UNIVERSITÀ di VERONA
Dipartimento di NEUROSCIENZE, BIOMEDICINA E MOVIMENTO

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

WG4

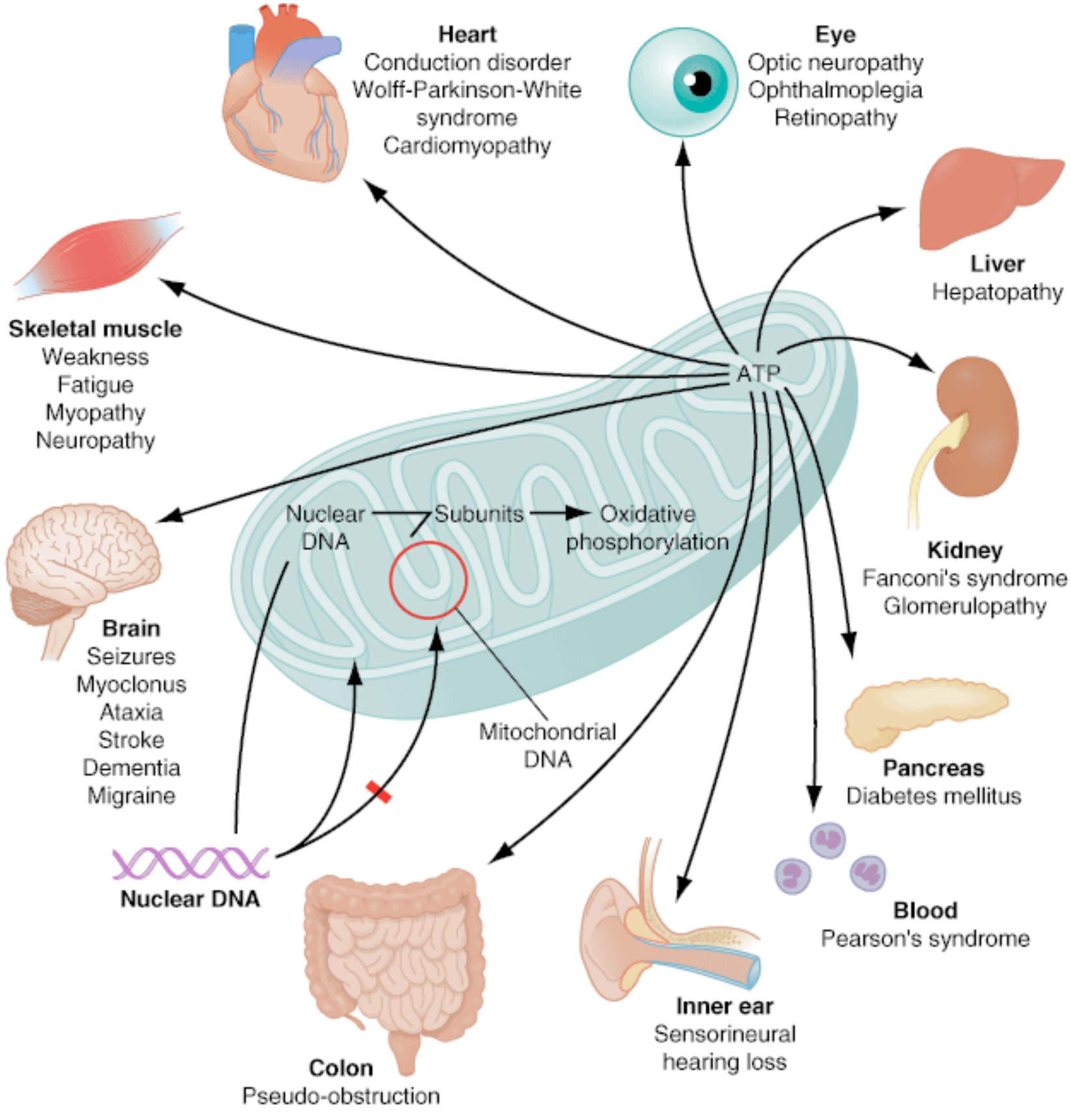
MITOEAGLE DATA REPOSITORY FOR BLOOD CELLS AND CULTURED CELLS

THERE IS A HUGE VARIETY OF CELL TYPES

WG4

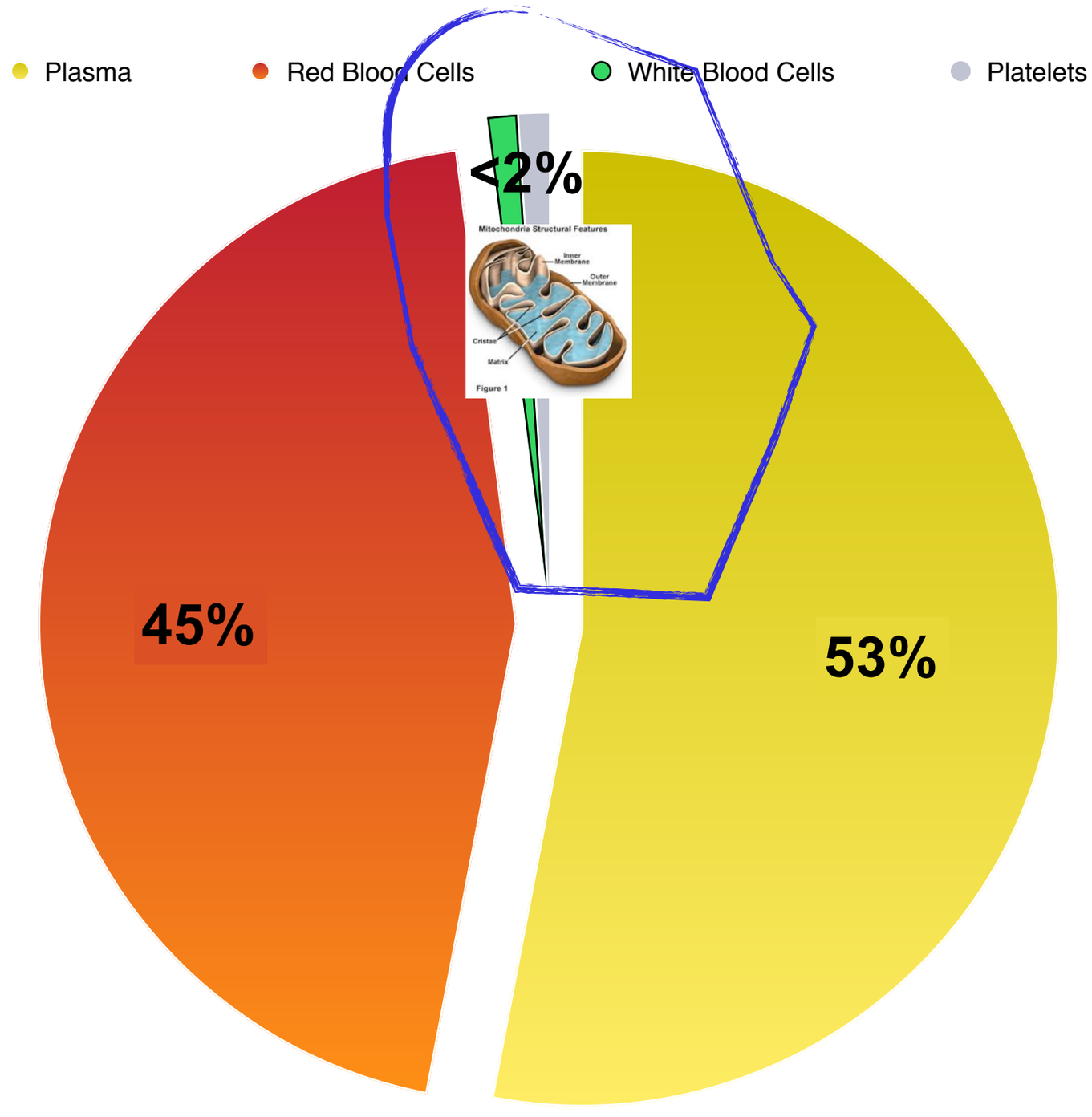
PBMCs

Platelets



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

BLOOD FRACTIONS



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)

RELEVANCE OF BLOOD CELLS IN THE STUDY OF THE MITOCHONDRIAL FUNCTION

Measuring mitochondrial function in blood cells is attractive and less invasive than a biopsy alternative for mitochondrial diagnostics, although blood sampling, storage and sharing still has some ethical aspects.

MANAGEMENT TASKS

* Kick-off meeting to develop a working plan for the establishment of consensus protocols, reporting schemes, and the assignment of specific study tasks.
* Development of SOPs for cell separation.
* Development of laboratory protocols for individual blood cell types (and other cell types) for mitochondrial studies on intact and permeabilized cells.
* Application of SOPs in experimental studies during Short Term Scientific Missions and feeding of data into the MITOEAGLE data repository.
* Publication of SOPs and study results.

MILESTONES

Consensus on protocols, reporting schemes and work assignments.

Completed SOPs for cell preparation & laboratory protocols.

Application study finished and data transmitted to MITOEAGLE data repository.

Publication finished.

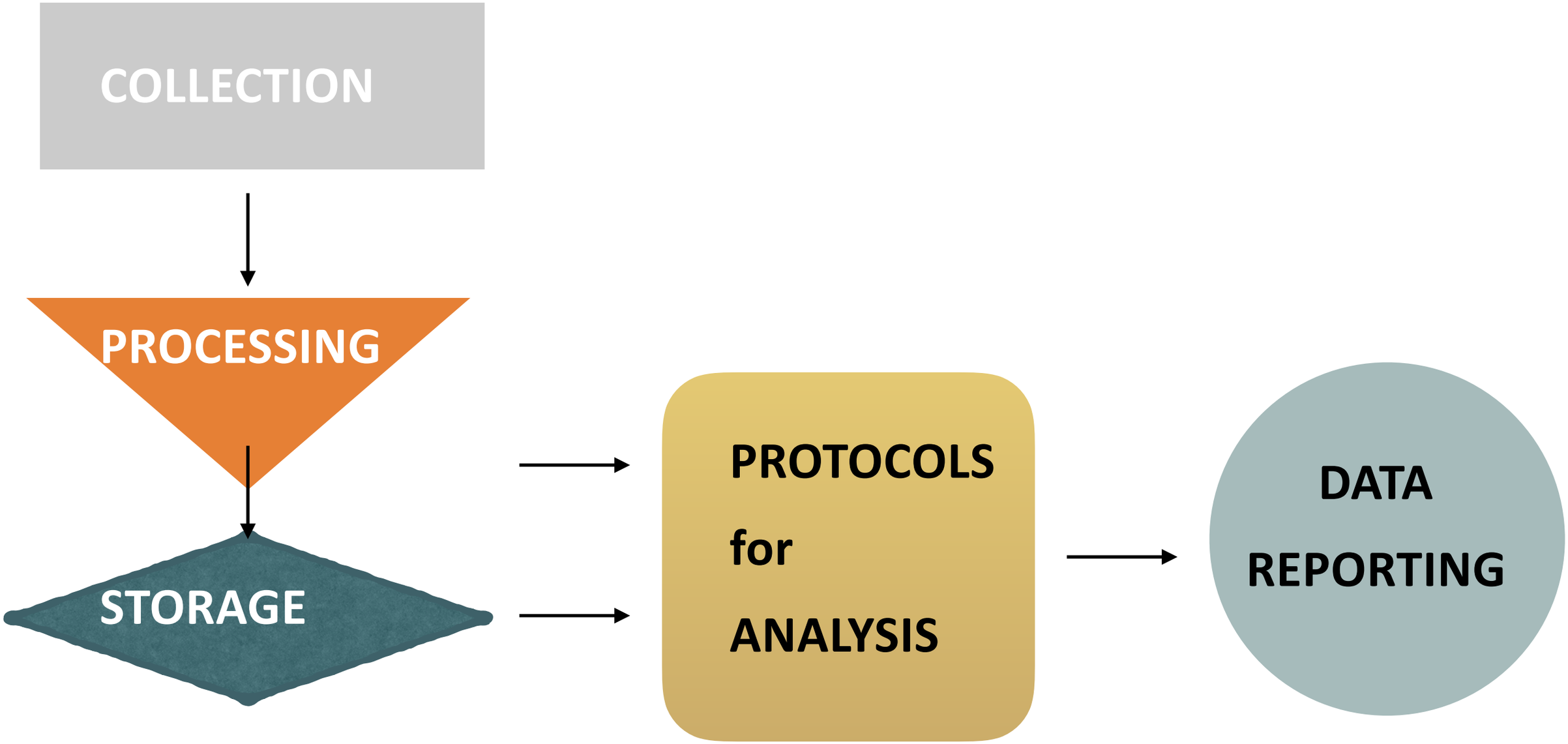
DELIVERABLES

SOPs for blood cell separation and respirometric characterisation open for the research community

MITOEAGLE data repository for comparative data evaluation, planning of future studies, data mining.

Publication with a set of reference data.

BLOOD TASKS


COLLECTION
PROCESSING
STORAGE
PROTOCOLS
for
ANALYSIS
DATA
REPORTING

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

STRATEGY

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

Subject/Patient data	informed consent for donating samples to the University/MITOEAGLE.		
Blood collection	tubes with anticoagulants are properly identified		
Date and time of withdrawal.			
Type of anticoagulant.			
Incidents not related to the protocol.			

STRATEGY

COLLECTION OF PERIPHERAL BLOOD MONONUCLEAR CELLS <u>USING LEUCOSEP TUBES</u>

LEUCOSEP TUBES PREPARATION	Add 15 ml of Ficoll to each labeled Leucosep tube (empty tubes 50 ml)	Centrifuge the Leucosep tube containing Ficoll at 1000xg for 1 min. Remove excess Ficoll by decanting or pipetting.	
PBMCs SEPARATION	Pool the contents of all blood collection tubes into one 50ml Falcon tube to homogenize the blood. Dilute 1:1 with a balanced salt solution (RPMI/PBS)	Add the diluted blood to the Leucosep tube.	Centrifuge the tubes at 800xg for 10 min. at 22ºC without using the brake

STRATEGY

COLLECTION OF PERIPHERAL BLOOD MONONUCLEAR CELLS <u>WITHOUT USING LEUCOSEP TUBES</u>

FALCON TUBES PREPARATION	Add 12,5 ml of Ficoll (Ficoll: Blood 1:2) to each labeled Falcon tube (empty tubes 50 ml)		
PBMCs SEPARATION	Pool the contents of all blood collection tubes into one 50 ml Falcon tube to homogenize the blood. Dilute 1:1 with a balanced salt solution (RPMI/PBS)	Slowly transfer the diluted blood in the Ficoll tube without perturbation the gradient	Centrifuge the tubes with blood at 300xg for 30 min. at 22ºC without using the brake

STRATEGY

COLLECTION OF PERIPHERAL BLOOD MONONUCLEAR CELLS

Remove the white layer of PBMCs and transfer it to a sterile tube. Make up the volume with PBS/RPMI.	Centrifuge the tube at 80xg for 15 min. at 22ºC	Remove the supernatants of the tubes by decanting, taking care not to break the cell pellet. Resuspend the obtained pellet in PBS/ RPMI.	Make up the volume with PBS/RPMI. Centrifuge the tube at 80xg for 15 min. at 22ºC. Remove the supernatants of the tubes by decanting, taking care not to break the cell pellet.
	Resuspend the obtained pellet in the final volume of PBS/RPMI/Mir05 For freezing (RPMI/FBS and CryoSURE)	Remove a 10-25 µl sample and count cells	Transfer in the O2K chamber 2,25 ml of cells (at least 1*10^6 cells/ml)
	Make the appropriate dilution to achieve a desired concentration:		

STRATEGY

TEMPORARY CRYOPRESERVATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS

CRYOPRESERVATION	Resuspend the cells in 0,5 ml of RPMI/FBS. Transfer in a cryovial and put on ice. Add drop by drop 0.5ml of pre-chilled CryoSURE solution. For freezing 5-15 x 10^6cells/ml. .	Freeze at -80 with a Freezing Container.	

STRATEGY

MAINTAINING TRACEABILITY AND DATA ASSOCIATED TO A SAMPLE:

Biobanks advise to gather the maximum amount of information possible concerning the sample, both at the time of receipt and after processing and storage, and depending on the studies for which they will be used, for example:			
Date and time of receipt and/or processing			
Degree of hemolysis			
Volume of blood received			
Degree of lipemia			
Degree of coagulation			
Incidents during processing			

STRATEGY

REFERENCE DOCUMENTATION

- *Standard ISO 9001:2008. Quality management systems. Requirements.*

- *Standard ISO 6710 which establishes the color code for tubes according the anticoagulant used.*

RELATED DOCUMENTATION

Karabtasiakis et al., Zumbalova et al. (??)

STRATEGY – ANALYSIS

We need one or few protocols, to detect the major key points in the cell metabolism.

We need a consensus on the principal data or “biomarkers” that should be reported in a a “standard form” to accompaign samples and experiments, also in view of a shared database.

STRATEGY – ANALYSIS

Could we consider a **basic protocol** in intact cells as a first step to define the **reference bioenergetic signature** for a cell line?

Furthermore we need **standard protocols** to **compare** different conditions: control/treated, WT/mt, ….

CULTURED CELLS

Cultured cells —-> cells derived from animal/plant cells, and grown under controlled conditions, outside of their natural environment

Primary cells

freshly isolated from tissue for ex-vivo culture

Cell lines

a population of cells descended from a single cell and containing the same genetic background

Bioenergetic signature

PRIMARY CELLS

IDENTIFICATION → **SOURCE**

- **Species and Gender**
- **Tissue of origin**

FORMAT

- **Proliferating**
- **Differentiated**
- **Cryopreserved**

TIME

Cross-contamination: primary cells can be contaminated by other cells present in the tissue of origin

CELL LINES

IDENTIFICATION → **SOURCE**

(Name)

- **Species and Gender**
- **Tissue of origin**

PROPERTIES

FORMAT

- **Proliferating**
- **Differentiated**
- **Cryopreserved**

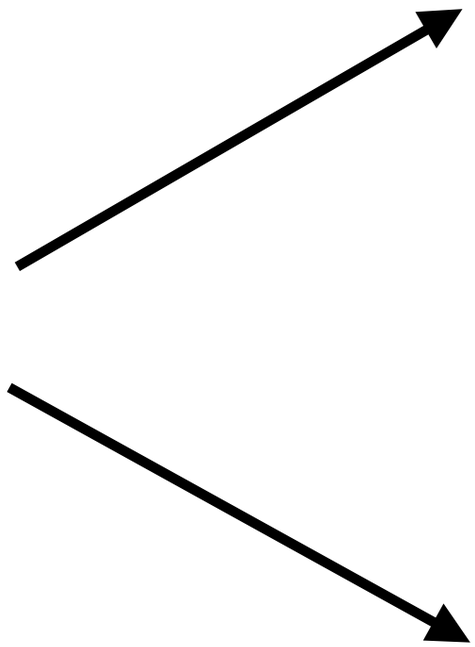
TIME - Passage

Quality control

Cross-contamination: 15-20% of the time cell lines can be contaminated by other cells

BIOENERGETIC SIGNATURE

For most cells cultures there is no **reference bioenergetic signature**



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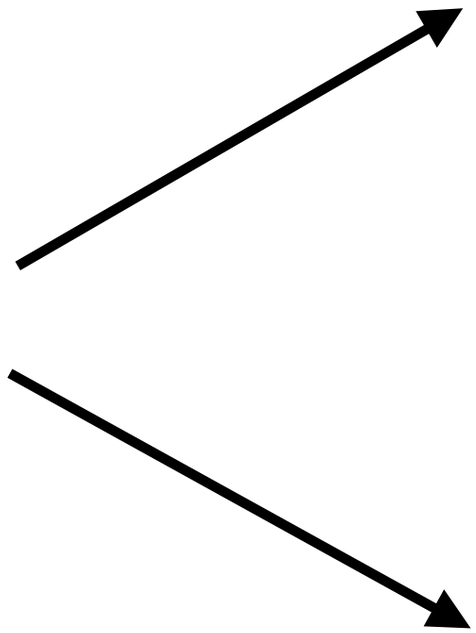
STRATEGY – DATA REPORTS



DATA SHARING between research of the same died

COMMUNICATION of results to the scientific community

STATE OF THE ART??

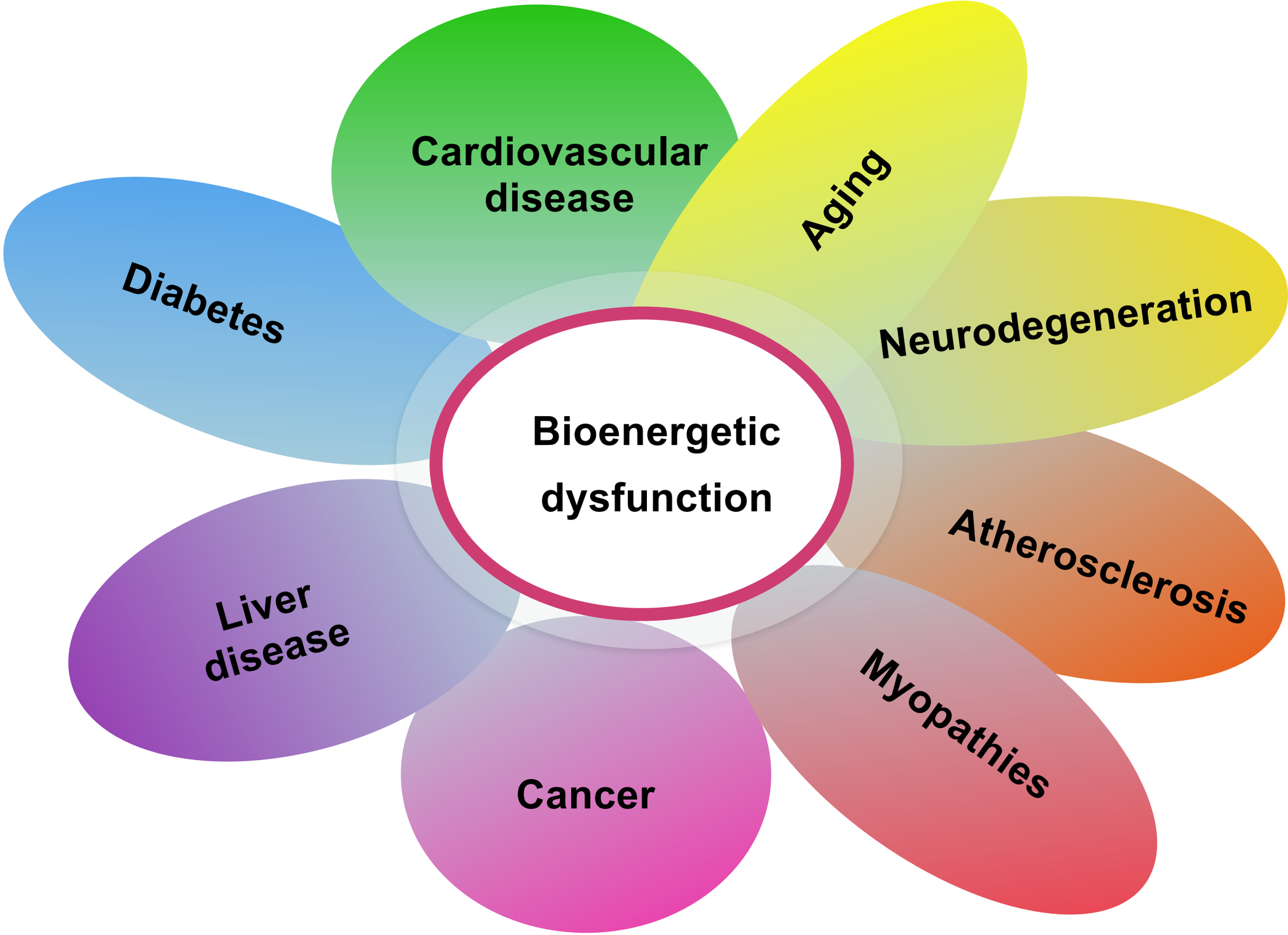
Consensus on cell preparation procedures

Update on respiromety protocols

How should we report our data?? —> nomenclature

Make the point for a publication

Thank you


Cardiovascular disease
Aging
Diabetes
Neurodegeneration
Bioenergetic dysfunction
Atherosclerosis
Liver disease
Cancer
Myopathies

BLOOD CELLS AND METABOLISM

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 ATD production in skeletal muscle has been associated to reduced physical function and aerobic capacity.

Recent studies proposed the use of blood cells to assess systemic mitochondrial health.