

## Platelets mitochondrial function depends on CoQ<sub>10</sub> concentration in winter, not in spring season

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**Abstract.** Seasonal variations in temperature may influence the physiological and pathological metabolic pathways, concentrations of antioxidants, degree of oxidative stress and mitochondrial function. The aim of this study was to evaluate platelet mitochondrial function in human subjects during seasonal variations in temperature. Two groups of healthy young subjects were enrolled in the study. Winter group, mean outside temperature was 4.77°C and Spring group, mean outside temperature was 24.32°C. High-resolution respirometry method was used for determination of mitochondrial respiration and oxidative phosphorylation in platelets. Concentrations of coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) and tocopherols were determined in platelets, blood and plasma. Our data showed slightly (not significantly) reduced respiration in intact platelets, basal and ADP-stimulated mitochondrial respiration at Complex I, as well as CoQ<sub>10</sub>-TOTAL and  $\alpha$ -tocopherol concentrations in winter. The concentration of  $\gamma$ -tocopherol was higher in winter. Platelet mitochondrial ATP production depended on platelet CoQ<sub>10</sub>-TOTAL concentration in winter, not in spring. We conclude that seasonal temperature participates in the mechanism of platelet mitochondrial respiratory chain function and oxidative phosphorylation that depends on their CoQ<sub>10</sub>-TOTAL concentration at lower winter outside temperature. CoQ<sub>10</sub> supplementation may improve platelets mitochondrial ATP production at winter season. High-resolution respirometry offers sensitive method for detection of changes of platelets mitochondrial respiratory function.

**Key words:** Seasonal temperature — Platelets — Mitochondria — Coenzyme Q<sub>10</sub>-TOTAL — High-resolution respirometry

### Introduction

Seasons, their duration and variations in temperature, duration of exposure to sun light, humidity and rainfall, may influence the physiological and pathological metabolic pathways (Schwartz and Andrews 2013).

Seasonal variations were found in antioxidant defence system and oxidative stress in mammals. Increased lipop-

eroxidation in heart of patients, rats and guinea pigs during summer compared to winter was observed (Mujkosová et al. 2008; Konior et al. 2011). Increased oxidative stress and low levels of antioxidant enzymes were found in rat erythrocytes during high temperature in summer in comparison with winter season (Bernabucci et al. 2002; Bhat et al. 2008). Conversely, increased oxidative stress was recorded in tissues of crocodile during winter in comparison to summer (Fuldato-Filho et al. 2007). Seasonal variations in antioxidant system and oxidative stress were documented in birds from Seychelles, when the availability of food was too low (Van de Cromennacker et al. 2011). Several studies showed an effect of seasonal variations on metabolic activities and mitochon-

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drial function in mammals (Heim et al. 2017; Ballinger and Andrews 2018).

Mitochondria are subcellular organelles present almost in all cells. During excessive ROS production mitochondrial function is impaired in various organs and participates in the development and progression of many diseases.

Platelets (PLT) are short-lived (7–10 days) circulating anucleate fragments with size 1.5–3  $\mu\text{m}$  generated from megakaryocytes in the bone marrow. They contain endoplasmic reticulum, Golgi apparatus and small amount of mitochondria (Garcia-Souza and Oliveira 2014; Ravera et al. 2018), important for energy production and for regulation of intracellular signaling through reactive oxygen species (ROS). Platelets are cells with high energy consumption. In resting state of PLT approximately 60% of adenosine triphosphate (ATP) is derived from glycolysis and 30–40% ATP from oxidative phosphorylation (OXPHOS) (Clemenson 2012; Kramer et al. 2014).

Platelets play an integral role in intracellular communication, they have the capacity to interact with almost all immune cells. Their main function in the blood stream is rapid binding to damaged blood vessels. Under pathological conditions, they are involved in processes of various diseases. However, limited studies are about PLT mitochondrial function in pathological states (Wang et al. 2017) and the effect of the seasonal temperature on platelet mitochondrial function.

Platelets are an easily obtainable source of viable mitochondria (Sjovall et al. 2010, 2013). High-resolution respirometry method offers sensitive diagnostic tests of mitochondrial respiratory chain function and OXPHOS in PLT mitochondria (Gnaiger et al. 2000; Pesta and Gnaiger

2012; Doerrier et al. 2016; Yun et al. 2016; Sumbalová et al. 2018), as well as in peripheral blood mononuclear cells in humans (Pecina et al. 2014; Hsiao and Hoppel 2018).

In this study we tested the hypothesis that platelets mitochondrial function, oxygen consumption, ATP production, CoQ<sub>10</sub> concentration and oxidative stress could be affected by seasonal temperature in humans.

## Material and Methods

### Participants and seasonal temperature

The study was carried out according to the principles expressed in the Declaration of Helsinki and the study protocol was approved by the Ethical Committee of the Academic Ladislav Dérer's Hospital, Bratislava, Slovakia (2018). Written informed consent from each subject was obtained prior to inclusion.

Two groups of healthy young subjects were enrolled in the study: *Winter group*: From February to March 2018, mean of outside temperature was 4.77°C. Winter group represented 13 healthy young volunteers, men ( $n = 3$ ) and women ( $n = 10$ ), mean age was 23 years. *Spring group*: From April to May 2018, mean outside temperature was 24.32°C. Healthy young 19 volunteers, men ( $n = 9$ ), women ( $n = 10$ ) were included in the study, mean age was 22 years. Figure 1 shows daily outside temperature during winter and spring 2018 in Bratislava, Slovakia ([www.shmu.sk](http://www.shmu.sk)).

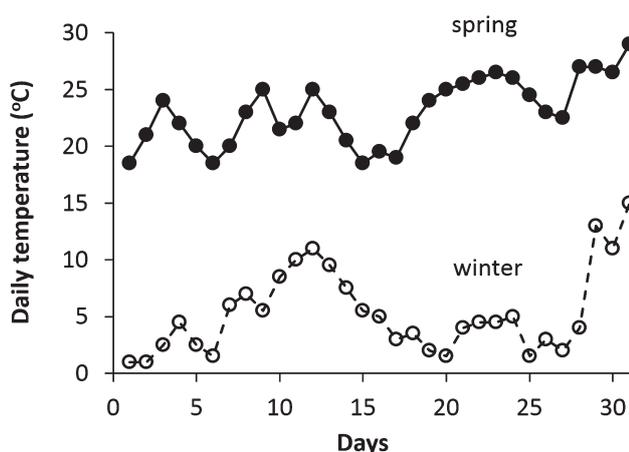
## Methods

### Metabolic parameters

Body height, weight, body mass index (BMI) and metabolic blood parameters were measured: hemoglobin, leukocytes and platelets count, CRP, triacylglycerol, LDL-cholesterol, HDL-cholesterol, total cholesterol, liver enzymes: AST, ALT, GMT, as well as glucose concentration. Determined kidney parameters include: creatinine, uric acid and glomerular filtration (in biochemical laboratory, using standard methods).

### Antioxidants and oxidative stress

Antioxidants (coenzyme Q<sub>10</sub>-TOTAL = ubiquinol+ubiquinone;  $\alpha$ -tocopherol,  $\gamma$ -tocopherol) in whole blood, plasma and isolated platelets were determined using HPLC method with UV detection (Lang et al. 1986) modified by authors (Kucharská et al. 1998). Total CoQ<sub>10</sub> concentrations were determined after oxidation with 1,4-benzoquinone (Mosca et al. 2002). A parameter of oxidative stress – thiobarbituric acid reactive substances (TBARS) was estimated by spectrophotometric method (Janero and Bughardt 1989).



**Figure 1.** Daily outside temperature recorded during the period of blood sampling: February to March 2018 (winter), April to May 2018 (spring). For the graph, publically available data from Slovak Hydrometeorological Institute ([www.shmu.sk](http://www.shmu.sk)) were applied.

### Platelets preparation

For platelets (PLT) isolation 18 ml of venous blood was collected to K<sub>3</sub>EDTA (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 200 × g 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-aminoethylether)-N,N,N',N'-tetraacetic acid) to final concentration 10 mmol/l. Next centrifugation in swing-out rotor without breaking at 1200 × g for 10 min resulted in a sediment containing PLT, which was washed with 4 ml of Dulbecco's Phosphate Buffered Saline (DPBS, Sigma-Aldrich, D8537), DPBS+10 mmol/l EGTA. After centrifugation at 1200 × g for 5 min, the sediment was resuspended in 0.4 ml of DPBS+10 mmol/l EGTA and used for the respirometric measurements (Sumbalová et al. 2016). 10 µl of PLT suspension was 10× 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, and 20 µl of PLT suspension was used for determination of mitochondrial marker – the activity of citrate synthase.

### Citrate synthase activity

The activity of mitochondrial enzyme citrate synthase was determined by spectrophotometric method (Srere 1969; Eigentler et al. 2015).

### High-resolution respirometry analysis

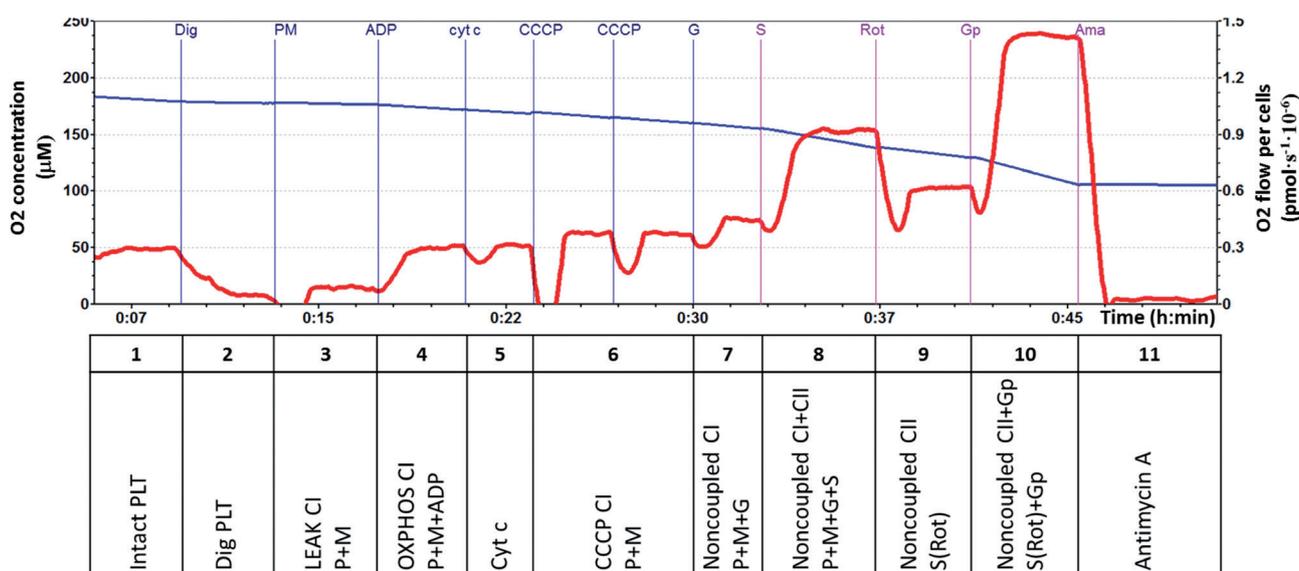
#### Platelets mitochondrial respiration and oxidative phosphorylation

For mitochondrial respirometric analysis, 200×10<sup>6</sup> PLT was used in 2 ml chamber of O2k-Respirometer (Oroboros Instruments, Austria) (Gnaiger et al. 2000; Pesta and Gnaiger 2012; Lemieux et al. 2017). The respiration was measured at 37°C in mitochondrial respiration medium MiR05+20 mM creatine using SUIIT (Substrate-Uncoupler-Inhibitor-Titration) protocol RP1 (Doerrier et al. 2016; Figure 2).

#### SUIIT protocol

SUIIT protocol for determination of respiration and OXPHOS in mitochondria of human PLT includes several steps (see Figure 2):

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<sup>-6</sup> 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) (P, 5 mM pyruvate; M, 2 mM malate) reflects LEAK rate of mitochondrial respiration compensating for proton leak, proton slip, cation cycling, and electron leak.



**Figure 2.** High-resolution respirometry of OXPHOS in platelets mitochondria. The effect of seasonal temperature on platelets mitochondrial respiration and OXPHOS of young subjects. Trace from the measurement of PLT respiration at 37°C in respiration medium MiR05+20 mM Cr following SUIIT protocol RP1. Blue line represents oxygen concentration, the red trace represents oxygen consumption as flow per cells (see online version for color figure). All steps and parameters are described in the section SUIIT protocol.

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: uncoupling of OXPHOS (P+M)*: After CCCP titration, *maximal oxidative capacity* at Complex I was measured.
7. *Noncoupled respiration at CI = (P+M+G)*: Addition of exogenous substrate G (10 mM glutamate) supported further Complex I-linked respiration.
8. *Noncoupled respiration at CI + CII = (P+M+G+S)*: Addition of exogenous substrate S (10 mM succinate) for Complex II (CII) 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 tables are expressed as mean  $\pm$  SEM.

#### Results

##### *Metabolic characteristics of young subjects during winter and spring seasons 2018*

During winter season, glucose concentration and the concentration of uric acid were significantly lower in comparison with spring season (glucose  $4.52 \pm 0.13$  vs.  $5.08 \pm 0.07$  mmol/l,  $p < 0.001$ ; uric acid  $254.77 \pm 10.48$  vs.  $297.27$

**Table 1.** Outside temperature, physical and metabolic characteristics of young participants involved in the study in winter and spring seasons 2018

Parameter	Winter	Spring	Reference values
Outside temperature (°C)	4.77	24.32***	–
<i>n</i> (number of participants)	16	23	–
Gender (M/F)	4/12	8/15	–
Age (years)	23 $\pm$ 0.7	22 $\pm$ 0.3	–
Height (cm)	168 $\pm$ 2.34	174 $\pm$ 2.14	–
Body weight (kg)	61.3 $\pm$ 1.89	67.1 $\pm$ 2.45	–
BMI (kg/m <sup>2</sup> )	22 $\pm$ 0.3	22 $\pm$ 0.5	–
Hemoglobin (g/l)	138 $\pm$ 3.43	139 $\pm$ 3.25	(130 – 180)
Leucocytes (cells $\times 10^9$ /l)	5.97 $\pm$ 0.41	6.95 $\pm$ 0.27	(3.8 – 10.6)
Platelets (cells $\times 10^9$ /l)	246 $\pm$ 20	269 $\pm$ 10	(150 – 400)
CRP (mg/l)	negative	negative	(0 – 5)
Triacylglycerols (mmol/l)	0.93 $\pm$ 0.11	0.89 $\pm$ 0.08	(0.10 – 1.70)
LDL-Cholesterol (mmol/l)	2.68 $\pm$ 0.13	2.60 $\pm$ 0.14	(0.26 – 2.60)
HDL-Cholesterol (mmol/l)	1.61 $\pm$ 0.11	1.47 $\pm$ 0.07	(0.90 – 1.45)
Cholesterol (mmol/l)	4.61 $\pm$ 0.18	4.21 $\pm$ 0.29	(2.90 – 5.00)
AST (µkat/l)	0.34 $\pm$ 0.02	0.36 $\pm$ 0.02	(0.00 – 0.85)
ALT (µkat/l)	0.514 $\pm$ 0.01	0.900 $\pm$ 0.10	(0.5 – 2.0)
GMT (µkat/l)	0.23 $\pm$ 0.02	0.300 $\pm$ 0.02	(0.00 – 0.92)
Glucose (mmol/l)	4.52 $\pm$ 0.13	5.08 $\pm$ 0.007**	(4.1 – 5.9)
Creatinine (µmol/l)	70.2 $\pm$ 2.82	74.5 $\pm$ 2.55	(64 – 104)
Uric acid (µmol/l)	254.8 $\pm$ 10.5	297.3 $\pm$ 14.9*	(208 – 428)
eGFR (ml/s/1.73m <sup>2</sup> )	1.84 $\pm$ 0.05	1.84 $\pm$ 0.04	(1.5 – 5.0)

BMI, body mass index; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GMT, gamma glutamyl transferase; eGFR, estimated glomerular filtration rate. Statistically significant differences are marked with asterisks: \*  $p < 0.05$ , \*\*  $p < 0.001$ , \*\*\*  $p < 0.0001$  vs. winter season.

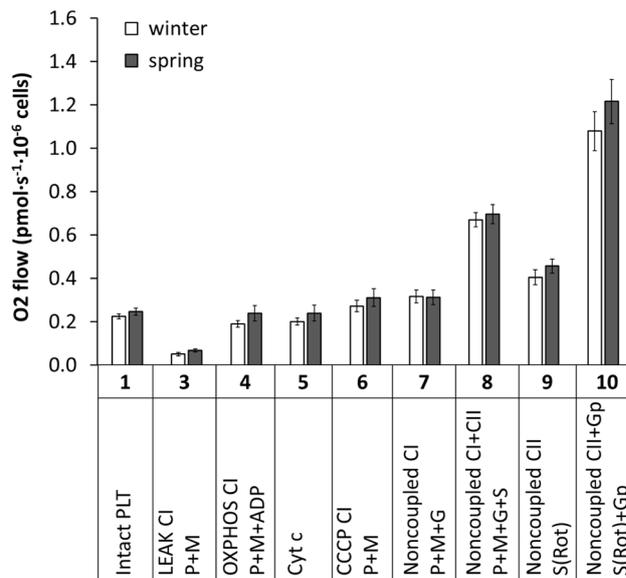
$\pm 14.95 \mu\text{mol/l}$ ,  $p < 0.05$ ), the levels were in reference values. Other metabolite parameters were not significantly affected by seasons (Table 1).

#### Antioxidants and oxidative stress

Winter outside temperature was associated with lower coenzyme Q<sub>10-TOTAL</sub> level in PLT in comparison with spring temperature by 29.24%, in whole blood by 10.10% and in plasma by 3.60%. Platelets  $\alpha$ -tocopherol concentration was lower in winter by 31.28%, not significantly. The concentration of this antioxidant was not affected by season temperature in whole blood or plasma. During winter temperature  $\gamma$ -tocopherol concentration was increased in PLT by 22.25%, in whole blood by 29.83% ( $p < 0.05$ ) and in plasma by 24.03% ( $p < 0.05$ ). The parameter of oxidative stress TBARS was slightly higher (+9.38%) during winter in comparison with spring (Table 2).

#### Mitochondrial respiration and OXPHOS in permeabilized platelets

All parameters of platelets oxygen consumption evaluated in  $\text{pmol}\cdot\text{s}^{-1}\cdot 10^{-6}$  cells were slightly, not significantly lower during winter outside temperature. Oxygen consumption of intact PLT was decreased by 8.8%, mitochondrial LEAK respiration in permeabilized PLT with CI-linked substrates (P+M) and OXPHOS at CI (P+M) were decreased by 25.2% and 16.2%, respectively, and noncoupled respiration at CI was decreased by 12.5% during winter. Noncoupled respiration after glutamate addition and noncoupled mitochondrial respiration at CI+CII were similar in both



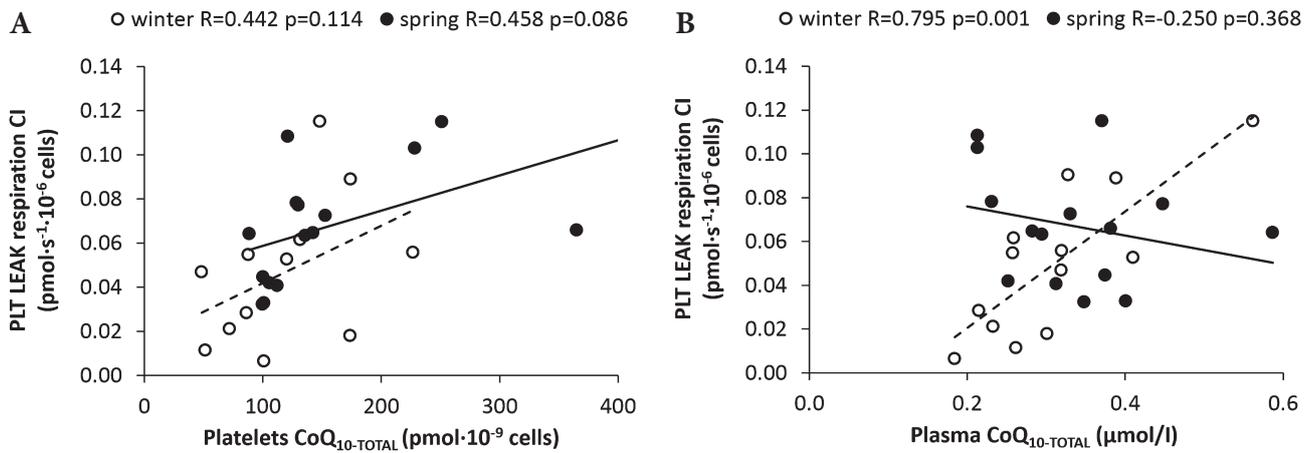
**Figure 3.** The effect of seasonal temperature on platelets mitochondrial respiration and OXPHOS of young subjects. Parameters of PLT respiration evaluated in  $\text{pmol}\cdot\text{s}^{-1}\cdot 10^{-6}$  cells are expressed as mean  $\pm$  SEM of measurements performed on PLT preparations from winter and spring groups of healthy young volunteers. All parameters are described in the section SUIT protocol.

groups. The electron transport capacity at CII measured after CI inhibition with rotenone and oxygen consumption after glycerophosphate addition were slightly lower in winter (-11.3% and -11.2%, respectively; Figure 3). The activity of citrate synthase in PLT was similar in both

**Table 2.** Concentration of CoQ<sub>10-TOTAL</sub>,  $\alpha$ -tocopherol,  $\gamma$ -tocopherol in platelets, whole blood and plasma of healthy young volunteers during winter and spring outside temperature

Parameter	Winter	Spring	Reference values
<b>CoQ<sub>10-TOTAL</sub></b>			
Platelets ( $\text{pmol}\cdot 10^{-9}$ cells)	128.8 $\pm$ 14.3	167.1 $\pm$ 17.2	-
Whole blood ( $\mu\text{mol/l}$ )	0.208 $\pm$ 0.012	0.229 $\pm$ 0.014	-
Plasma ( $\mu\text{mol/l}$ )	0.335 $\pm$ 0.025	0.347 $\pm$ 0.021	(0.4 - 1.0)
<b><math>\alpha</math>-Tocopherol</b>			
Platelets ( $\text{pmol}\cdot 10^{-9}$ cells)	2429 $\pm$ 268	3189 $\pm$ 300	-
Whole blood ( $\mu\text{mol/l}$ )	15.1 $\pm$ 0.565	15.0 $\pm$ 0.740	-
Plasma ( $\mu\text{mol/L}$ )	21.5 $\pm$ 0.899	20.8 $\pm$ 1.0	(15 - 40)
<b><math>\gamma</math>-Tocopherol</b>			
Platelets ( $\text{pmol}\cdot 10^{-9}$ cells)	279.6 $\pm$ 42.3	217.4 $\pm$ 24.0	-
Whole blood ( $\mu\text{mol/l}$ )	1.14 $\pm$ 0.116	0.800 $\pm$ 0.100*	-
Plasma ( $\mu\text{mol/l}$ )	1.64 $\pm$ 0.187	1.17 $\pm$ 0.091*	(2 - 7)
<b>TBARS</b>			
Plasma ( $\mu\text{mol/l}$ )	5.12 $\pm$ 0.435	4.64 $\pm$ 0.094	(< 4.5)

Statistically significant differences are marked with asterisks: \*  $p < 0.05$  vs. winter season.



**Figure 4.** Correlation between platelets mitochondrial LEAK respiration at CI and CoQ<sub>10-TOTAL</sub> concentration in platelets (A) and in plasma (B) of healthy young volunteers during winter and spring season. PLT, platelets; CI, complex I; CoQ<sub>10-TOTAL</sub>, total concentrations of coenzyme Q<sub>10</sub>.

groups ( $0.331 \pm 0.012 \text{ nmol}\cdot\text{min}^{-1}\cdot 10^{-6}$  cells in winter and  $0.330 \pm 0.016 \text{ nmol}\cdot\text{min}^{-1}\cdot 10^{-6}$  cells in spring). Normalization of respiration for this mitochondrial marker supported the conclusion about no significant difference in PLT respiration between winter and spring group (data are not shown).

*The correlation between platelets mitochondrial function and concentration of CoQ<sub>10-TOTAL</sub>*

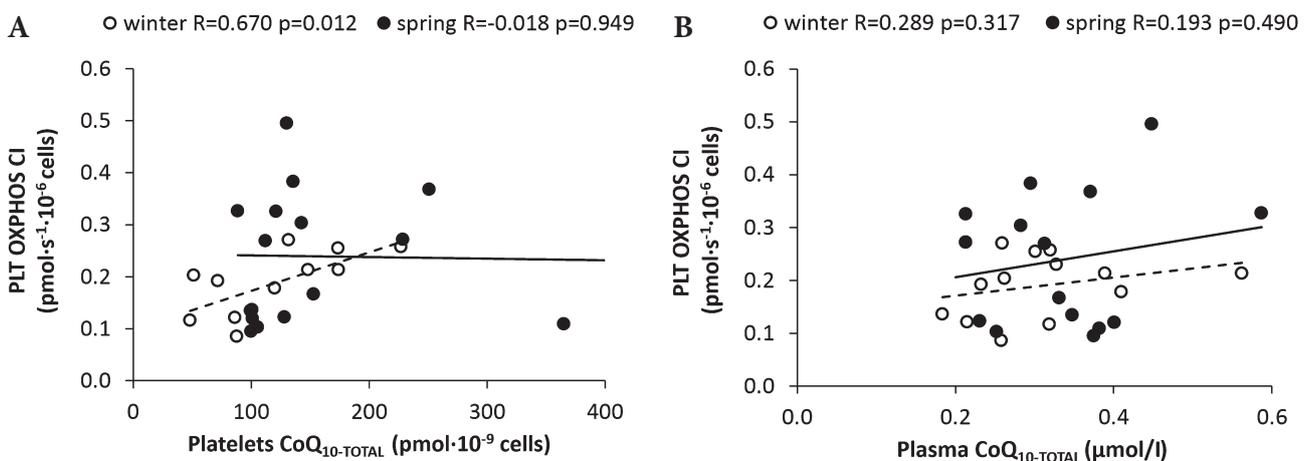
The correlation between LEAK respiration at CI in PLT mitochondria and PLT CoQ<sub>10-TOTAL</sub> concentration was not significant in winter (Figure 4A), but LEAK respiration at

CI in winter significantly correlated ( $p = 0.001$ ) with plasma CoQ<sub>10-TOTAL</sub> concentration (Figure 4B).

PLT mitochondrial OXPHOS at CI significantly depended on PLT CoQ<sub>10-TOTAL</sub> concentration ( $p = 0.012$ ), Figure 5A, not on plasma CoQ<sub>10-TOTAL</sub> concentration at winter temperature (Figure 5B).

**Discussion**

Seasonal factors, as temperature, diseases, hormonal changes, reproduction and growth, may regulate metabolic function and contribute to the development of diseases. Most of the



**Figure 5.** Correlation between platelets mitochondrial OXPHOS at CI and CoQ<sub>10-TOTAL</sub> concentration in platelets (A) and in plasma (B) of healthy young volunteers during winter and spring season. PLT, platelets; CI, complex I; CoQ<sub>10-TOTAL</sub>, total concentrations of coenzyme Q<sub>10</sub>.

available information about the effect of the season on mitochondrial function and antioxidants are focused on various animals and birds.

The activities of antioxidant enzymes (glutathione peroxidase, superoxide dismutase, glutathione reductase, total antioxidant status) and malondialdehyde concentration were found higher during summer season than in winter in Karan Fries bull (Soren et al. 2018).

#### *Antioxidants and lipid peroxidation*

In our study we did not find significant seasonal differences in CoQ<sub>10-TOTAL</sub> and  $\alpha$ -tocopherol concentration in platelets, whole blood and plasma as well as plasma lipid peroxidation in human subjects. However, CoQ<sub>10-TOTAL</sub> concentration and  $\alpha$ -tocopherol in platelets were reduced in winter by 29.24% and 31.28%, respectively. On the other hand, the concentrations of  $\gamma$ -tocopherol in blood and plasma were significantly higher ( $p < 0.05$ ) in winter in comparison with spring season (Table 2). Other authors in a Swiss population found higher concentrations of  $\gamma$ -tocopherol in winter and spring than in other seasons (Winklhofer-Roob et al. 1997). High concentrations of two tocopherol isomers ( $\gamma$  and  $\delta$ ) found in tissues of insects in winter as protection against the higher risk of oxidative damage to PUFA in membranes (Košťál et al. 2013). Concentrations of  $\gamma$ -tocopherol in blood and tissues are dependent on cytochrome P<sub>450</sub> metabolism in the liver (Abe et al. 2007). We suppose that the inhibition of cytochrome P<sub>450</sub> activity due to the slightly increased lipid peroxidation and decreased antioxidant protection in winter could affect concentrations of  $\gamma$ -tocopherol.

#### *Platelets mitochondrial respiration and oxidative phosphorylation*

Seasonal variations on mitochondrial function in mammals, in various animals and birds were documented (Bernabucci et al. 2002; Fultado-Filho et al. 2007; Bhat et al. 2008; Mujkosová et al. 2008; Konior et al. 2011; Van de Cromennacker et al. 2011). An effect of higher temperature and hibernation during season on mitochondrial function was found in fish of Adriatic sea (Chainy et al. 2016). In mitochondria isolated from the eurythermal bivalve *Mya arenaria* from a low-shore intertidal population of the German Wadden Sea respiration was measured between 5–15°C and 20–25°C. The highest mitochondrial respiration (state 3 and state 4) and respiratory control ratios (RCR) were found at 15°C, at higher temperature RCR decreased, and released ROS were doubled between 15 and 25°C (Abele et al. 2002). In oysters a strong effect of seasonality on mitochondrial function was found (Cherkasov et al. 2010). Low temperature is characterized

by lower metabolism. Hypothermia suppresses platelets metabolism, increases volume and deformation of platelets, may enhance platelets aggregation (Poucke et al. 2014). Decreased mitochondrial ATP production, lower membrane potential and increased free oxygen radicals production were reported at hypothermia (Hendriks et al. 2017).

Increased mitochondrial respiration in peripheral blood mononuclear cells was associated with low concentration of vitamin D in healthy adults during winter season with lower intensity of UV sunlight (Calton et al. 2016, 2017). This effect on bioenergetics of immune cells corresponded to activation of immune system by insufficient vitamin D concentration (Calton et al. 2015).

Our data showed no significant difference in respiration of intact and permeabilized PLT between winter and spring season in young healthy humans. Although all parameters of PLT respiration were slightly lower in winter season, the differences between the two groups of young healthy participants examined in winter and spring were not significant. In our previous study we did not find significant differences in PLT respiration between healthy young and elderly people (Gvozdjaková et al. 2018).

Together these findings support the findings of others that significant changes in PLT respiration indicate disease-related mitochondrial dysfunction. Impaired platelet mitochondrial function has been found in various human diseases, in critically ill patients with sepsis and cardiogenic shock (Protti et al. 2015), in patients with type 2 diabetes, Alzheimer's (Bosetti et al. 2002; Shi et al. 2008), Huntington's disease (Parker et al. 1990) and migraine headaches (Sangiorgi et al. 1994). Lower platelet mitochondrial Complex I and Complex II-III activity was seen in early untreated Parkinson's disease patients (Haas et al. 1995), while patients with schizophrenia showed an increase in Complex I activity (Zharikov and Shiva 2013). In platelets of patients with septic shock and cardiogenic shock activities of mitochondrial complexes were lower in comparison with 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). 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 I, III and IV, and citrate synthase (Bohm et al. 2007). A decrease of platelets mitochondrial Complex I-linked respiration was found in humans after statins treatment (Vevera et al. 2016).

### *The correlation between platelet mitochondrial function and coenzyme Q<sub>10</sub> concentration*

Figure 3 shows slightly decreased oxygen consumption by intact platelets and slightly decreased platelets mitochondrial respiration at LEAK and OXPHOS state with CI-linked substrates. In winter season LEAK respiration at CI positively correlates with plasma CoQ<sub>10-TOTAL</sub> concentration ( $p = 0.001$ ), not with CoQ<sub>10-TOTAL</sub> concentration in platelets (Figures 4A, B).

Opposite, platelets mitochondrial respiration associated with ATP synthesis via oxidative phosphorylation stimulated by saturating concentration of ADP at Complex I in winter season significantly depended on CoQ<sub>10-TOTAL</sub> concentration in platelets ( $p = 0.012$ ), not on CoQ<sub>10-TOTAL</sub> concentration in plasma (Figures 5A, B). These correlations were not found in spring season. We suppose that concentration of coenzyme Q<sub>10</sub> as a key component of mitochondrial respiratory chain necessary for ATP synthesis is important for platelets mitochondrial function in winter season. In our previous study we found significant dependence of platelets mitochondrial OXPHOS function on their CoQ<sub>10</sub> concentration in young, not in elderly humans (Gvozdjaková et al. 2018). These findings suggest that insufficient CoQ<sub>10</sub> concentration in platelets could adversely affect platelet mitochondrial function, however, limiting concentrations are not known.

Coenzyme Q<sub>10</sub> supplementation at winter season is recommended. The mechanism of hypothermia on platelets function have not been fully elucidated.

### **Conclusion**

Our data showed no significant difference in platelets mitochondrial function between two groups of healthy young people examined in winter and spring season, although in the winter season platelets mitochondrial respiration associated with ATP production as well as coenzyme Q<sub>10</sub> concentration were slightly decreased. The importance of CoQ<sub>10-TOTAL</sub> for platelets mitochondrial respiration and oxidative phosphorylation during winter season was demonstrated.

We conclude that seasonal temperature participates on the mechanisms of platelets mitochondrial respiratory chain function and oxidative phosphorylation, which depend on their CoQ<sub>10-TOTAL</sub> concentration at lower winter outside temperature. CoQ<sub>10</sub> supplementation may improve platelet mitochondrial respiration associated with ATP production in the winter season. High-resolution respirometry offers a sensitive method for detecting slight changes in platelet mitochondrial function.

**Disclosure.** This work was not published before; part of the results were presented at the 9<sup>th</sup> Conference of the International Coenzyme

Q<sub>10</sub> Association, New York, USA, June 21–24, 2018, (Nemec et al. 2018). This publication was approved by all coauthors.

**Conflict of interest.** The authors declare that they have no conflict of interests.

**Author's contribution.** AG prepared design, managed this study and wrote manuscript; JK measured and evaluated antioxidants and revised the manuscript; ZS and MN evaluated respirometric data and revised the manuscript; ZS measured and evaluated citrate synthase activity, prepared figures and tables; ACh managed blood collection and evaluated biochemical parameters; OV, ZR, MK, ZK performed respirometric measurements; VM managed blood collection, biochemical parameters and revised the manuscript.

**Acknowledgements.** Grant KEGA Ministry of Education. 063UK-4/2017 and Grant VEGA Ministry of Education, No. 1/0039/19.

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Received: November 26, 2018

Final version accepted: March 20, 2019

First published online: June 26, 2019