

Longitudinal PBMC respirometry reveals preserved electron-transfer capacity but constrained bioenergetic flexibility in Parkinson's disease

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Introduction: In Parkinson's disease (PD), peripheral mitochondrial changes may reflect reduced electron-transfer (ET) capacity, altered respiratory control, or both [1,2]. Protein-normalized flux does not show how respiration uses available ET capacity; internally normalized indices provide complementary information on respiratory partitioning [3]. We used longitudinal PBMC respirometry to assess these features and explore shared bioenergetic profiles.

Methods: This two-year cohort comprised 114 participants: 57 with PD (PD1, $N = 29$; PD2, $N = 28$) and 57 matched controls. Diagnosis followed Movement Disorder Society criteria; baseline Hoehn–Yahr ranges were 0.5–2.0 (PD1) and 2.0–3.5 (PD2). Exclusions were other neurological disease, diabetes, relevant renal/hepatic disease, inflammation, infection, or serious systemic illness. Blood was sampled at baseline and annually; usual dopaminergic treatment continued. Ethics approval and written consent were obtained.

PBMCs were isolated from EDTA blood by Histopaque density-gradient centrifugation and assessed fresh at 37 °C in MiR05 using an Oroboros O2k (about $6 \cdot 10^6$ cells in each 2 mL chamber). SUI titrations recorded routine and pyruvate-supported respiration, oligomycin-induced leak (L), CCCP-stimulated ET capacity (E), rotenone and succinate states, digitonin permeabilization, cytochrome c response, and antimycin A residual oxygen consumption rox [1]. Fluxes were rox -corrected and protein-normalized. Indices were $j_{RLE} = (R-L)/E$, $j_{E-R} = (E-R)/E$, $j_{R-L} = (R-L)/R$, and $j_{E-L} = (E-L)/E$. Age- and sex-adjusted mixed models tested Year $\times$ Group with participant

random intercepts and Tukey-adjusted contrasts. Baseline clustering/PCA used j_{RLE} , j_{R-L} , and j_{E-R} in 113 complete cases; PERMANOVA used 999 permutations. Clusters were exploratory, without stability validation.

Results and discussion: Protein-normalized routine, leak, and ET-capacity fluxes showed no consistent group differences across the three assessments. The routine ATP-coupling ratio (j_{RLE}) was higher in PD1 than HC1 at baseline, year 1, and year 2 (Tukey-adjusted $p = 0.008$, 0.006 , and 0.010); in PD2 it was higher than HC2 at baseline and year 1 ($p = 0.006$ and <0.001), but not year 2 ($p = 0.060$). The $E-R$ excess ratio (j_{E-R}) was lower in PD1 than HC1 throughout follow-up ($p = 0.015$, 0.013 , and 0.020) and in PD2 at years 1 and 2 ($p = 0.001$ and 0.015). Other coupling indices showed only transient changes.

Clustering of 113 baseline cases identified high j_{E-R} /lower j_{RLE} (C1, $N = 42$), intermediate (C2, $N = 41$), and constrained j_{E-R} /higher j_{RLE} profiles (C3, $N = 30$). Controls were enriched in C1 and PD samples more frequent in C3, although groups overlapped. PCA/PERMANOVA supported group separation along a shared bioenergetic gradient ($p = 0.001$). Results support sustained redistribution toward greater routine engagement and lower $E-R$ excess despite preserved ET capacity. PBMC profiles reflect systemic, not neuronal, bioenergetics; cell composition may contribute.

Keywords: Parkinson's disease; PBMCs; mitochondrial respiratory control; bioenergetic flexibility; high-resolution respirometry

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Conflicts of interest: EG is CEO of Oroboros Instruments and provided methodological advice and manuscript review. The company had no role in funding, data collection, data ownership, or submission. The other authors declare no competing interests.

References

1. Schirinzì T, Salvatori I, Zenuni H, Grillo P, Valle C, Martella G, Mercuri NB, Ferri A (2022) Pattern of mitochondrial respiration in peripheral blood cells of patients with Parkinson's disease. *Int J Mol Sci.* 23:10863. <https://doi.org/10.3390/ijms231810863>
2. Zharikov AD, Cannon JR, Tapias V, Bai Q, Horowitz MP, Shah V, El Ayadi A, Hastings TG, Greenamyre JT, Burton EA (2015) shRNA targeting α -synuclein prevents neurodegeneration in a Parkinson's disease model. *J Clin Invest.* 125:2721–35. <https://doi.org/10.1172/JCI64502>
3. Gnaiger E (2026) Mapping metabolic flexibility of respiratory flux control: Bioenergetic cluster analysis of mitochondrial fitness. *BEC preprints 2026.5.v2.* <https://doi.org/10.26124/becprep.2026-0005.v2>
4. Gnaiger E (2025) Mitochondrial respiratory function in living cells. *Bioenerg Commun* 2025.5. <https://doi.org/10.26124/bec.2025-0005>

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