

Seasonal variation in mitochondrial bioenergetics of human peripheral blood mononuclear cell

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Introduction

Peripheral blood mononuclear cell (PBMC) bioenergetics is a potential biomarker of systemic mitochondrial function [1,2]. Altered PBMC mitochondrial function has been associated with pathological conditions including neurodegenerative, metabolic, and inflammatory diseases [2,3]. However, findings across studies remain inconsistent [4,5]. These discrepancies may partly reflect methodological differences in PBMC isolation and bioenergetic assessment [6], although physiological adaptations of PBMCs may contribute to the observed variability. Consequently, additional studies are needed before PBMC bioenergetics can be established as a reliable clinical biomarker.

Seasonal variation is known to influence numerous blood parameters through changes in circadian rhythms, melatonin secretion, vitamin D status, and other environmental and physiological factors [7,8]. Several groups have reported seasonal fluctuations in mitochondrial DNA content in PBMCs [9-11]. However, whether PBMC bioenergetics is influenced by seasonality remains unclear. Understanding the impact of seasonal variation is essential for establishing standardized clinical protocols and distinguishing disease-related mitochondrial dysfunction from physiological adaptations.

In this study, we assessed PBMC mitochondrial respiration in 20 healthy volunteers from Innsbruck (Tyrol, Austria) at five time points over one year, with the first and last visits in winter, to evaluate whether PBMC bioenergetics exhibits seasonal variation within individuals. In parallel, PBMC bioenergetics was evaluated in an independent cross-sectional cohort of approximately 800 participants from the same region (Tyrol, Austria) enrolled in the VASCage Innsbruck Health Promotion Program (INN.HEALTH). PBMCs were isolated, cryopreserved, and mitochondrial activity was analyzed by high-resolution respirometry to establish reference values for PBMC bioenergetics and to evaluate the effects of age, sex, and season.

Methods

Study design and participants

Seasonal variation in PBMC mitochondrial function was assessed in 20 healthy volunteers (37 ± 8.9 years; 1:1 female-to-male ratio) sampled at five time points over a 12-month period. Whole blood was collected at the Department of Neurology, Medical University of Innsbruck, and transported to the Technical University of Innsbruck for PBMC isolation and cryopreservation, as previously described [11]. Additional blood samples were collected for hematological analyses, and demographic, nutritional, and sleep-related data were recorded at each visit. In parallel, 783 participants enrolled in the INN.HEALTH study were included. Samples were collected between May 2025 and April 2026. Mean age was 54.3 years (SD 18.4; median 57.0 y, range 18–90 y). The cohort comprised 397 women (50.7%) and 386 men (49.3%). The study was approved by the Ethics Committee of the Medical University of Innsbruck (EK 1354/2023), and all participants provided written informed consent.

PBMC isolation and high-resolution respirometry

PBMCs were isolated from whole blood by density gradient centrifugation and cryopreserved at -80°C for one week prior to analysis. Bioenergetic assessments were performed by high-resolution respirometry (HRR) using an Oroboros O2k system, following the SUIT-003_D115 protocol [6]. Samples were collected after HRR measurement for posterior analysis of mitochondrial content.

Data analysis

Seasonal variation in each respiratory state was modelled using linear mixed-effects models with sine/cosine terms for day-of-year. Seasonal effects were tested via likelihood-ratio tests against null models, with Benjamini–Hochberg–adjusted p -values and marginal r^2 reported. Model-independent variance decomposition (between- vs within-participant variance) validated mixed-model intraclass correlation coefficient estimates.

Results and discussion

PBMC bioenergetic analyses from both, the longitudinal (small) cohort and the INN.HEALTH (large) cohort, demonstrated stable routine and leak respiration throughout the year. In contrast, oxidative electron transfer (ET) capacity exhibited significant seasonal differences.

In living PBMCs, ET capacity was significantly higher during summer (May to August) than winter (October to February) in both cohorts, with the seasonal decline being more pronounced in the larger cohort. This seasonal effect became even more evident following plasma membrane permeabilization with digitonin, indicating that substrate-specific ET pathways are influenced by season. In particular, respiration

supported by the succinate (CII-linked) pathway showed the most pronounced seasonal variation.

Our findings demonstrate that while routine mitochondrial respiration remained stable across the year, the reserve respiratory capacity, specifically CII-linked oxidative phosphorylation, was subject to seasonal modulation. This highlights the importance of considering the timing of sample collection in studies assessing mitochondrial function in PBMCs and suggests that seasonal effects represent a relevant biological confounder in clinical and translational bioenergetic studies.

Standardization of sampling time, or recording and adjusting for season, improve the reliability of PBMC bioenergetics as a biomarker in mitochondrial diagnostics, prevention, and therapy. Further studies are required to explore the mechanisms underlying these seasonal adaptations, including potential roles of vitamin D, melatonin, and physical activity.

Keywords: PBMC; mitochondrial respiration; high-resolution respirometry; seasonality; ET capacity; biomarkers.

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