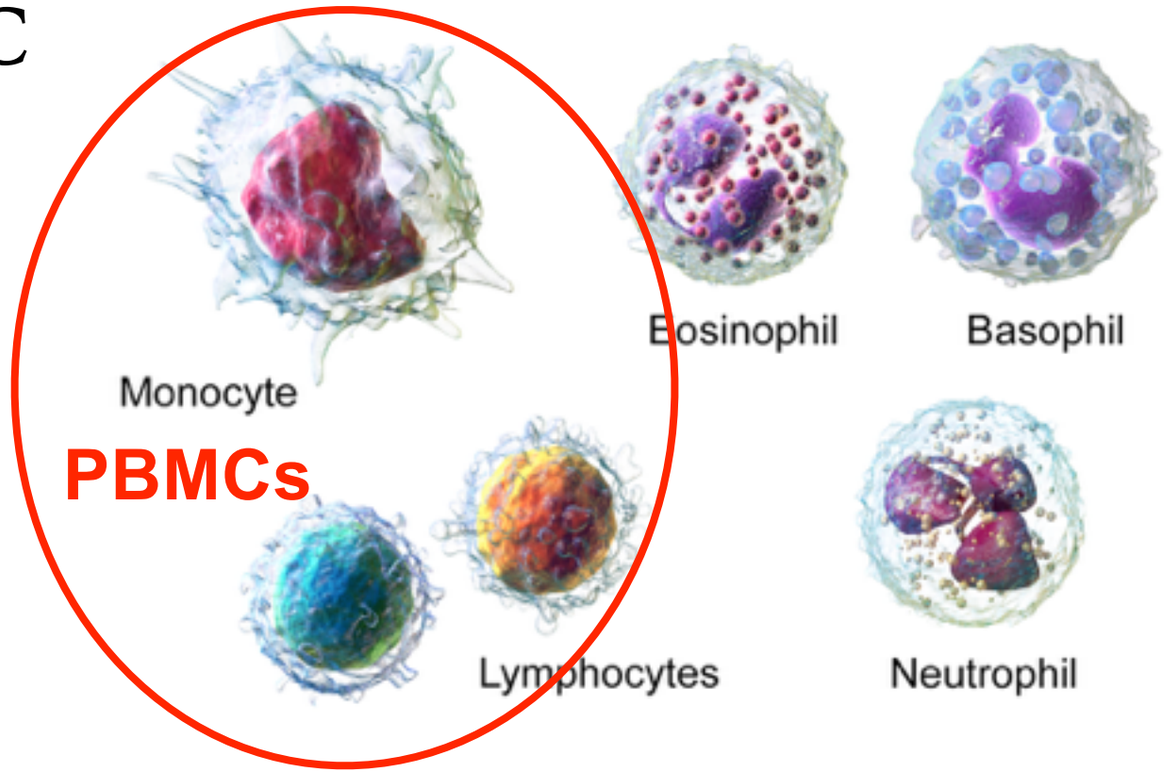
BLOOD IS A SOURCE OF INFORMATION

We can consider blood as a fluid tissue, in which several cell types are present.

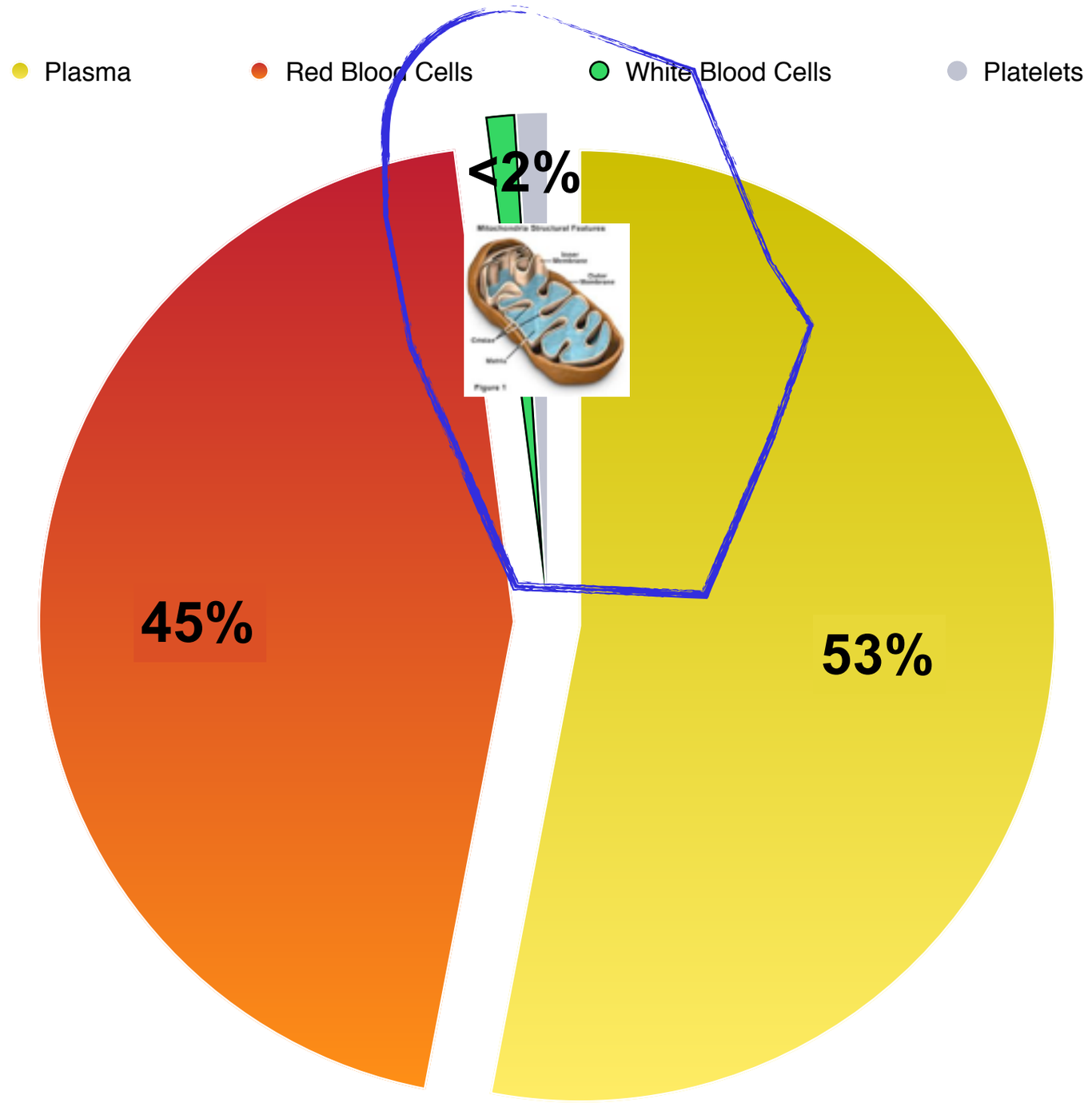
- RBC no nucleus, no mitochondria



- WBC

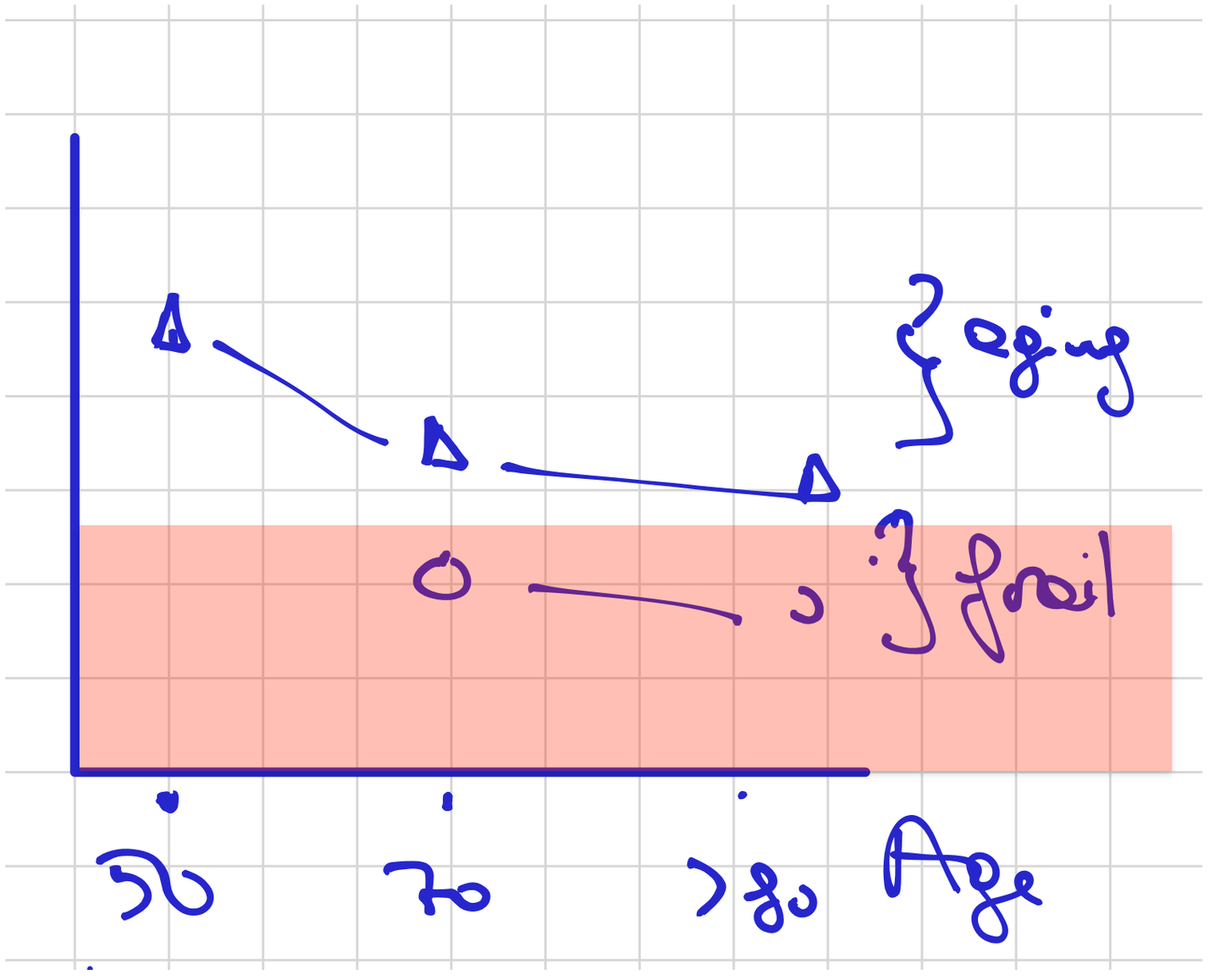


THE BLOOD TISSUE



EXPERIMENTAL SCHEME

We are recruing 80 subjects of both genders



For each subject we are evaluating:

- ***ISAR***: Identification of Seniors Risk (McCusker),

- ***SPPB***: Short Physical Performance Battery (Guralnik) to evaluate mobility/functionality.

- ***cardiorespiratory function***: oxidative capacity (VO2max),

- ***strenght evalutation tests***: Maximum handgrip strength,

- ***body composition***: dual-energy X-ray absorptiometry (DEXA) scan.

- ***cognitive function***: Mini mental Test, M-GOC.

- ***blood samples:*** * hematological analysis,

- * mitochondrial bioenergetics (O2+H2O2)

STRATEGY

COLLECTION, PROCESSING AND STORAGE OF PERIPHERAL BLOOD MONONUCLEAR CELL (PBMC) SAMPLES

- SUBJECT/PATIENT DATA

- BLOOD COLLECTION

COLLECTION OF PERIPHERAL BLOOD MONONUCLEAR CELLS **USING** LEUCOSEP TUBES

COLLECTION OF PERIPHERAL BLOOD MONONUCLEAR CELLS **WITHOUT USING** LEUCOSEP TUBES

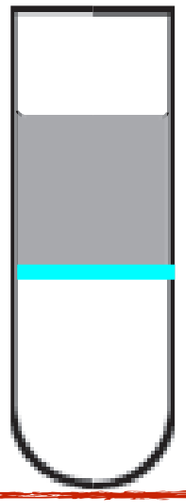
COLLECTION OF PERIPHERAL BLOOD MONONUCLEAR CELLS

HRR ANALYSIS

TEMPORARY CRYOPRESERVATION

* PROTOCOLS

Red Biöbancos
Instituto de Salud Carlos III

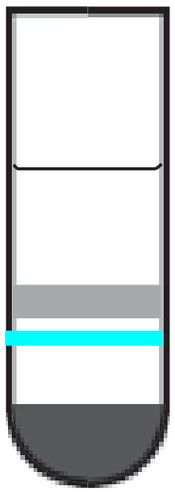


Leucosep tubes

Blood sample

Ficoll-Paque PLUS

centrifuge at 800g for
10 min —> PBMCs



Plasma

Lymphocytes Monocytes Platelets

Ficoll-Paque PLUS

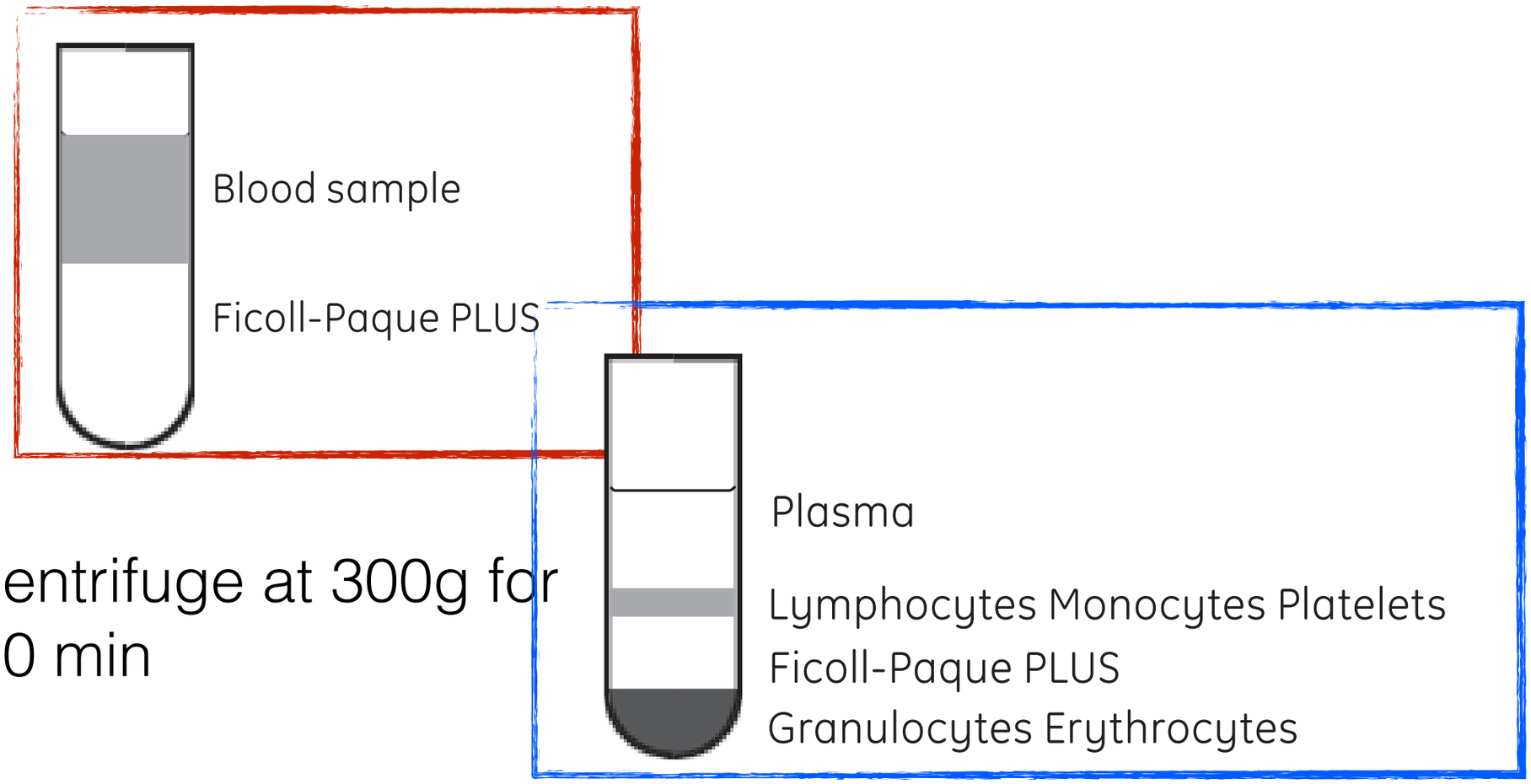
Granulocytes Erythrocytes

2X dilute in RPMI
and centrifuge at 80g
for 10 min

resuspend the
pellet

HRR

cryopreservation



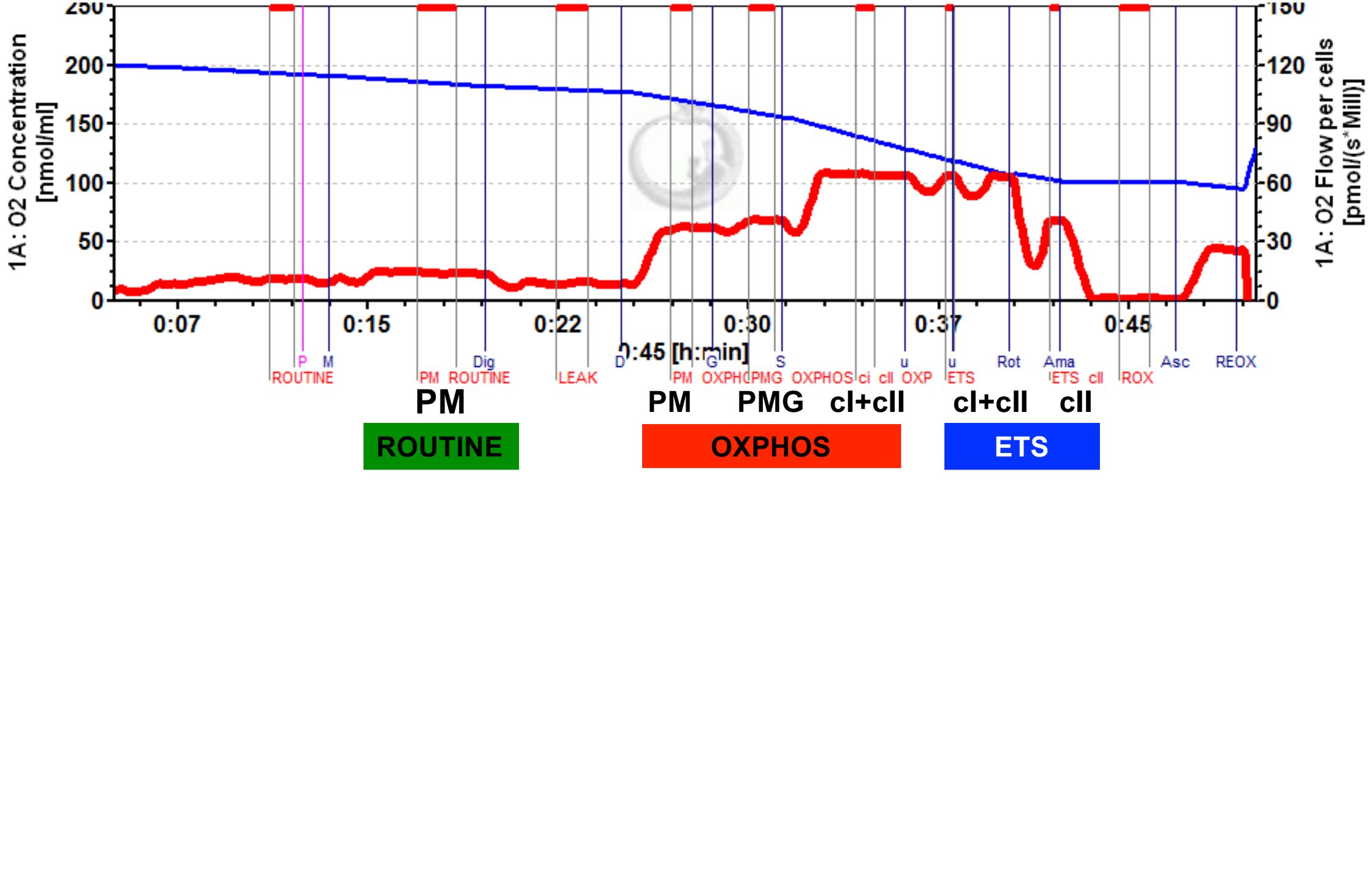
centrifuge at 300g for 30 min

transfer lymphocyte payer to a new tube,
add 3 volumes of HBBS,
2X centrifuge 10 min at 100g

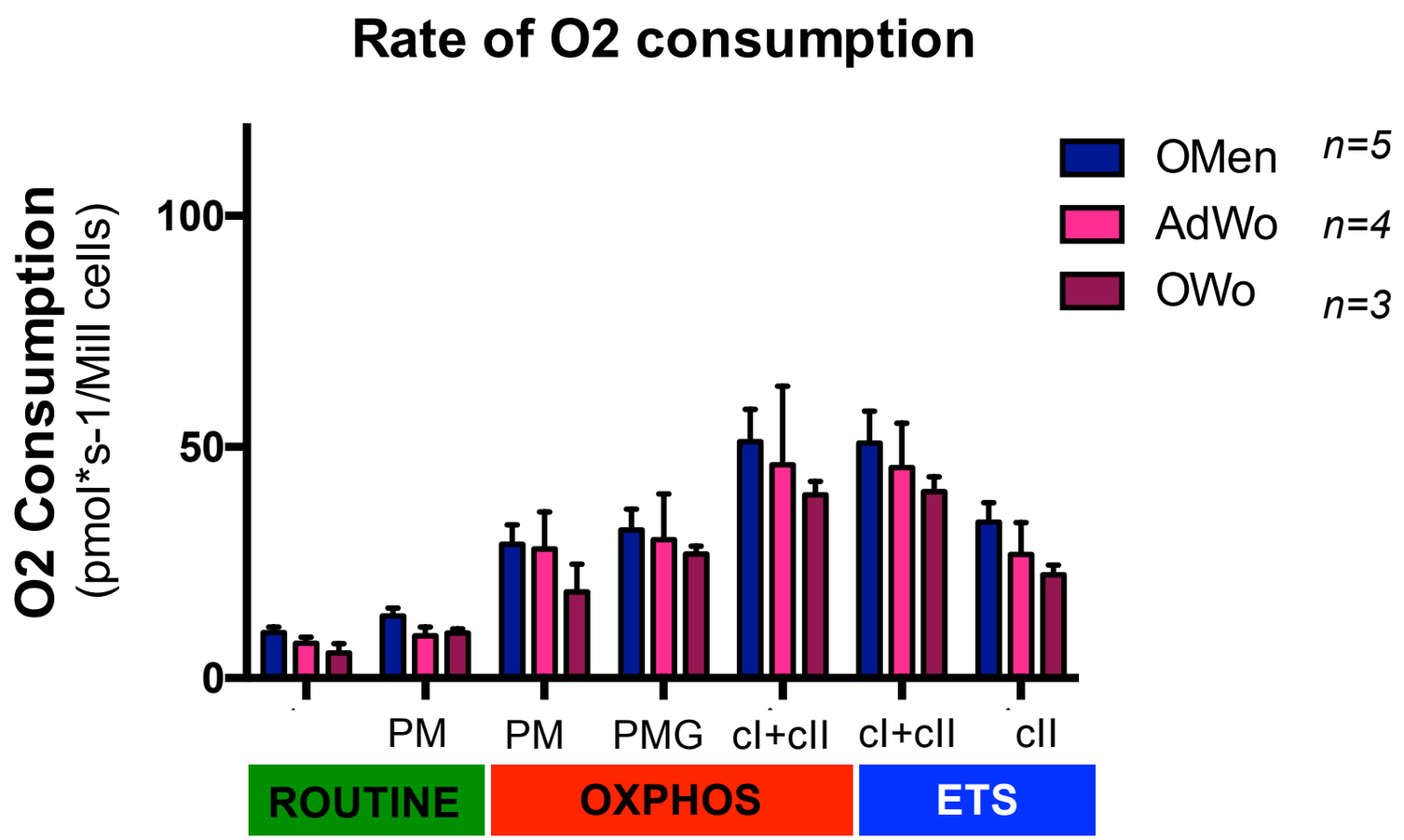
WE ARE IN THE VERY BEGINNING

Group	n	age
OM	*n=5*	*68*
AdW	*n=4*	*40*
OW	*n=3*	*79*

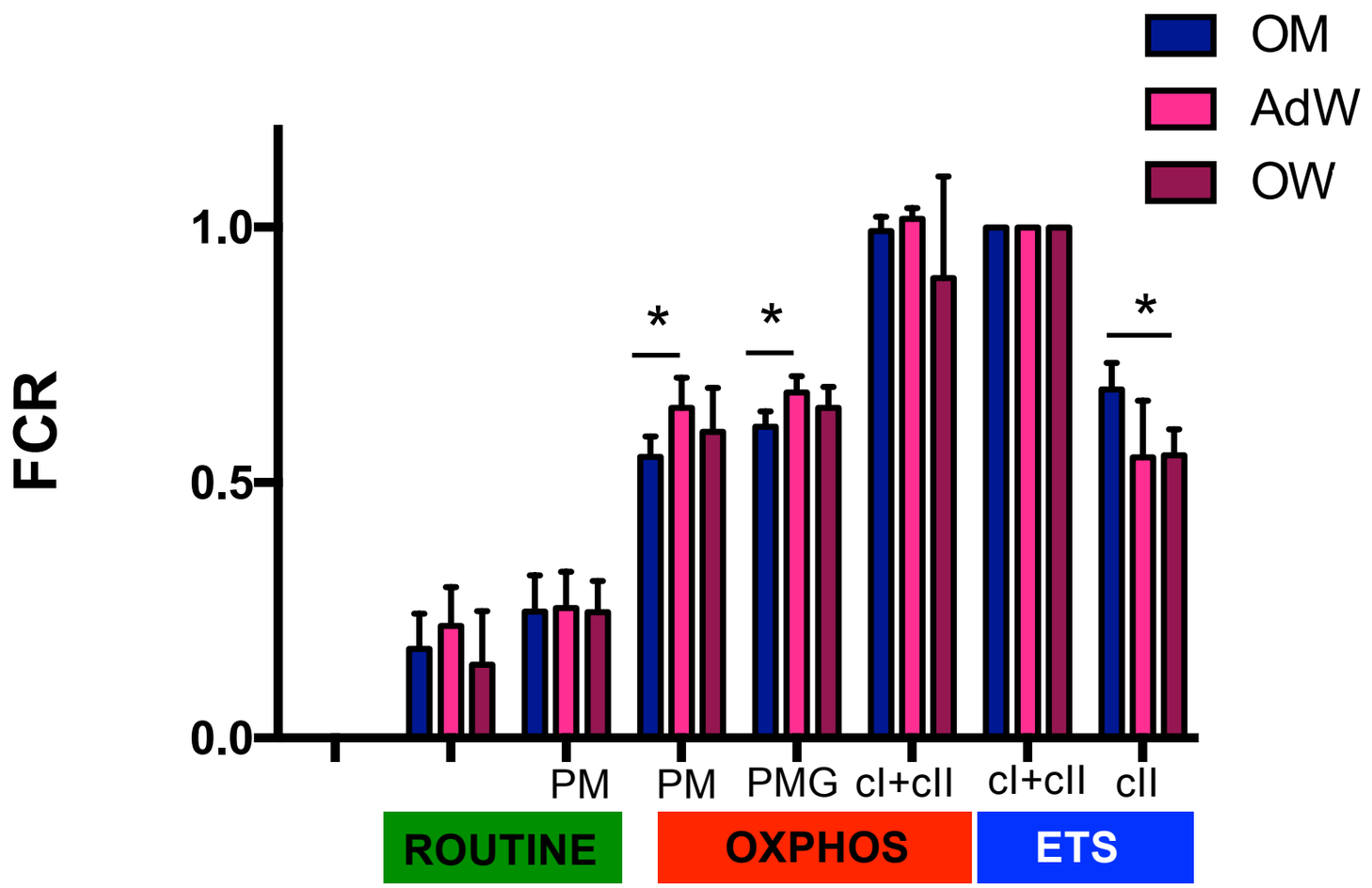
RESPIRATORY CAPACITY OF BLOOD CELLS IN AGING



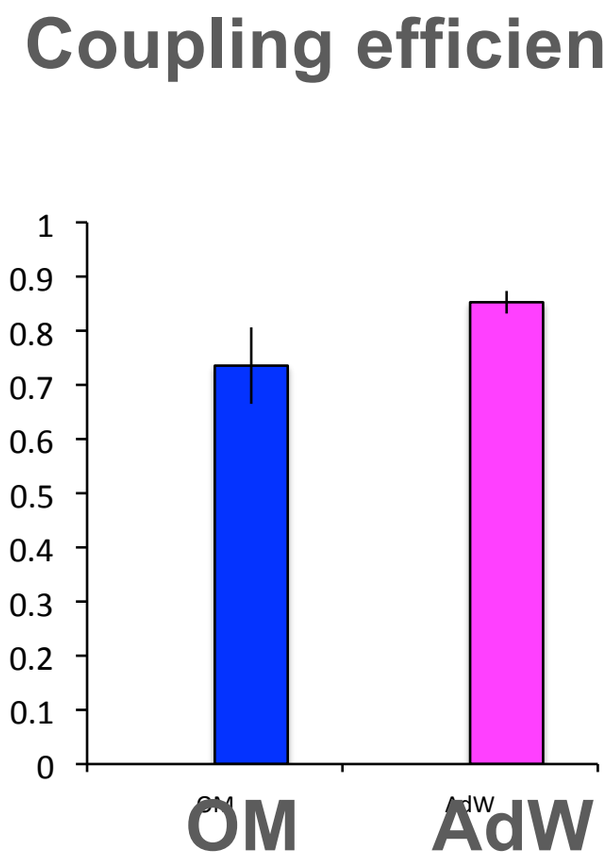
RESPIRATORY CAPACITY OF BLOOD CELLS IN AGING



RESPIRATORY CAPACITY OF BLOOD CELLS IN AGING



CORRELATION BETWEEN AGE AND HEART RATE, AND COUPLING EFFICIENCY



HR <—> Age *r=-0.877 p 0.004*

Age <—> 1-(L/E) *r=-0.740 p 0.036*

VO2max <—> 1-(L/E) *r=-0.720 p 0.044*

Weight <—> 1-(L/E) *r=-0.777 p 0.023*

BMI <—> 1-(L/E) *r=-0.793 p 0.019*

CONCLUDING REMARKS

We do not have conclusions, since we're at the beginning …….

- the data collected at the moment suggest that healthy aging seems to affect at the same time whole body physiological parameters and intracellular mechanisms

- these data also confirm that beside a change at the molecular level blood cells mitochondria undergo also a decline in function

- It's important to properly shape results since the early steps to favor data sharing and communication

BLOOD CELLS AND METABOLISM

At the present time no clinical test is available to assess “energetic health".

Through circulation leucocytes and platelets can act as sentinels of mitochondrial function and early biomarkers of metabolic stress/ dysfunction in other tissues.

Integrated approach in cells isolated from human blood to establish a quantitative assay of mitochondrial function that will have the power to **predict disease progression** and **response to treatment**

A quantitative assay of mitochondrial function in blood cells will have the power to **predict health/frailty, disease progression** and **response to treatment** or **intervention**

Elisa Calabria
Federico Schena
Carlo Capelli
Massimo Venturelli
Cantor Tarperi
Chiara Milanese
Giuseppe Lippi
Gianluca Salvagno

Erich Gnaiger

PBMCs freezing for sample storage and sharing

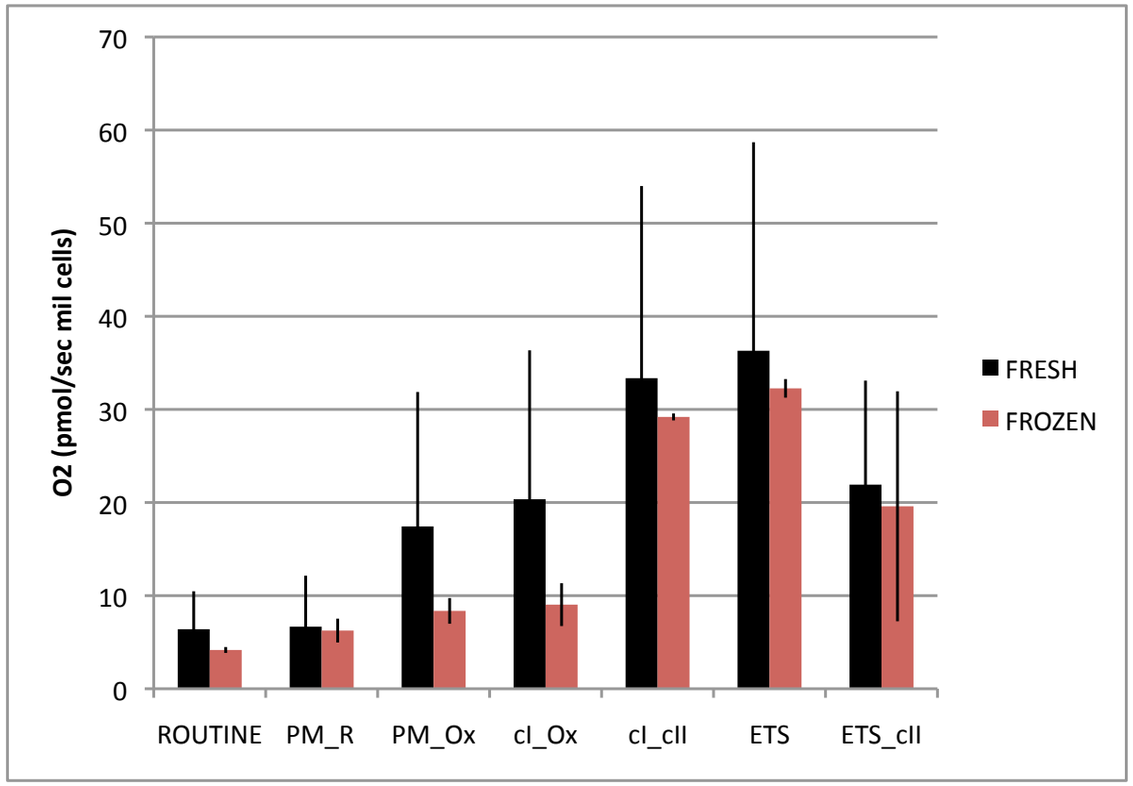
Cryopreservation is the most used method to preserve structure and function of biological samples.

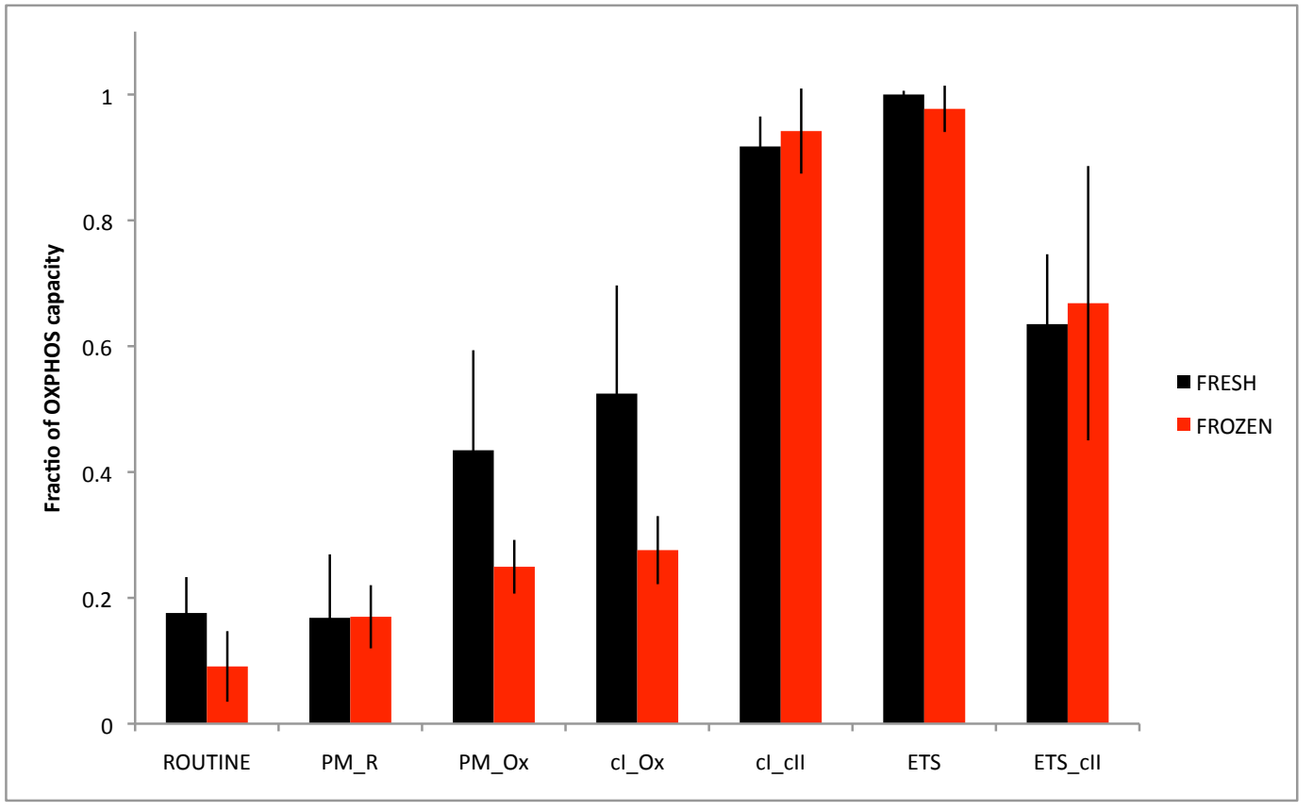
Unfortunately, after a freeze–thaw cycle **cell** survival and mitochondrial function can be limited.

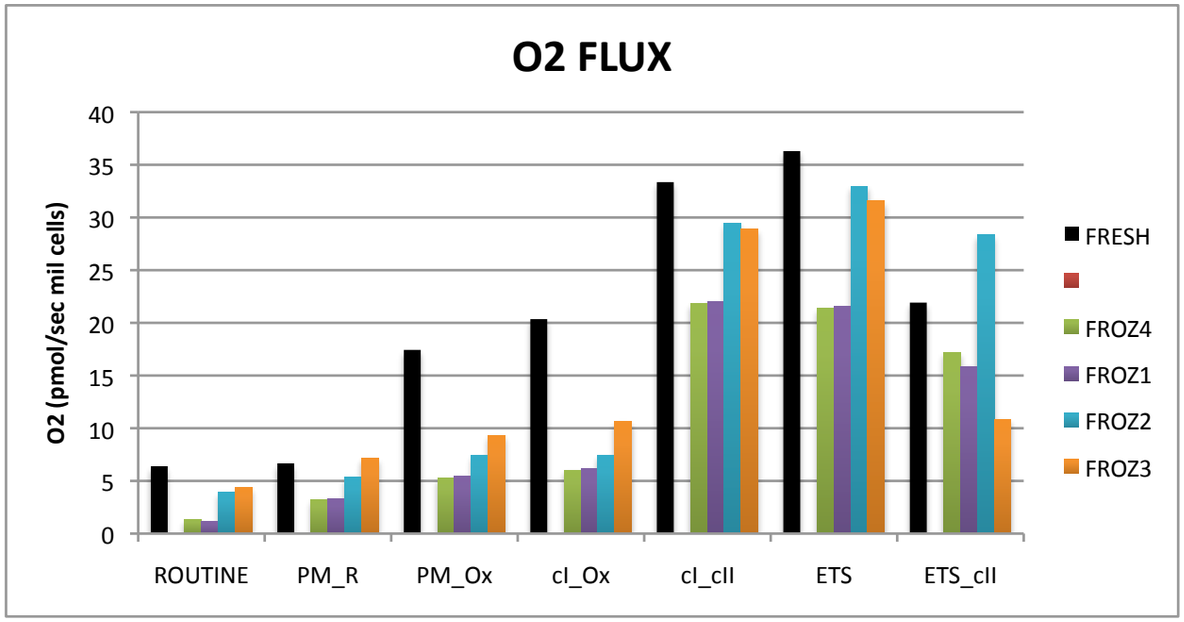
Injury events: ice formation —> integrity of membranes —> dehydration.

Optimal cooling/heating rates for each cell type.

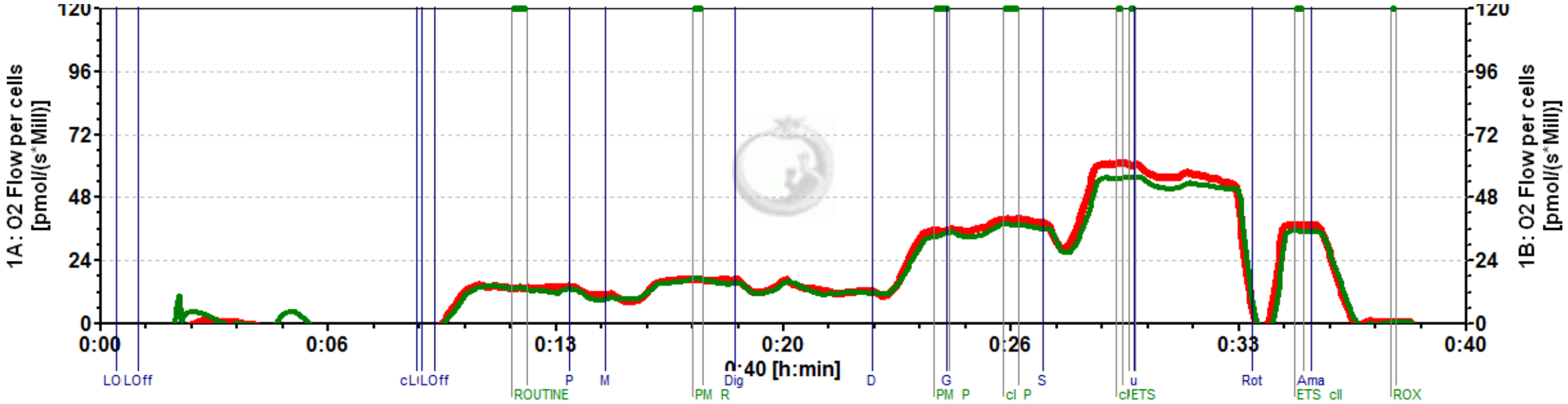
Cryo-Preserving Agents —> CryoSure (DMSO+Dextran)


O2 (pmol/sec mil cells)
70
60
50
40
30
20
10
0
ROUTINE
PM_R
PM_Ox
cI_Ox
cI_cII
ETS
ETS_cII
FRESH
FROZEN

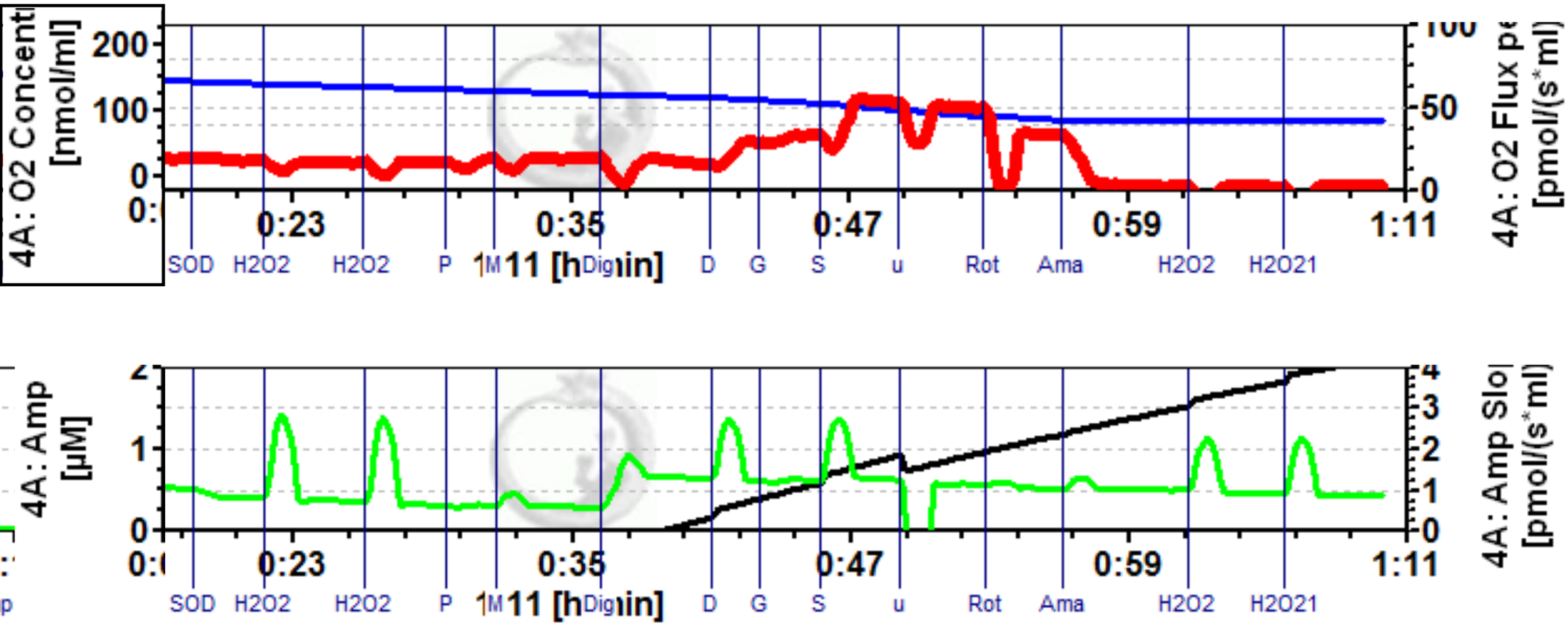

Fractio of OXPHOS capacity
1
0.8
0.6
0.4
0.2
0
ROUTINE
PM_R
PM_Ox
cI_Ox
cI_cII
ETS
ETS_cII
FRESH
FROZEN


O2 FLUX
O2 (pmol/sec mil cells)
40
35
30
25
20
15
10
5
0
ROUTINE
PM_R
PM_Ox
cI_Ox
cI_cII
ETS
ETS_cII
FRESH
FROZ4
FROZ1
FROZ2
FROZ3

PBMCs


1A: O2 Flow per cells [pmol/(s*Mill)]
1B: O2 Flow per cells [pmol/(s*Mill)]
120
96
72
48
24
0
0:00
0:06
0:13
0:20
0:26
0:33
0:40
0:40 [h:min]
LO
LOff
cLi
LOff
ROUTINE
P
M
PM
R
Dig
D
G
PM
P
cl
P
S
cl
ETS
u
Rot
Ama
ETS
cll
ROX

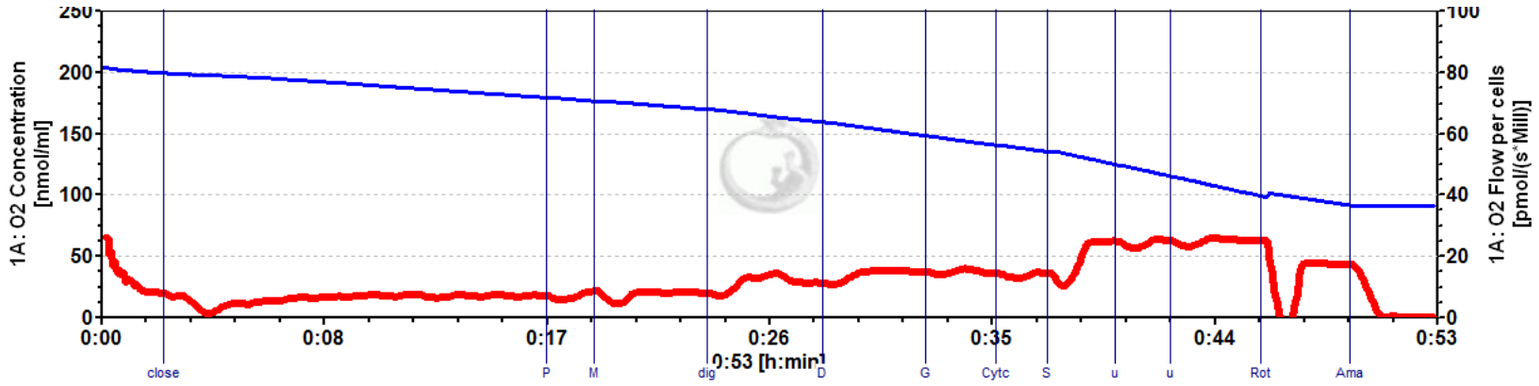
Parallel evaluation of cellular oxidative capacity and ROS (H2O2) production,



Oxygen flux

H2O2 production -ROS

Fresh PBMCs



Frozen PBMCs