Platelets Mitochondrial Function Depends on Coenzyme Q_{10} Concentration in Human Young, Not in Elderly Subjects

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Abstract: Ageing is characterized by a progressive decline in the physiological functions of various organs. Mitochondrial alterations occurring in senescence. Antioxidants, including coenzyme Q_{10} concentration, fall with ageing and contribute to enhanced oxidative stress age-related diseases. The impairment of platelet mitochondrial function occurs in a broad spectrum of diseases.

The aim of this study was to evaluate mitochondrial function in platelets in elderly and young human controls and correlate it with a concentration of coenzyme Q_{10}. Platelets mitochondrial function was determined by the use of High-Resolution Respirometry method.

We did not find significantly decreased platelet mitochondrial function in elderly subjects. Dependence of platelets mitochondrial respiratory chain function and ATP production at Complex I on a concentration of coenzyme Q_{10} in platelets and whole blood in young not in elderly human volunteers was documented. This dependence was not found for Complex II in any group. Platelet mitochondrial coenzyme Q_{10} concentration was insufficient for improving platelet mitochondrial function in elderly human subjects. Recommending supplementation with coenzyme Q_{10} in elderly and aged humans is waranted.

High-Resolution Respirometry method offers a perspective to diagnose mitochondrial energy metabolism which might be useful for further studies in patients with mitochondrial disorders. Our results could contribute to the explanation of platelets mitochondrial function in elderly and aged human subjects.

Keywords: Platelets, mitochondria, High-Resolution Respirometry, coenzyme Q_{10}, age.

INTRODUCTION

Mitochondria are subcellular organelles present almost in all cells involved in many physiological metabolic pathways, directly in energy and redox metabolism, cell signalling and apoptosis. Mitochondria play an important role in the ageing metabolism and in the pathological processes of human diseases [1-3].

Mitochondrial oxidative phosphorylation (OXPHOS) is the main source of energy production in the cell. Coenzyme Q_{10} (CoQ_{10}) is a crucial part of respiratory chain of a inner mitochondrial membrane (IMM) important for electron transport from Complex I and Complex II to Complex III, as well as for energy production – adenosine triphosphate (ATP) via OXPHOS. Increased concentration of reactive oxygen species (ROS) and decreased antioxidant protection participate in the impairment of mitochondrial function and in the development of mitochondrial diseases, such as age-associated mitochondrial disorders (Parkinson´s disease, Alzheimer´s disease, diabetes). However little is know about mitochondrial function in platelets during ageing [4].

Platelets, circulating anucleate fragments, contain a small amount of mitochondria. They are generated from megakaryocytes in the bone marrow and live between 7 and 10 days [5]. Platelets are metabolically active cells with high energy consumption which is supplied mainly by mitochondria during thrombus formation. In the resting state of platelets, approximately 60% of ATP is derived from glycolysis and 30-40% energy from OXPHOS [6]. Under pathological conditions, platelets are involved in processes as atherosclerosis, cardiovascular diseases, inflammation, tumor metastasis, and neurodegenerative diseases [7].

For the assessment of mitochondrial respiratory chain function and energy production, biopsy of skeletal muscle is commonly used. In the last years,

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non-invasive method was developed - the analysis of mitochondrial respiration and energy production by High-Resolution Respirometry in human blood cells, such as platelets and peripheral blood mononuclear cells. This method offers sensitive diagnostic tests for mitochondrial respiratory chain function and oxidative phosphorylation in platelets mitochondria [8-12].

In this study, we tested the hypothesis that platelet mitochondrial function depends on concentration of coenzyme Q_{10} in platelets and that both these parameters will be lower in elderly than young people.

MATERIAL AND METHODS

Two groups of human subjects were enroled in the study.

Young group: Young volunteers, number of 37 (12 men, 25 women), age from 22 - 23 years (22.62 ± 0.33, mean ± sem).

Erderly group: Erderly volunteers, number of 19 (7 men, 12 women), age from 56 to 82 years, (68.37 ± 1.32, mean ± sem).

Antioxidants (coenzyme $Q_{10\text{-TOTAL}}$ = ubiquinol+ubiquinone); α-tocopherol, γ-tocopherol) in whole blood, plasma and isolated platelets were determined using HPLC method with UV detection [13] modified by authors [14]. Total CoQ_{10} concentrations were determined after oxidation with 1,4-benzoquinone [15]. A parameter of oxidative stress – thiobarbituric acid reactive substances (TBARS) was estimated by spectrophotometric method [16].

Isolation of Platelets

For platelets (PLT) isolation 18 mL of venous blood was collected to K_3EDTA (tripotassium ethylenediaminetetraacetic acid) tubes each day between 7:00 – 8:00 a.m. at 25°C room temperature. Fresh blood was centrifuged at room temperature at 200g for 10 min using swing-out rotor without breaking. Platelets rich plasma (PRP) was transferred into a new plastic tube and mixed with 100 mM EGTA (ethylene glycol-bis(2-aminoethyl ether)-N,N,N′,N′-tetraacetic acid) to final concentration 10 mmol/L. Next centrifugation in swing-out rotor without breaking at 1000g for 10 min resulted in sediment containing PLTs, which was washed with 4 ml of Dulbecco’s Phosphate Buffered Saline (DPBS, Sigma-Aldrich, D8537), DPBS+10 mmol/L EGTA. After repeated centrifugation, the sediment was resuspended in 0.4 mL of DPBS+10 mmol/L EGTA and used for the respirometric measurements [17]. 10 µL of PLT suspension was 10x diluted with DPBS+10 mmol/L EGTA and used for cell counting on hematological analyzer Mindray BC-2800 (Mindray, China). 100-200 µL of PLT suspension was used for determination of antioxidants.

Mitochondrial Respiration and Oxidative Phosphorylation in Platelets

For mitochondrial respirometric analysis, 200×10^6 PLT was used in 2 mL chamber of O2k-Respirometer (Oroboros Instruments, Austria) [8, 9, 18]. The respiration was measured at 37°C in mitochondrial respiration medium MiR05+20 mM creatine using SUIT *(Substrate-Uncoupler-Inhibitor-Titration)* protocol RP1 [12], Figure **1**.

SUIT Protocol

SUIT protocol for determination of respiration and OXPHOS in mitochondria of human platelets (PLT) includes several steps (Figure **1**):

1. ***Intact PLT***: Oxygen consumption rate in intact PLT (ROUTINE respiration) was measured.

2. ***Dig – PLT***: After addition of digitonin into the chamber (Dig – final concentration of 0.20 µg·10^{-6} cells), respiration rate of mitochondria in permeabilized PLT was measured.

3. ***LEAK respiration at CI*** = **(P+M)** (State 4 at CI): The oxidation of exogenous substrates for Complex I (CI) (PM: 5 mM pyruvate + 2 mM malate) reflects LEAK rate of mitochondrial respiration compensating for proton leak, proton slip, cation cycling, and electron leak.

4. ***OXPHOS at CI = (P+M+ADP)*** (State 3 at CI): ADP was added in saturating concentration (1.0 mM). ADP-stimulated respiration ~ oxidation of substrates chemiosmotically coupled to the phosphorylation of ADP to ATP; At saturating ADP represents maximum capacity of OXPHOS with given substrates at CI.

5. ***Cyt c***: Addition of 10 µM cytochrome *c* is a test for the integrity of the outer mitochondrial membrane.

6. ***CCCP at CI***: uncoupler of OXPHOS (P+M): After CCCP titration, maximal oxidative capacity at Complex I was measured.

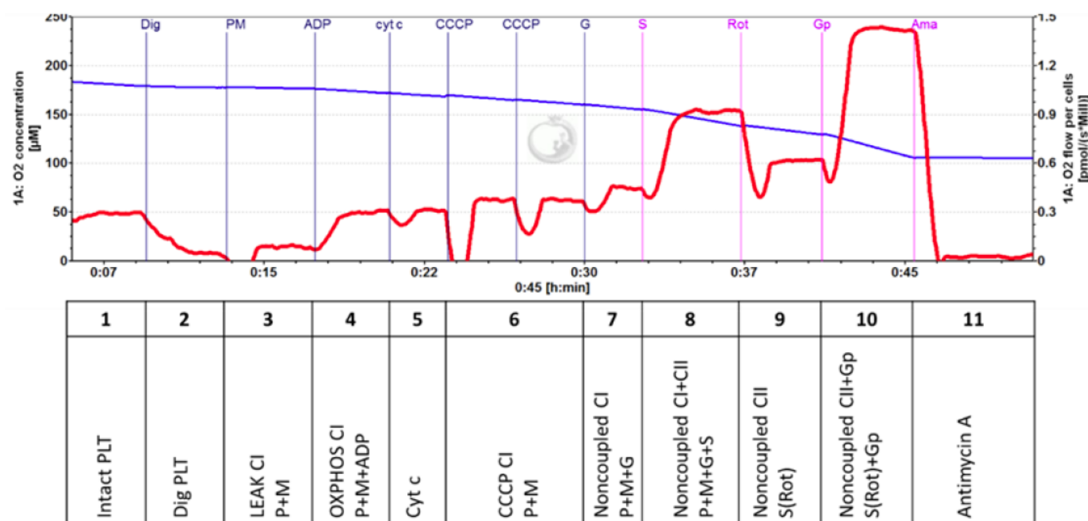

Figure 1: High-Resolution Respirometry of OXPHOS in platelets mitochondria.

Legend: The oxygen consumption rate is expressed in pmol $O_2 \cdot s^{-1} \cdot 10^{-6}$ plts.

1. Oxygen consumption rate of intact PLT- ROUTINE respiration.
2. Respiration rate of mitochondria in permeabilized PLTs with digitonin.
3. LEAK at Complex I (State 4) reflects rate of mitochondrial respiration with exogenous substrates (pyruvate + malate).
4. OXPHOS at Complex I (State 3) after ADP addition reflects ATP production.
5. Cytochrome c addition – a test for the integrity of outer mitochondrial membrane.
6. The rate after CCCP addition (uncoupled of OXPHOS) represents maximal oxidative capacity at Complex I with substrates pyruvate + malate.
7. Noncoupled oxygen consumption at Complex I after addition of substrate glutamate.
8. Noncoupled oxygen consumption at Complex I and Complex II after addition of CII substrate succinate.
9. Noncoupled oxygen consumption at Complex II after addition of rotenone – inhibitor of Complex I.
10. Noncoupled oxygen consumption at Complex II after addition of glycerophosphate (Gp) – test of glycerophosphate dehydrogenase activity on electron transport (ET)-capacity.
11. Residual oxygen consumption (ROX) was determined after addition of Antimycin A - an inhibitor of Complex III.

7. ***Noncoupled respiration at CI = (P+M+G):*** Addition of exogenous substrate (10 mM glutamate) supported Complex I-linked respiration.

8. ***Noncoupled respiration at CI + CII = (P+M+G+S):*** Addition of exogenous substrate for Complex II (CII) succinate (10 mM) allowed determination of electron transfer capacity (ET-capacity) of convergent electron flow from Complex I and Complex II to coenzyme Q.

9. ***Noncoupled respiration at CII (rotenone – an inhibitor of CI)*** (ET- capacity at CII): Addition of rotenone (0.5 µM) inhibited Complex I and Complex II-linked respiration rate was measured.

10. ***Gp + CII = (ET-capacity):*** Noncoupled respiration after addition of 10 mM glycerophosphate (CII + Gp) was used to test the additional effect of glycerophosphate dehydrogenase activity on ET- capacity.

11. ***Antimycin A = (inhibitor CIII** – Complex III**):*** Addition of 2.5 µM Antimycin A blocked platelet mitochondrial respiration and allowed determination of residual oxygen consumption rate (ROX).

Statistics

Unpaired Student´s t-test was applied to evaluate the effect of age on determined parameters. Pearson's correlation analyses were performed on GraphPad Prism 6. The level of statistical significance was set at $p<0.05$. The results in figures and the Table **1** are expressed as mean±sem.

The study was carried out according to the principles expressed in the Declaration of Helsinki and

Table 1: The Effect of Ageing on Selected Antioxidants in Platelets, Blood and Lipid Peroxidation in Plasma

Parameter	Reference values	Young (Y)	Elderly (E)	Statistics	Y vs E (%)
$CoQ_{10\text{-TOTAL}}$					
Platelets (pmol.10^{-9} cells)		155.8±12.2	167.7±12.8	NS	↑7.64
Whole blood (µmol/L)		0.221±0.010	0.239±0.021	NS	↑8.14
Plasma (µmol/L)	0.4 - 1.0	0.344±0.016	0.373±0.022	NS	↑8.40
α-Tocopherol					
Platelets (pmol.10^{-9} cells)		2910.4±216	1763.8±151	P=0.006	↓39.4
Whole blood (µmol/L)		15.2±0.503	17.2±1.13	n.s.	↑13.1
Plasma (µmol/L)	15 – 40	21.3±0.710	26.1±1.48	P=0.003	↑22.5
γ-Tocopherol					
Platelets (pmol.10^{-9} cells)		245.6±22.4	180.6±28.9	n.s.	↓26.5
Whole blood (µmol/L)		0.991±0.064	1.06±0.119	n.s.	↑4.50
Plasma (µmol/L)	2 – 7	1.38±0.102	1.70±0.184	n.s.	↑23.2
TBARS					
Plasma (µmol/L)	< 4.5	4.71± 0.108	4.71± 0.134	n. s.	

the study protocol was approved by the Ethical Committee of the Academic Ladislav Dérer´s Hospital, Bratislava, Slovakia (24.06.2018). Written informed consent from each subject was obtained prior to inclusion.

RESULTS

Antioxidants and Oxidative Stress

$CoQ_{10\text{-TOTAL}}$ concentration in PLT, whole blood and plasma were slightly, not significantly increased in elderly human volunteers in comparison with young controls. The concentration of α-tocopherol in PLT was significantly decreased (p=0.006), in whole blood slightly and in the plasma significantly increased (p=0.003) in elderly subjects in comparison with young controls. The concentration of γ-tocopherol was decreased by 26.5% (n.s.) in PLT and nonsignificantly increased in whole blood and plasma in the elderly group in comparison with healthy young volunteers. TBARS – parameter of oxidative stress was similar in both groups (Table **1**).

Mitochondrial Respiration and OXPHOS in Permeabilized Platelets

All parameters of oxygen consumption are evaluated in pmol.s^{-1}.10^{-6}cells. Oxygen consumption of intact PLT (1R), mitochondrial LEAK respiration in permeabilized PLT with CI-linked substrates (1PM) and OXPHOS – ATP production (2D) were not significantly decreased in the elderly group compared to the young group. The integrity of OMM after addition of cytochrome *c* did not significantly differ between elderly and young group (2c). Noncoupled respiration at CI after uncoupler titration (3U) and glutamate addition (4G) were decreased in the elderly group by 13.6% and 8.9% in comparison with young controls (n.s.). Noncoupled mitochondrial respiration at CI+CII (5S) was decreased (n.s.) in elderly group. The electron transport capacity at CII (CII_E) measured after CI inhibition with rotenone (6Rot) was slightly (n.s.) stimulated in elderly group. Similar nonsignificant stimulation of oxygen consumption in the elderly group was observed after glycerophosphate (7Gp) addition (Figure **2**).

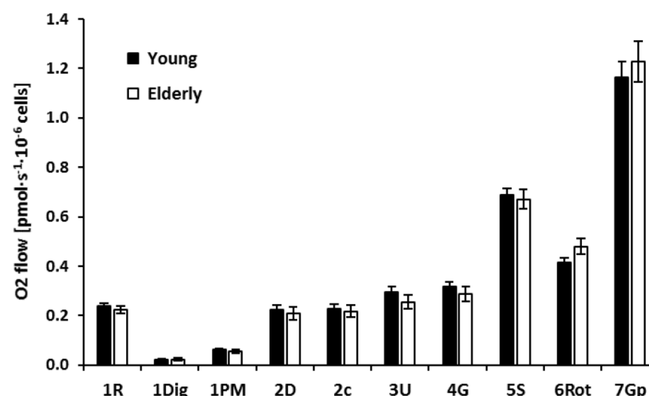

Figure 2: The effect of ageing on mitochondrial respiration and OXPHOS in platelets of human controls.

Calculated ET- capacity at Complex I = CI_E, (CI+CII) - CII) was decreased in elderly controls by 20.91% in comparison with young group (0.208±0.025 respectively 0.236±0.022 $pmol.s^{-1}.10^{-6}$ cells). Calculated ET- capacity at Complex II = CII_E, (CI+CII) - CI) was similar in both groups. The measure of integrity of IMM determined as the ratio of 2D/1PM was slightly (n.s.) damaged in elderly group, the measure of the integrity of OMM determined as the relative increase in respiration after addition of cytochrome *c* was similar in both groups.

The Correlation between Mitochondrial Function and of $CoQ_{10\text{-}TOTAL}$ Concentration

A positive correlation between LEAK respiration (1PM) in mitochondria of platelets (LEAK at CI) and $CoQ_{10\text{-}TOTAL}$ concentration in PLT was determined (p=0.0098) in young subjects. This correlation was nonsignificant in elderly controls (Figure **3A**). The correlation between LEAK at CI and $CoQ_{10\text{-}TOTAL}$ concentration in plasma was nonsignificant in both groups (Figure **3B**).

We found a positive correlation between OXPHOS at CI and $CoQ_{10\text{-}TOTAL}$ concentration (p=0.022) in PLT in young subjects, not in elderly controls (Figure **4A**); Similarly, the correlation of OXPHOS at CI with concentration of $CoQ_{10\text{-}TOTAL}$ in blood was significant only in the young group (p=0.022) (Figure **4B**); OXPHOS at Complex I did not depend on $CoQ_{10\text{-}TOTAL}$ concentration in plasma (Figure **4C**).

The noncoupled mitochondrial respiration at Complex II did not depend on $CoQ_{10\text{-}TOTAL}$ concentration in PLT (Figure **5A**), but there was marginally significant positive dependence of CII-linked noncoupled respiration and $CoQ_{10\text{-}TOTAL}$ concentration in whole blood in young group (Figure **5B**). We also found marginally significant negative correlation between CII_E and $CoQ_{10\text{-}TOTAL}$ concentration in plasma in both groups (Figure **5C**).

DISCUSSION

Ageing is characterized by a progressive decline in the physiological functions of various organs and an increased occurrence of various diseases [19]. Harman was one of the first authors postulating that mitochondria play a central role in ageing [20].

Mitochondrial dysfunctions as respiratory chain diseases originate primarily at the genetic level from nuclear or mitochondrial DNA mutations. The secondary cause of mitochondrial diseases includes a wide variety of factors, as ischemia, reperfusion, cardiovascular diseases, neurodegenerative diseases, renal failure, diabetes, infections, oncological diseases, mitochondria immune weakness, infertility, stress and ageing [21, 22]. Mitochondrial dysfunction contributes to ageing related diseases [2, 21, 23, 25].

Ageing impairs mitochondrial OXPHOS, causes the decrease in activity of CIII and CIV, and decreases mitochondrial respiration. Age induces defects in electron transport. The transfer of electrons down the redox potential gradient from NADH or $FADH_2$ to oxygen is coupled to the active transport of hydrogen ions from the matrix to the cytosolic side of the IMM by CI, CIII, and CIV. CI oxidizes NADH, with sequential electron flow to CoQ, CIII, cyt *c*, and ultimately to CIV, the later reducing oxygen to water. The complexes are organized into larger "supercomplexes", to optimize channeling of reducing equivalents between the

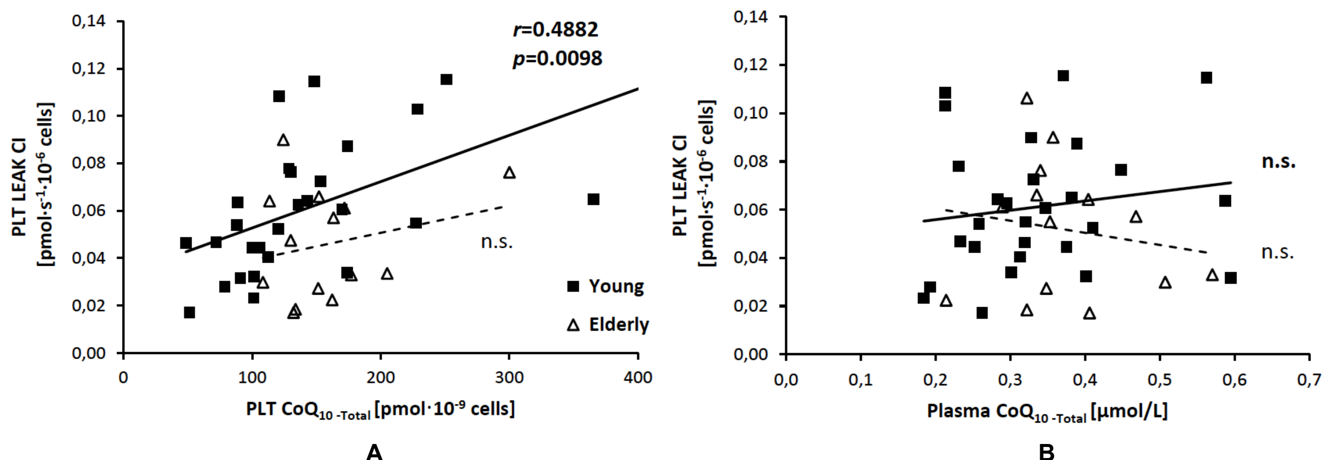

Figure 3: Correlation between platelets mitochondrial LEAK respiration at Complex I and $CoQ_{10\text{-}TOTAL}$ concentration in platelets and plasma.

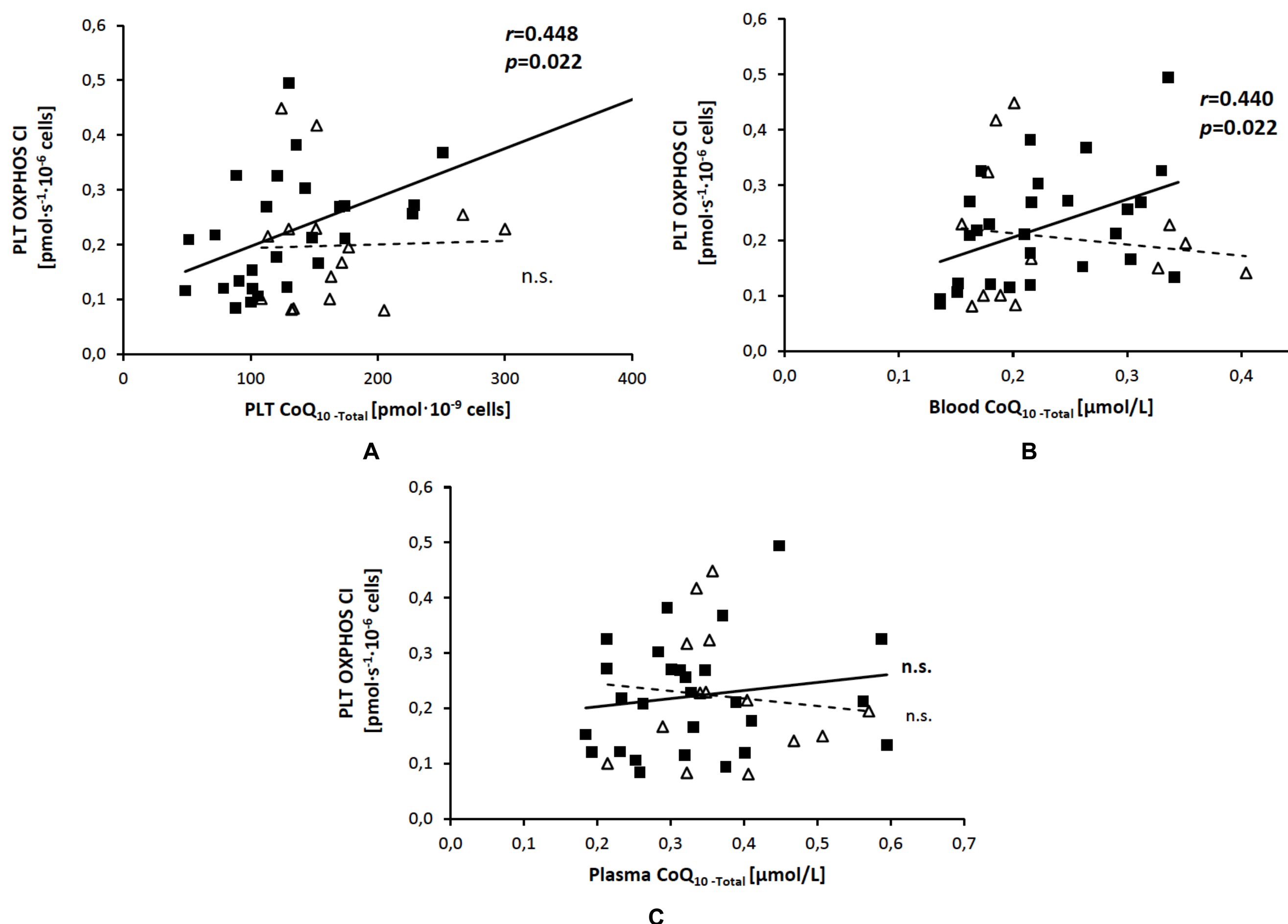

Figure 4: Correlation between platelets mitochondrial OXPHOS and concentration of $CoQ_{10\text{-TOTAL}}$ in platelets, blood and plasma.

components. The activity of CV, and potentially the coupling of OXPHOS decreases with age [3].

The study of physiological and pathological mitochondrial function is essential for the diagnostics and targeting therapy of mitochondrial diseases. Platelet mitochondrial function is an important clinical target for the pathophysiology of many diseases [10, 23]. The analysis of mitochondrial respiration and energy production by High-Resolution Respirometry in human blood cells offers a sensitive diagnostic test of mitochondrial respiratory chain function and oxidative phosphorylation [8, 9, 11, 12]. It was shown that circulating platelets and leucocytes can act as different sensors of the inflammatory and metabolic stresses associated with cardiovascular disease, neurodegeneration, diabetes, and other chronic diseases [27]. Impaired platelet mitochondrial function has been found in various human diseases: in patients with type 2 diabetes, Alzheimer´s, Parkinson´s, Huntington´s disease and migraine headaches. Decreased activity of mitochondrial CI in platelets of patients with Parkinson´s disease was found, while patients with schizophrenia showed an increase in CI activity [26]. The effect of age on platelet metabolism and mitochondrial function is not completely clear [28].

Antioxidants and Lipids Peroxidation

In this study, PLT isolated from venous blood were used for respirometric analysis of mitochondrial function. PLT count in the blood remains relatively stable between 25-60 years of age and declines after 60 years of age [29]. We did not find significant differences in selected antioxidants between an elderly and young group of healthy volunteers. It is known that $CoQ_{10\text{-TOTAL}}$ concentration decreases in higher age. In our study, we found a slightly increased $CoQ_{10\text{-TOTAL}}$ concentration in PLT, blood and plasma in elderly human controls. Reduction in PLT α-tocopherol concentration in elderly controls (-39.4%, p=0.006) was compensated by the increased α-tocopherol concentration in whole blood (+13.15%, n.s.) and plasma (+22.5%, p=0.003). The concentration of γ-tocopherol in PLT was decreased by 26.47%, and in whole blood and in plasma slightly increased (+4.5%,

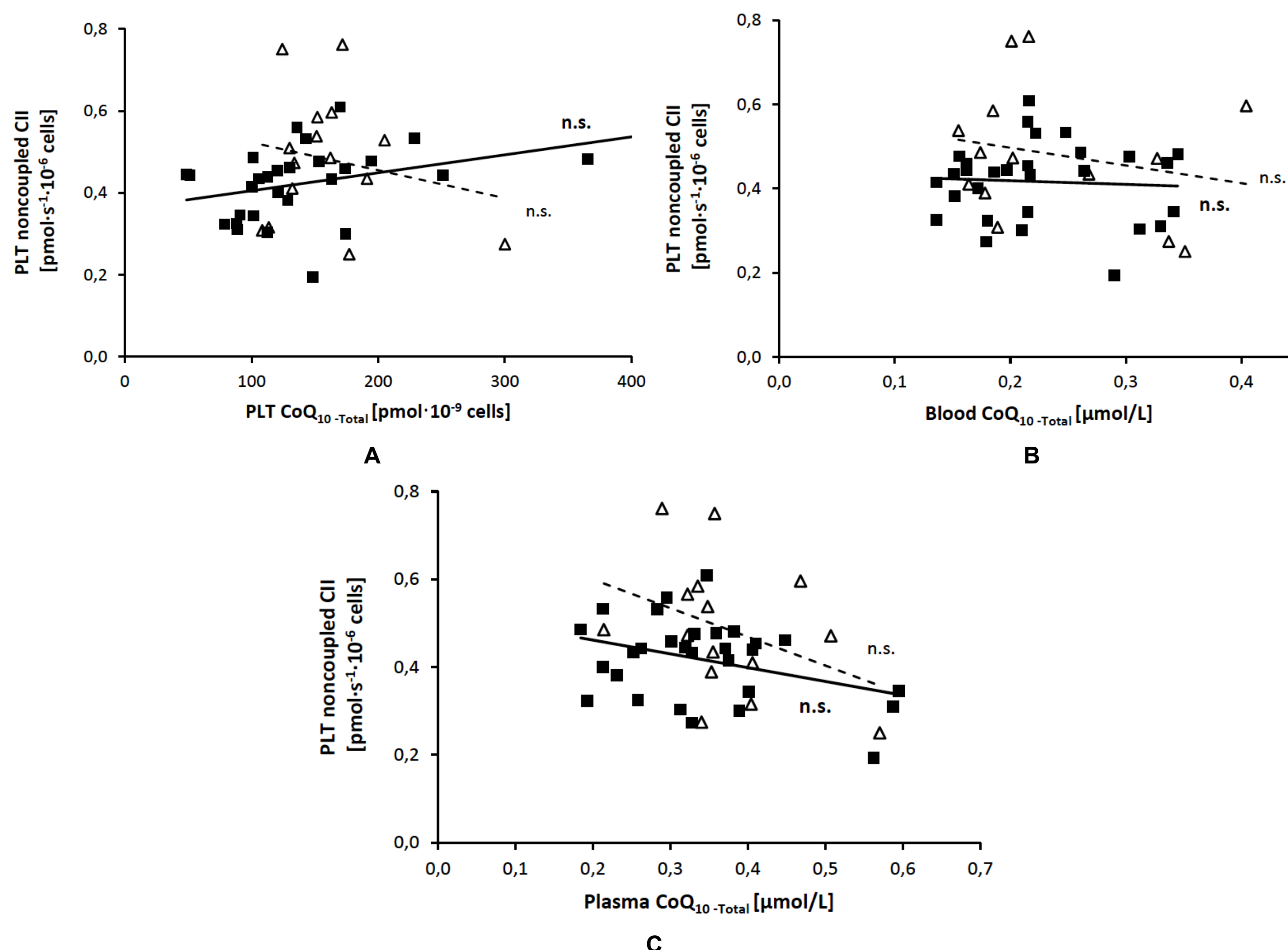

Figure 5: Correlation between Complex II-linked noncoupled respiration in platelets and $CoQ_{10\text{-TOTAL}}$ concentration in platelets, blood and plasma.

and +23.19% respectively) in elderly controls. The concentrations of γ-tocopherol in plasma and tissues depend on cytochrome P_{450} metabolism in the liver [30]. Increased $CoQ_{10\text{-TOTAL}}$ and α-tocopherol concentrations may improve cytochrome P_{450} metabolism, liver function, and stimulate degradation and elimination of γ-tocopherol that leads to a reduction of its concentration in platelets by 26.5% (Table **1**).

During ageing, ROS concentration is increased leading to increased cell loss [3]. In our study, we did not find changes in lipids peroxidation in the elderly group in comparison with the young group (Table **1**).

Mitochondrial Respiration and OXPHOS in Human Platelets

High-Resolution Respirometry offers the possibility to use peripheral blood mononuclear cells and platelets for studying mitochondrial bioenergetic metabolism in humans. This non-invasive method was applied in various diseases demonstrating the high potential of isolated lymphocytes for diagnostics of OXPHOS disorders [31].

In pediatric patients, High-Resolution Respirometry was used for the study of mitochondrial function in lymphocytes. Based on the results the authors proposed the best biochemical parameters predictive for defects of respiratory CI, CIV and CV manifested in peripheral blood lymphocytes. Peripheral blood mononuclear cells were used for respirometric analysis of mitochondrial function in patients with prostate cancer [32].

In platelets of patients with septic shock and cardiogenic shock the activities of mitochondrial complexes were lower in comparison with the control group: NADH – nicotinamide adenine dinucleotide dehydrogenase (20 to 25% reduction, $p<0.0001$), Complex I (NADH-ubiquinone reductase - 30% reduction), Complex I + III (NADH-cytochrome c reductase - 30 to 35% reduction), Complex IV (cytochrome c oxidase - 60 to 65% reduction). Platelets

of patients with sepsis had also lower succinate dehydrogenase activity (20% reduction) than those of controls [33]. Lower platelet mitochondrial Complex I and II/III activity was seen in early untreated Parkinson´s disease patients [34]. The activities of respiratory chain Complexes I and II in isolated platelets were significantly higher in females with anorexia nervosa in comparison with control group. No differences were found in the activities of Complexes IV and I+III, and citrate synthase [35]. Decreased platelets mitochondrial oxygen consumption at CI was found in depressive patients [36] as well as in patients treated with statins [37]. Platelet-dependent thrombus formation may be altered by endogenous or exogenous antioxidants, as well as by formation of ROS and NOS [38]. Mechanisms of the platelet mitochondrial function are not completely known [28].

Our data showed no significant differences in platelet mitochondrial respiration and ATP production at CI between young and elderly subjects, all parameters at CI were slightly decreased in elderly group. Respiration of intact PLT (1R) in elderly subjects was decreased by 6,28%, mitochondrial respiration of permeabilized PLT at CI (State 4) by 9.8% and ATP production by 4.13% at CI (2D) in elderly group (Figure **2**) in comparison to healthy young humans. Maximal oxidative capacity of mitochondrial electron transport system at CI (3U) and noncoupled mitochondrial respiration at CI after glutamate addition (4G) in elderly controls were decreased (by 13.56% and 8.9%, respectively, Figure **2**). Decreased mitochondrial function stimulate glycolysis and lactate production. We suppose that slight alterations in PLT mitochondria occuring in senescence may signal bioenergetic deficiencies.

Increased electrons transport capacity from CII to coenzyme Q (CII_E) in erderly subjects by 15.3% indicated increased inner mitochondrial membrane permeability (Figure **2**). Slightly higher (n.s.) ET-capacity at CII (6Rot) and at CII+ Gp in elderly subjects could be seen as compensation to slightly decreased CI-linked activities (3U and 4G) in elderly group with average of age 68. These subjects were in a good mental and physical conditions without any diseases.

Although the differences in the parameters of PLT respiration were not significant, we found a negative correlation of ROUTINE respiration of intact PLT (1R) with age in elderly group (p=0.0422, Figure **6**) indicating the reduced respiratory function of PLT in older age. Positive correlation of PLT ROUTINE respiration and negative correlation of noncoupled CII respiration with age in healthy people between 0 and 65 years was reported previously [10]. Other study found reduced glutamate uptake in platelets from old (74 years) compared to young (41 years) human subjects [40] indicating the reduced ability of platelet activation and thrombus formation in aged humans [41].

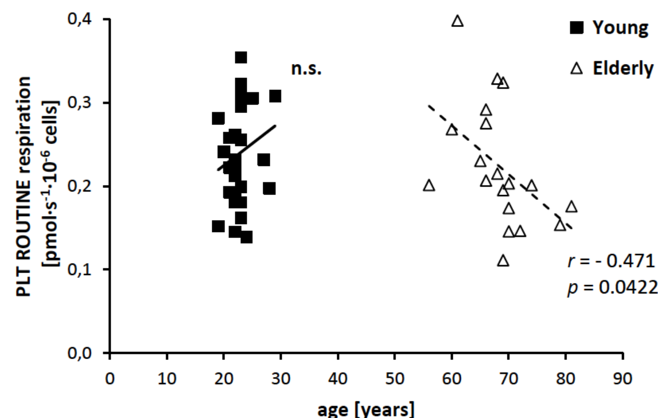

Figure 6: Correlation of ROUTINE respiration of intact platelets with age.

In our previous study platelets mitochondrial function in young humans was not affected by season temperature [39]. Overall, our results indicate that changes in respiratory rates in intact and permeabilized PLT could be used as a marker of mitochondrial disorders independently of age and season.

The Correlation between Mitochondrial Function and of $CoQ_{10\text{-}TOTAL}$ Concentration

We found a dependence of PLT mitochondrial oxygen consumption at CI (LEAK CI) on $CoQ_{10\text{-}TOTAL}$ concentration (p=0.0098) in PLT in the young group, not in an elderly group (Figure **3A**). Platelets mitochondrial ATP production at CI (OXPHOS) depended on $CoQ_{10\text{-}TOTAL}$ concentration in PLT and blood in young group (p=0.022; Figures **4A**, **4B**), not in the elderly group. Mitochondrial CII function did not depend on the concentration of $CoQ_{10\text{-}TOTAL}$ in PLT, blood or plasma in any group (Figures **5A**; **5B**; **5C**).

We found positive correlation between PLT mitochondrial function on their CoQ_{10} concentration in young subjects, not in elderly group. We suppose that in elderly subjects several factors can contribute to slightly lower PLT mitochondrial function: a/ increased permeability of platelet and inner mitochondrial membrane; b/ decreased concentration of α- and γ-tocopherol in PLT can indicate age-related changes in

PLT redox balance which could participate in slightly lower PLT in Complex I mitochondrial function in elderly subjects PLT; c/ and insufficient PLT coenzyme Q_{10} concentration for improving PLT mitochondrial function in ageing. Recommending of mitochondrial targeting CoQ_{10} supplementation in ageing could be warranted.

CONCLUSION

We present the first data showing significant dependence of platelets mitochondrial OXPHOS function on their CoQ_{10} concentration in young humans. Although we found slight differences in mitochondrial respiration of PLT between young and elderly group of healthy volunteers indicating decreased activity of CI and increased activity of CII in older age, the differences were not statistically significant. We suppose that in PLT mitochondrial function differences in elderly subjects could participate increased PLT and mitochondrial membrane permeability, decreased PLT antioxidants protection and insufficient coenzyme Q_{10} concentration. CoQ_{10} supplementation in ageing could be warranted.

High-Resolution Respirometry method offers a sensitive diagnostic test for mitochondrial energy metabolism which might be useful for further studies in patients with mitochondrial disorders. Our results indicate that changes in respiratory parameters in intact and permeabilized PLT could be used as a biological marker of mitochondrial disorders independently of age.

DISCLOSURE

This work was not published before; part of the results was presented at the 9th Conference of the International Coenzyme Q_{10} Association, New York, USA, June 21- 24, 2018 [39]. This publication was approved by all coauthors.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

AUTHOR´S CONTRIBUTION

Anna Gvozdjáková prepared the design, managed this study and wrote manuscript; Zuzana Sumbalová evaluated respirometric data, prepared figures and revised the manuscript; Jarmila Kucharská measured and evaluated antioxidants and revised the manuscript; Anežka Chládeková managed patients, blood collection and evaluated biochemical parameters; Zuzana Rausová performed respirometric analysis and revised the manuscript, Oľga Uličná evaluated platelets count and revised manuscript; Michal Nemec, Oľga Vančová, Mária Kubalová, Zuzana Kuzmiaková performed respirometric measurements; Viliam Mojto managed blood collection, biochemical parameters and revised the manuscript.

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