Sampling, transport, storage and characterization of whole blood / plasma

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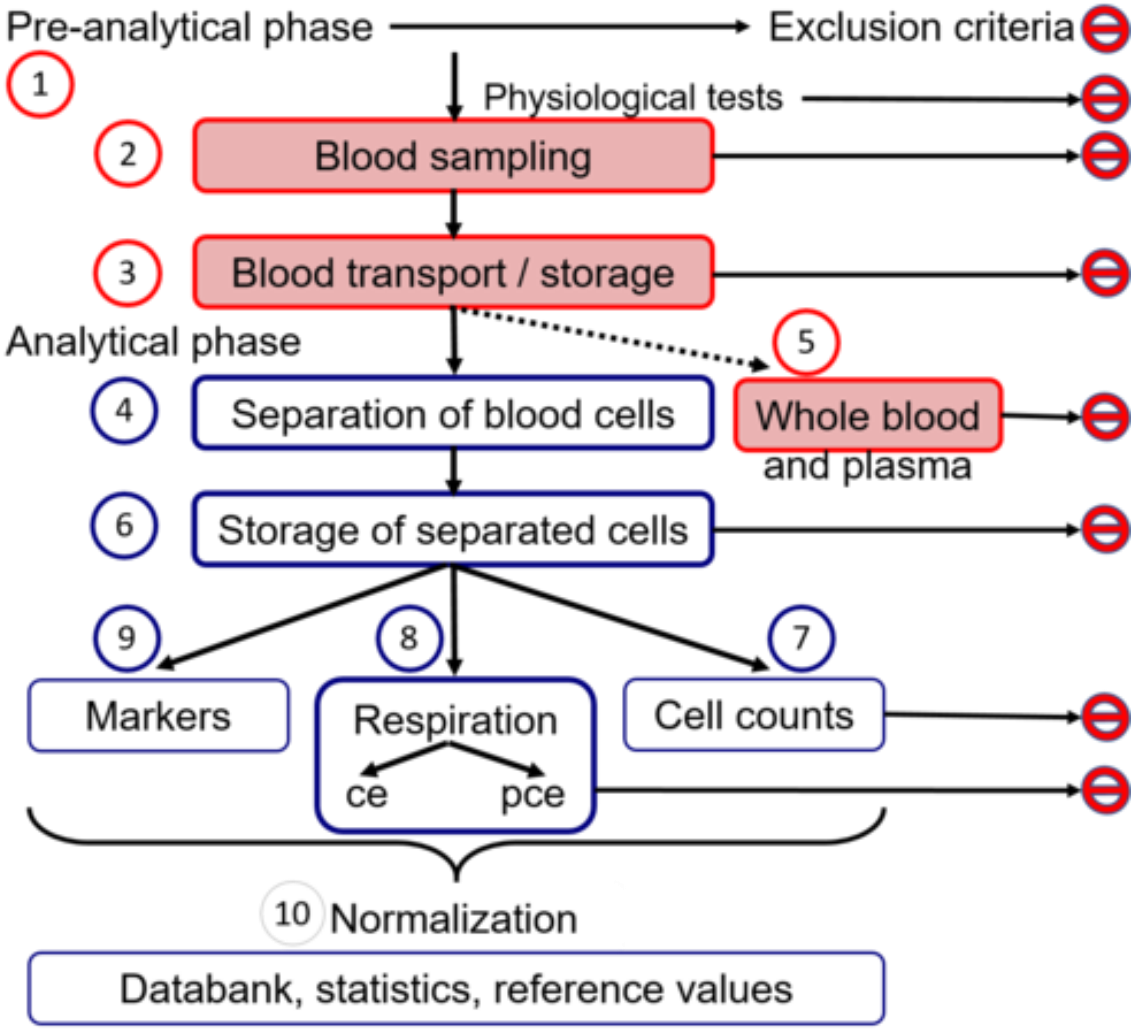


Fig. 1. Workflow and topics - Innsbruck – Poznan manuscript
http://www.mitoeagle.org/index.php/MitoEAGLE_Poznan_2018

Pre-analytical phase

A. Exclusion criteria	**- smoking** **- alcohol intake** **- BMI** **- lifestyle intervention** **- consider medication**
B. Matching factors	**- sex** **- age** **- lifestyle** **- ethnicity (genetic background)**

Pre-analytical phase – effect of smoking

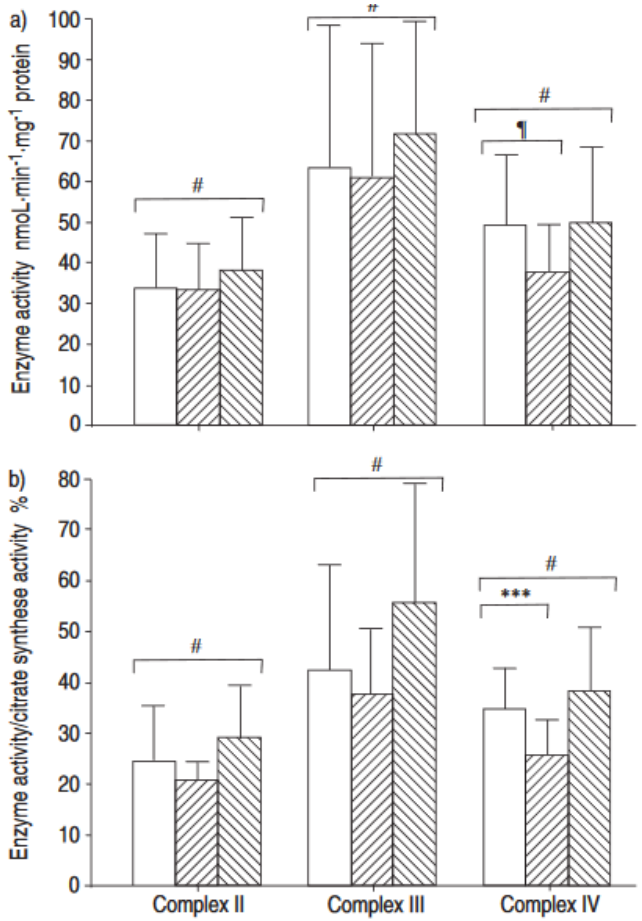


Fig. 1.–a) Absolute enzyme activities and b) relative enzyme activities. □: before smoking; ▨: immediately after smoking; ▧: 24 h after smoking. #: nonsignificant difference; ¶: p=0.002; ***: p<0.001.

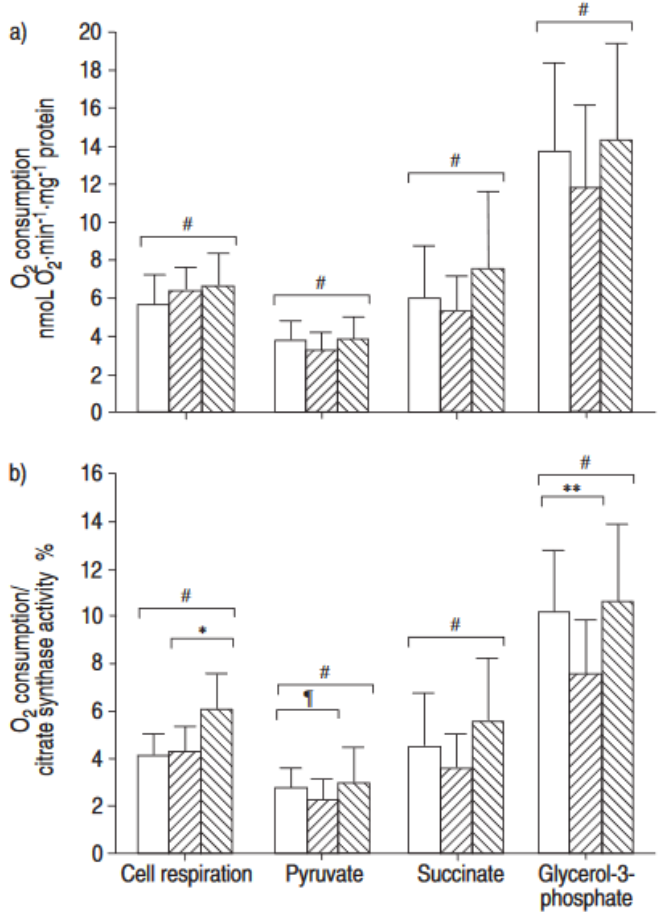


Fig. 2.–a) Absolute oxidation rates and b) relative oxidation rates. O_2: oxygen. □: before smoking; ▨: immediately after smoking; ▧: 24 h after smoking. #: nonsignificant difference; ¶: p=0.01; *: p<0.05; **: p<0.01.

Alonso J-R et al. Eur Respir J 2004; 23: 214–218

Pre-analytical phase – effect of smoking

- healthy, non-smokers
- all mitochondrial changes disappeared after 24 h of smoking abstinence when blood COHb and COEA (carbon monoxide in exhaled air) levels had returned to basal values

Alonso J-R et al. Eur Respir J 2004; 23: 214–218

- PBMCs from smokers release IL-1α, IL-18 and IL-33 after exposure to combustion-generated ultrafine particles

De Falco G et al., Sci Rep. 2017; 7: 43016

Sampling

- tubes, anticoagulants

EDTA K3 tube (Sarstedt, Germany)
is pre-dosed as a liquid preparation in an average concentration of
1.6 mg EDTA/ml blood
ca. 3.85 mM

Heparin tube (Sarstedt, Germany), contains heparin at an average concentration of 16 I.U./ml blood

Sampling - anticoagulants

Ethylenediaminetetraacetic acid (EDTA)

- widely used in laboratory practice for chelation of divalent ions, particularly Ca^{2+} and Mg^{2+}
- applied as anticoagulant in blood samples, but also in media used for mitochondria isolation
- reported to prevent mitochondria swelling and involved in mitochondria contraction
- can cause swelling of both the intracristal and perimitochondrial space and increase in density of the matrix in aged mitochondria
- restores mitochondrial ATP
- inhibits activity of mitochondrial ATPase

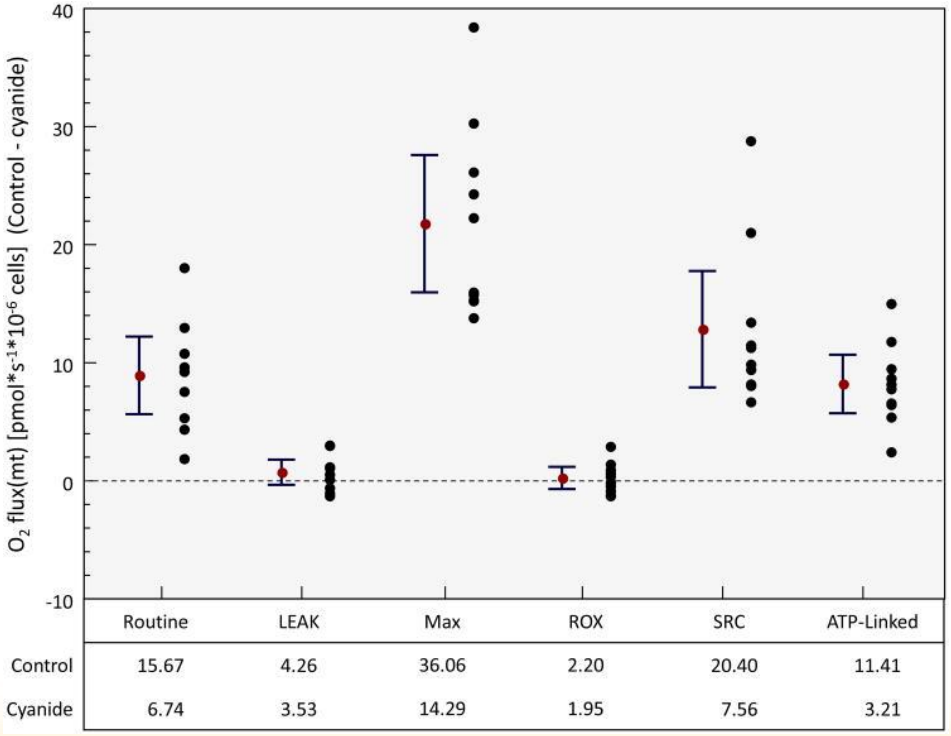
Sampling - anticoagulants
Heparin

- member of the family of polysaccharides, the glycosaminoglycans
- may inhibit the phosphorylation of numerous enzymes in signaling pathways, including protein kinase C (PKC), mitogen activated protein kinase (MAPK) and casein kinase II (CKII)
- is competitive inhibitor of phoshorylases
- has antiproliferative and proapoptotic effects
- stimulates Mg^{+2} – ATPase activity
- found to stabilize mitochondrial membranes

Sampling – anticoagulants
Citrate

- chelator of physiologically important cations such as Ca^{2+}, Zn^{2+} and Mg^{2+}
- monocytes depletion - with the use of citrated (ACD=Acid Citrate Dextrose) blood
- < 3% of the cells were stained by trypan blue after 5 or 30min

Boyum A et al. Scand J Immunol 2002; 56: 76-84



	Routine	LEAK	Max	ROX	SRC	ATP-Linked
Control	15.67	4.26	36.06	2.20	20.40	11.41
Cyanide	6.74	3.53	14.29	1.95	7.56	3.21

Jang DH et al. Clin Toxicol (Phila). 2016;54(4):303-7

Transport

- blood samples transported immediately to respirometry laboratory are delivered within 30 minutes in room temperature (RT)

- for the transportation time longer than 30 minutes blood samples are packed in thermo-insulating containers (RT) and protected from light

- open questions – data lacking:
  - RT vs ice transport of blood for PLT – PBMCs activation
  - Boxes with ice-packs for PBMCs transport only

Whole blood treatment before cells separation

Solutions for whole blood samples diltution before PBMCs isolation

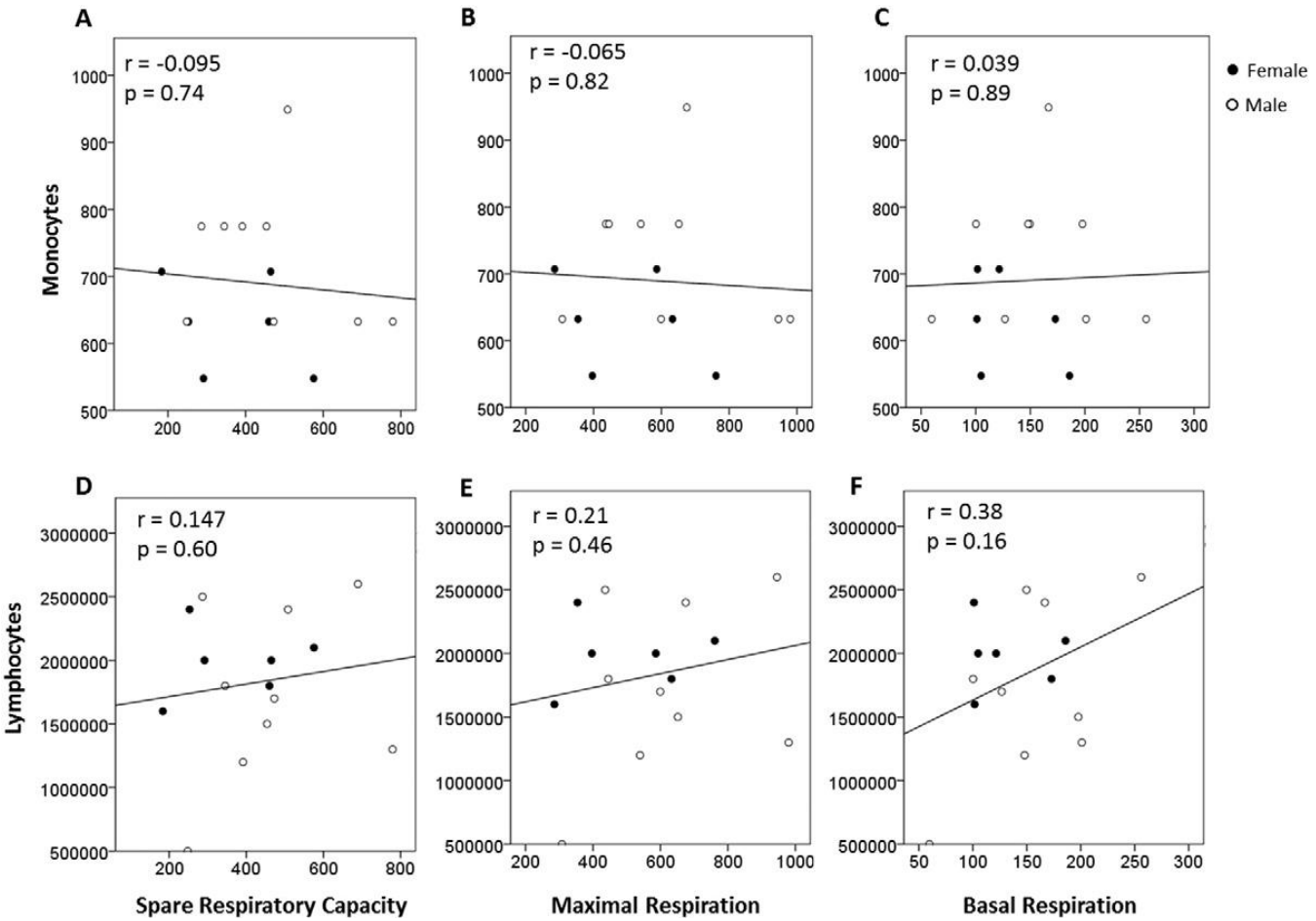
- 1:1 vol/vol
- PBS
- balanced salt solution – Hank's balanced salt solution
  - anhydrous d-glucose 5.5mM
  - $CaCl_2$ 5mM
  -$MgCl_2$ 0.98mM
  - KCl 5.4mM
  - Tris 145mM
  - and NaCl 140mM, pH = 7.6

  Jang DH et al. Clin Toxicol (Phila). 2016;54(4):303-7
- composition of HBSS :
  6 mM glucose, with / without 4 mM $NaHCO_3$,
  4 mM KH_2PO_4 , 3 mM Na_2HPO_4

Storage

- whole blood is processed immediately
- storage, when neeeded:
  * at RT
  * 37 ° C
  * 4 ° C

Characterization of blood



absolute number of lymphocytes or monocytes was not associated with bioenergetic function measured in PBMCs from older, overweight/obese, adults

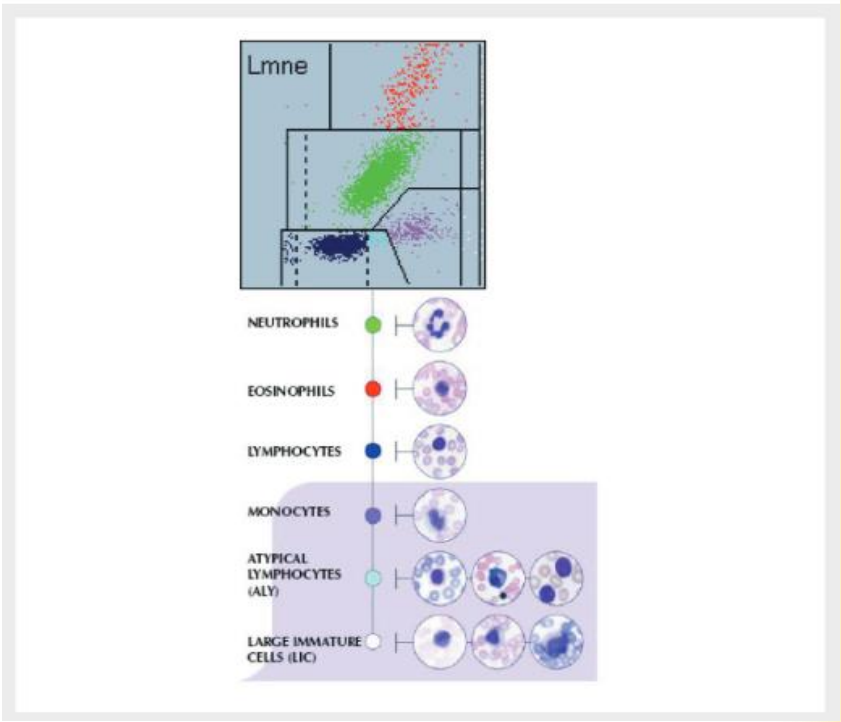
Tyrrell DJ et al., Exp Gerontol 2015; 70:84-91

Characterization of blood

Extended-5 DIFF:
classification of 5 lineages of leukocytes (lymphocytes, monocytes, neutrophils, eosinophils, basophils) including the count of **L**arge **I**mmature **C**ells (**LIC**)
a specific differential including :
IMM (Immature Monocytes),
IML (Immature ymphocytes) and
IMG (Immature Granulocytes)

- LIC parameter – flags samples containing abnormal leukocytes – HORIBA analyzer

- **l**arge **u**nstained **c**ells (LUCs) - activated lymphocytes, myeloid and lymphatic blasts, and plasma cells - Siemens Advia 120 analyzer



Nerin P, HORIBA Technical Reports, English EditionNo.39 September 2012

Characterization of blood

Detection of Immature Cells by Analyzer Flags: Sensitivity and Specificity of Immature Cell Flagging[a]

	Blasts		Immature Granulocytes		Left Shift	
	Sensitivity, %	Specificity, %	Sensitivity, %	Specificity, %	Sensitivity, %	Specificity, %
Sapphire	65	86	62	82	14	88
Advia 120	71	67	56	89	29	89
XE-2100	94	80	56	84	47	85
DxH 800	82	80	73	84	51	79
Microscope[b]	17/186		43/186		72/186	

[a] Sensitivity and specificity of the respective flags using microscopic evaluation as reference.
[b] Number of samples with the respective abnormality out of all samples evaluated.

Abbott – Sapphire
Siemens - Advia 120
Sysmex XE-2100
Beckman Coulter - DxH 800

Meintker L et al. Am J Clin Pathol 2013; 139 (5): 641–650

Characterization of blood

Platelets activation

- during platelet activation and secretion, the rate of both glycolysis and aerobic respiration increase significantly

Zharikov S, Shiva S. Biochem Soc Trans 2013; 41 (Pt1):118-123

- platelet indices :
  MPV – mean platelet volume
  PDW – platelet distribution width
  P-LCR – platelet-large cells ratio

} indicate PLT activation

Characterization of plasma

- hemolysis
- plasma / blood lactate concentration > 3-4 mmol/L
- plasma C reactive protein concentration
- other plasma compounds (to consider)

Characterization of plasma

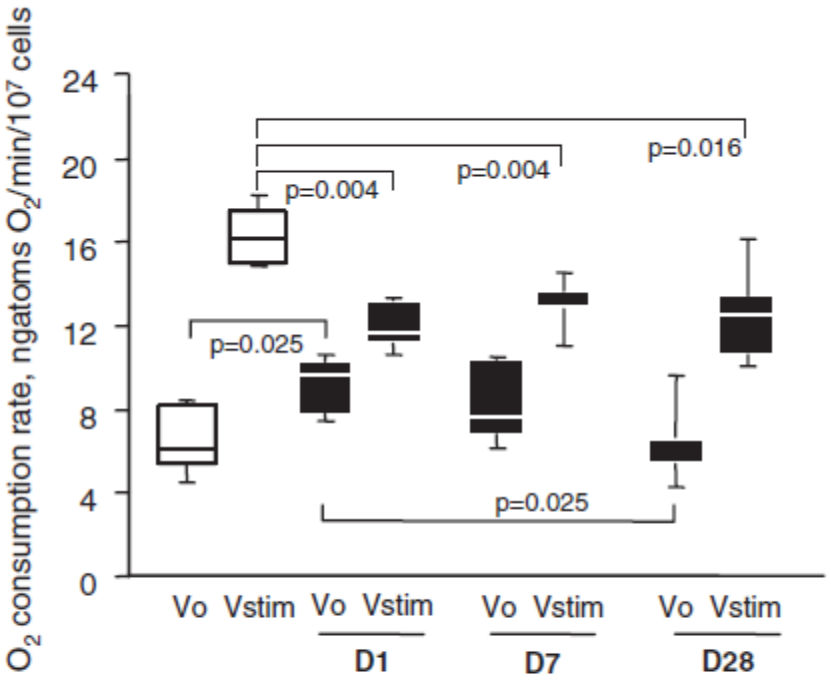


Figure 4. Effect of incubation of healthy cells with septic plasma from different time points of sepsis (days 1, 7, 28) on oxygen consumption (n = 6). V_o is baseline oxygen consumption and V_{stim} is adenosine diphosphate-stimulated oxygen consumption. *Unfilled bars* are healthy volunteer oxygen consumption values; *filled bars* are oxygen consumption in septic plasma. Data displayed as *box and whisker plot* (median, interquartile ranges and range).

Belikova I et al. Crit Care Med 2007; 35:2702–2708

Characterization of plasma

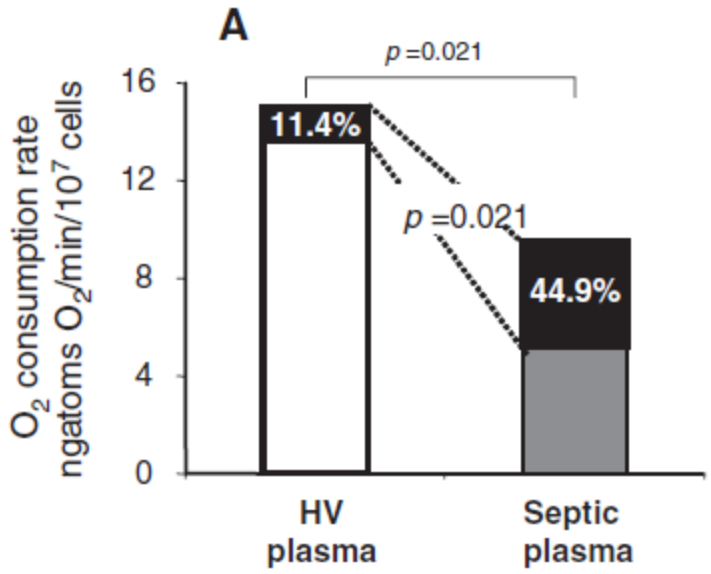


Figure 5. *A*, inhibition of peripheral blood mononuclear cell oxygen consumption by oligomycin. Healthy cells were incubated for 3 hrs in their own plasma (*HV*) and in pooled day 1 septic plasma (n = 6). Oligomycin was added in the chamber after inducing maximal mitochondrial respiration by adenosine diphosphate (*ADP*). *Black bars* are percentage of oxygen consumption not inhibited by oligomycin. Data displayed as mean of four separate experiments. *B*, effect of carbonylcyanide

Belikova I et al. Crit Care Med 2007; 35:2702–2708

Characterization of plasma

Table 2
Correlations between PBMC bioenergetics (spare respiratory capacity, maximal respiration, and basal respiration) with physical function, strength, lean mass, muscle quality, and interleukin-6.

Correlation	Spare respiratory capacity		Maximal respiration		Basal respiration	
	r	p-Value	r	p-Value	r	p-Value
Plasma interleukin-6						
Pearson	**−0.55**	**0.05***	**−0.58**	**0.04***	**−0.61**	**0.03***
Partial (age)	**−0.61**	**0.03***	**−0.64**	**0.03***	**−0.63**	**0.03***
Partial (sex)	**−0.60**	**0.04***	**−0.65**	**0.02***	**−0.69**	**0.01***
Partial (BMI)	−0.53	0.08^	−0.56	0.06^	**−0.59**	**0.04***

Notes: N = 13–15; SPPB = short physical performance battery; BMI = body mass index.
^ $p \leq 0.1$.
* $p \leq 0.05$.
** $p \leq 0.01$.

- negative correlation between IL-6 and PBMCs respiration
- remained statistically significant when independently adjusting for age and sex
- adjusted for BMI, only the association between basal respiration and plasma IL-6 remained significant

Belikova I et al. Crit Care Med 2007; 35:2702–2708

Characterization of plasma

- hyperglycemia, insulinresistance

- the mitochondria of patients with type 2 diabetes - smaller, occupy less cell area, are more spherical
- mitochondrial membrane potential – expressed as the absolute red:green ratio, in the isolated mononuclear cells of patients with T2DM was significantly greater in magnitude than that measured in nondiabetic subjects

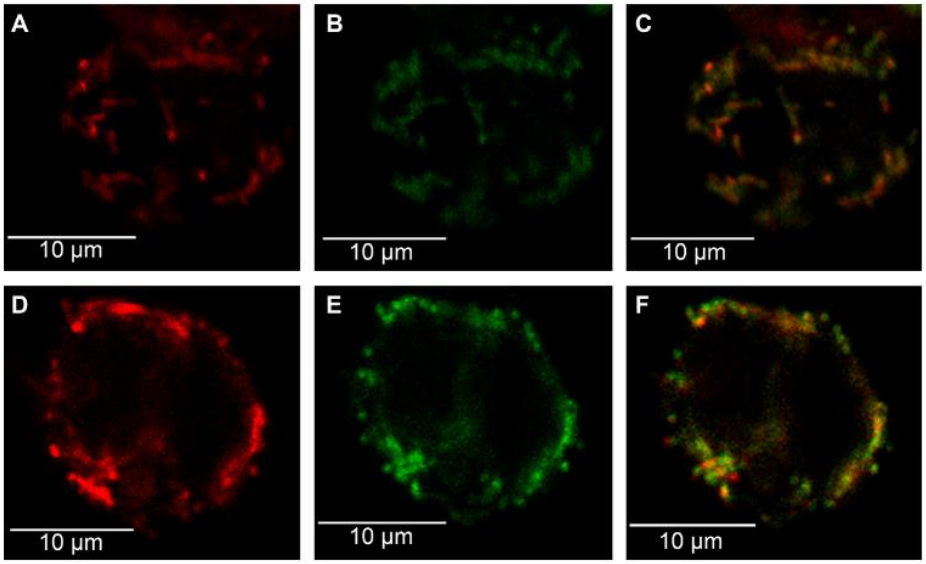


Fig 1. JC-1 localization in representative confocal images of mononuclear cells from a subject with T2DM (**A, B, and C**) and a subject without diabetes (**D, E, and F**). (**A, D**) Localization of red JC-1 fluorescent intensity after excitation with a wavelength of 488 nm. (**B, E**) Green JC-1 fluorescence intensity after excitation with the same wavelength. (**C, F**) Overlay for green and red JC-1 fluorescence, showing colocalization to the same area of the cells. The red:green fluorescence ratio for the diabetic and nondiabetic cells were 1.88 and 1.19, respectively.

Widlansky ME et al., Transl Res 201; 156: 15–25

Characterization of plasma

- hyperglycemia, insulinresistance

- mitochondrial mass as estimated by NAO (acridine orange 10-nonyl bromide) - metachromic dye that binds to cardiolipin - fluorescent intensity was lower in patients with T2DM compared with control subjects

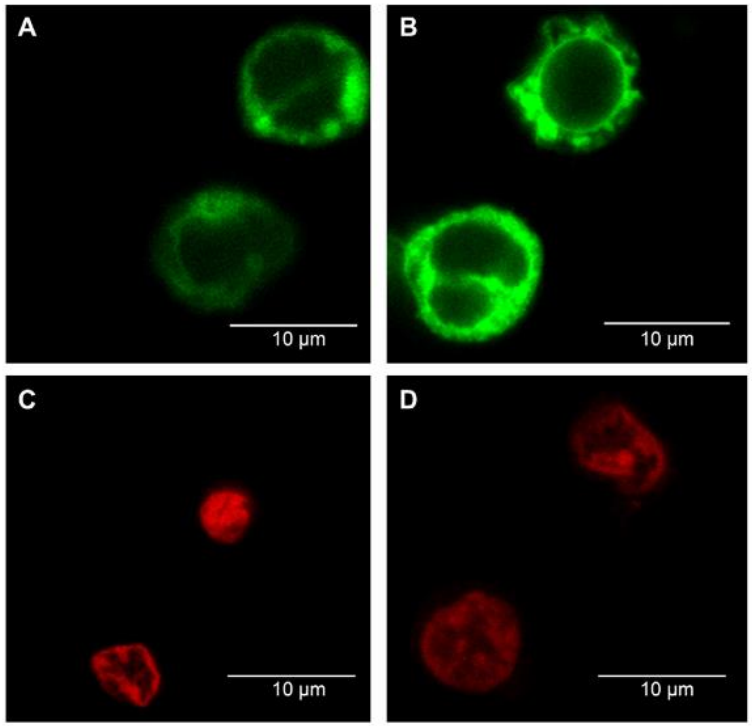


Fig 2. Representative fluorescent confocal images of human mononuclear cells with NAO (**A, B**) and MitoSox (**C, D**) fluorophores for a patient with T2DM (**A, C**) and without diabetes (**B, D**). (Color version of figure is available online.)

Widlansky ME et al., Transl Res 201; 156: 15–25