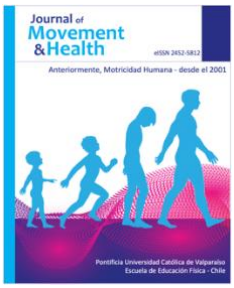
Journal of Movement and Health 2026,23(2):1-11.
DOI: https://doi.org/10.5027/jmh-Vol23-Issue2(2026)art277




eISSN: 2452-5812
http://jmh.pucv.cl/

**Received:** 16/12/2025
**Accepted:** 04/03/2026
**Available:** 18/03/2026
**Published**: 01/07/2026

## Original article

# Mitophagy Markers and Mitochondrial Oxidative Phosphorylation in Peripheral Blood Mononuclear Cells after Endurance Exercise under Normobaric Hypoxia: A Pilot study

Marcadores de mitofagia y fosforilación oxidativa mitocondrial en células mononucleares de sangre periférica tras ejercicio de resistencia en hipoxia normobárica: Estudio piloto

**Alarcón-Cortés, JP[1], Vargas-Foitzick, R[2]; Lang, M[2]; Gutiérrez, J[2]; Viscor, G[3]; Figueroa, A[2]; Castro-Sepúlveda, M[2]**

Correspondence✉
MSc. Juan Pablo Alarcón Cortés
University of Playa Ancha, Department of Rehabilitation, Intervention, and Therapeutic Approach
juan.alarcon@upla.cl

**Abstract**
Hypoxia and endurance exercise (EE) independently induce cellular stress, affecting mitophagy and mitochondrial function. Their combined effect remains poorly understood in human peripheral blood mononuclear cells (PBMCs). This exploratory pilot study aimed to generate hypotheses regarding the acute effects of EE under normobaric hypoxia (NH) on markers of mitochondrial oxidative phosphorylation (OXPHOS) and mitophagy in PBMCs. **Methods:** Four men performed an acute bout of EE under normoxia and NH in a crossover design. Physiological parameters and PBMCs were collected before and after exercise. OXPHOS proteins and parkin were quantified in PBMCs. In addition, basal oxygen consumption rate (OCR) was assessed in PBMCs. **Results:** In PBMCs, NH tended to reduce Complex I ($p = 0.07$), whereas EE increased Complex II ($p = 0.04$). NH decreased parkin abundance ($p = 0.01$), while EE tended to increase it ($p = 0.07$), with no significant condition-by-intervention interaction ($p = 0.56$). **Conclusion:** This pilot study suggests differential modulation of parkin in PBMCs by EE and NH, without changes in basal OCR. The absence of a significant EE × NH interaction on parkin in this small sample supports the need for further investigation in larger cohorts.

**Keywords:** endurance; exercise; hypoxia.; mitophagy; normobaric

**Resumen**
La hipoxia y el ejercicio de resistencia (ER) inducen estrés celular independientemente, afectando la mitofagia y función mitocondrial. El efecto combinado aún no se comprende en células mononucleares de la sangre periférica (PBMCs) en humanos. Este estudio piloto exploratorio tuvo como objetivo generar hipótesis sobre los efectos del ER agudo en hipoxia normobárica (HN) sobre marcadores de fosforilación oxidativa mitocondrial (OXPHOS) y mitofagia en PBMCs **Métodos:** Cuatro varones realizaron ER agudo en normoxia y NH en un diseño cruzado. Se obtuvieron parámetros fisiológicos y PBMCs antes y después del ejercicio. Cuantificando OXPHOS y parkin en PBMCs. Además, se midió la tasa de consumo basal de oxígeno (OCR) en PBMCs. **Resultados:** En PBMCs, NH tendió a disminuir el Complejo I ($p = 0,07$) y ER incrementó el Complejo II ($p = 0,04$). NH disminuyó parkin ($p = 0,01$) y el ER tendió a aumentarla ($p = 0,07$), sin una interacción significativa entre ambos factores($p = 0,56$). **Conclusión:** Este estudio sugiere una modulación diferencial de parkin en PBMCs por ER y NH, sin afectar la OCR basal. La falta de interacción significativa entre ER y NH sobre parkin en esta pequeña muestra justifica mayor investigación en cohortes más amplias.

**Palabras clave:** resistencia; ejercicio; hipoxia; mitofagia; normobárica

DOI: https://doi.org/10.5027/jmh-Vol23-Issue2(2026)art277

## Key points

- Normobaric hypoxia induced by the hypoxic generator Everest Summit II elicits measurable reductions in peripheral oxygen saturation and triggers an autonomic/sympathetic response in healthy young adults.
- Acute endurance exercise produced an effect on OXPHOS Complex II abundance in PBMCs that was independent of hypoxic exposure.
- Normobaric hypoxia, independent of exercise, induced changes in parkin protein abundance in PBMCs in a small cohort of healthy subjects.

## Introduction

Hypoxia and physical exercise are both metabolic stressors that can trigger significant physiological adaptations[1,2]. In line with the mitochondrial quality-control framework described by Picca et al.[3], hypoxia alone robustly induces mitophagy through HIF-1–linked receptor pathways, including BNIP3/BNIP3L[4] and FUNDC1-mediated mechanisms[5]. Exercise alone has been shown to promote mitochondrial remodeling and activate mitophagy in skeletal muscle[6,7]. Collectively, these studies support the idea that either stressor independently engages mitophagy as a key adaptive pathway to maintain mitochondrial homeostasis under metabolic challenge[8,9].

Moderate hypoxia (altitudes of 1,800-3000 m) and exercise combined produce unique physiological effects that are not observed under normoxic conditions[10]. Normobaric hypoxia (NH) technologies, such as masks and tents that reduce oxygen fractional concentration (FiO2), have been shown to elicit similar physiological responses to hypobaric hypoxia, including increased ventilation[11], tachycardia, and altered heart rate variability (HRV)[12]. These NH technologies thus serve as valuable tools for understanding the effects of hypoxia on human physiology.

Consequently, our study contributes to filling the knowledge gap regarding the acute effects on mitochondrial dynamics, mitophagy, and Peripheral blood mononuclear cells (PBMCs) respiration under normobaric hypoxia conditions during moderate-intensity cardiorespiratory exercise. This is because it has been evidenced that both exercise and acute hypoxia have effects on enhancing mechanisms that regulate the first line of defense of our organism against a wide range of pathogens, through the increase of hormones that respond to the stress of both conditions[13].

However, to date, there is no evidence demonstrating that this physiological alteration attributable to simulated hypoxia with a decrease in the partial pressure of oxygen from the alveoli to the circulation generates a modification of mitochondrial dynamics in PBMCs. Studying PBMCs is a less invasive strategy than muscle biopsies for investigating mitochondrial bioenergetics[14] and systemic function[15].

PBMCs are essential components of the immune response and serve as a valuable, less invasive model for studying physiological and mitochondrial dynamics processes at the cellular level in humans[16]. This study aims to determine if endurance exercise under normobaric hypoxic (NH) conditions induces mitophagy, a process crucial for human health and a potential therapeutic target for diseases such as neurodegenerative disorders (such as Alzheimer's and Parkinson's disease), metabolic conditions (insulin resistance and type 2 diabetes), and various forms of cancer (including hepatocellular carcinoma and breast cancer)[3], and alters mitochondrial function in PBMCs of healthy young individuals. We hypothesize that a single session of endurance exercise under normobaric hypoxic conditions could modify mitophagy markers and mitochondrial function in the PBMCs of healthy young individuals.

DOI: https://doi.org/10.5027/jmh-Vol23-Issue2(2026)art277

## Methods

A quasi-experimental design conducted at the Exercise Physiology and Metabolism Research Laboratory of Universidad Finis Terrae (UFT). The research was descriptive and received approval from the UFT Scientific Ethics Committee in Research (ID-23-0), in accordance with the Declaration of Helsinki. Participants signed an informed consent form and could withdraw from the study at any time.

The present human study employed a repeated-measures experimental design to investigate the cardiovascular response, the expression of pro-mitophagic proteins, the capacity for oxidative phosphorylation, and the mitochondrial oxygen consumption rate (OCR), before and after an acute bout of endurance exercise under normoxic and normobaric hypoxic conditions

*Participants*

Six healthy human male volunteers were recruited to participate in the study, completing assessments and physical exercise tests under both normoxic and NH conditions. Each subject completed a pre-participation survey and underwent a resting electrocardiogram (characteristics of participants in Table 1). However, enough PBMCs samples of adequate quality and quantity for molecular biology analyses were only obtained from four participants. The sample size (n=6 recruited n=4 for molecular analyses) was determined based on the pilot nature of this study, which aimed to establish the feasibility of the experimental protocol and obtain preliminary data for future research.

**Table 1.** Anthropometry and aerobic fitness characteristics of participants.

| Subject | Age (years) | Weight (kg) | Height (m) | BMI (kg/m$^2$) | $VO_2$max (ml/kg/min) |
|---|---|---|---|---|---|
| 1 | 34 | 88.3 | 1.74 | 29.2 | 42.1 |
| 2 | 26 | 78 | 1.72 | 26.4 | 39.6 |
| 3 | 30 | 93.1 | 1.77 | 29.7 | 37.2 |
| 4 | 31 | 97.9 | 1.77 | 31.2 | 34.6 |
| Mean ± SD | 30 ± 2 | 89.3 ± 6.2 | 1.75 ± 0.02 | 29.1 ± 1.4 | 38.4 ± 2.5 |

Notes: BMI = Body Mass Index; VO2max = Maximum oxygen uptake

*Procedure*

*Day 1*: Each volunteer visited the lab for 3 days, separated by a minimum of 72 hours. The volunteers arrived at the laboratory after an overnight fasting period of 8 hours. Height and body mass were measured using a 700 dry mechanical scale with an SECA 220 telescopic height rod. Resting heart rate was recorded with a Polar H10 monitor (Polar Electro Oy, Kempele, Finland), while peripheral oxygen saturation was measured using a Nonin Onyx (Nonin Inc., Plymouth, MN, USA). Systolic/diastolic blood pressure was assessed with an Omron HEM-7120 monitor (OMRON Healthcare Co., Ltd., China). A 12-lead electrocardiogram (Ergocard CPX from Medisoft, Belgium) was also performed at rest. Additionally, maximal oxygen consumption ($VO_2$max) was assessed using a stepped incremental protocol. The initial workload was set at 50 watts, with subsequent increments of 25 watts applied every 120 seconds. with expired gas analysis (Ergocard CPX from Medisoft, Belgium), and the power output was measured on a cycle ergometer (Ergoline 800s; SensorMedics, Yorba Linda, CA).

*Day 2:* The second day involved a single session of endurance exercise on a cycle ergometer under Hypo conditions, simulating an altitude of 2,743 meters with a $FiO_2$ of 0,148 %, which is considered safe for health[17]. For the 'Hypoxia-Rest' condition, participants were exposed to hypoxia using a mask (Altitude Training Mask, Hypoxico Altitude Training Systems, USA), connected via a corrugated plastic tube to a hypoxic generator (Everest Summit II, Hypoxico Altitude Training Systems, USA) for 45 minutes while resting. At the end of this 45-minute period, venous blood was drawn from the antecubital

DOI: https://doi.org/10.5027/jmh-Vol23-Issue2(2026)art277

fossa. Next, the volunteers were positioned lying down, and HRV was assessed for 10 minutes using a portable electrocardiogram (Bittium Faros 180°; Bittium Inc., Oulu, Finland). Following this, participants began their 45-minute endurance exercise on a cycle ergometer at 50% of their $VO_2$ max. Hypoxic exposure during the exercise was induced using a mask (Altitude Training Mask, Hypoxico Altitude Training Systems, USA), connected via a corrugated plastic tube to a hypoxic generator (Everest Summit II, Hypoxico Altitude Training Systems, USA). At the end of the session, a second venous blood draw was performed immediately post-exercise.

Day 3: On the third day, the same measurements were replicated as on Day 2, but volunteers exercised under normoxic conditions. To control for the effect of wearing the mask and being connected to the equipment, participants wore the mask connected via a corrugated plastic tube to the hypoxic generator (Everest Summit II, Hypoxico Altitude Training Systems, USA), but the generator was turned off, so there was no exposure to hypoxia. As on Day 2, venous blood was drawn before and immediately after the 45-minute endurance exercise session. Days 2 and 3 were randomized.

*Instruments*

*Heart rate variability determination*

Heart rate variability (HRV) was registered while lying down for 10 minutes before and after each exercise session, obtaining beat-to-beat RR intervals (Bittium Faros 180°; Bittium Inc., Oulu, Finlandia) he inclusion of HRV measurement served as a positive control to confirm the expected increase in sympathetic nervous system activity in response to exercise, particularly under hypoxic conditions.

Data corresponding to at least 5 minutes of the recording were selected and processed using Kubios HRV software version 3.5.0 (Medical Biosignal and Image Analysis Group, Department of Physics, University of Kuopio, Kuopio, Finland) removing artefacts by automatic filtering with a 5% threshold. Time and frequency domains were analyzed. In the time domain, the primary markers of heart rate variability selected is RMSSD (root mean square of the sum of differences between adjacent RR intervals)[18]. In the frequency domain, low frequency (LF between 0.05 Hz and 0.15 Hz), high frequency (HF between 0.15 Hz and 0.4 Hz), and the LH/HF ratio were analyzed[19]. The high frequency band corresponds to the respiratory frequency that allows the identification of parasympathetic modulation of cardiac activity. The low frequency band is related to blood pressure oscillations. At rest, the LF/HF index is considered a marker of sympathetic-parasympathetic balance and may represent an index of sympathetic activity[20].

*PBMC isolation*

According to the protocol of Castro-Sepúlveda et al.[16], 10 mL of freshly drawn blood was used to isolate PBMCs under sterile conditions using a Ficoll-Histopaque 1077 gradient (Sigma, Milan, Italy) to obtain approximately 80% lymphocytes. To achieve this, blood was diluted with an equal volume of Dulbecco's phosphate-buffered saline (PBS) and gently layered onto an equal volume of Ficoll-Histopaque 1077 in centrifuge tubes. The samples were then centrifuged at 1500 rpm for 50 minutes. The resulting interface, containing the PBMCs, was carefully aspirated from the gradient and washed three times in PBS. PBMCs were subsequently divided into two tubes: one was stored at -80°C for future Western blot analysis, and the other was used for oxygen consumption rate evaluation.

*Oxygen Consumption Rate*

Basal oxygen consumption rate (OCR) was register in freshly isolated PBMCs was measured using a Clark-type oxygen electrode (Yellow Springs Instruments) at 37°C. Due to limitations in sample availability and processing time in this pilot study, a full mitochondrial respiration protocol was not

DOI: https://doi.org/10.5027/jmh-Vol23-Issue2(2026)art277

feasible. Viable PBMCs (10^6 cells/500 µL) were resuspended in glucose-free DMEM and transferred to a hermetically sealed respiration chamber. OCR was monitored over a 3-min interval[16]. Once the data was recorded, the cells were lysed and the amount of protein was quantified to adjust the OCR to the amount of protein.

*Western blotting*

PBMCs were homogenized in lysis buffer containing: 20 mM Tris-HCl (pH 7.5), 1% Triton X-100, 2 mM EDTA, 20 mM NaF, 1 mM Na2P2O7, 10% glycerol, 150 mM NaCl, 10 mM Na3VO4, 1 mM PMSF and a cocktail of protease inhibitors (Complete TM, Roche Applied Science). Then the proteins were separated by SDS-PAGE and transferred to PVDF membranes. The following antibodies and dilutions were used: mouse anti-parkin (1:1000, SC-32282, Santa Cruz Biotechnology), rabbit anti-BNIP3L (1:1000, 12396, Cell Signaling Technology), mouse anti-Total OXPHOS cocktail (1:1000, ab110413, Abcam), mouse anti-MFN2 (1:1000; Ab56889, Abcam), and mouse anti-GAPDH (1:10000, 2118, Cell Signaling Technology) which was used as a loading control. The membranes were then incubated with an appropriate secondary antibody: goat anti-mouse IgC-HRP (1:10000; 31430, Invitrogen, USA) or goat anti-rabbit IgG-HRP (1:10000; 7074P2, Cell Signaling, Danvers, MA, USA). Image acquisition and band quantification were performed using a ChemiDocTM MP System (Bio-Rad, CA, USA) and ImageJ software (Wayne Rasband, National Institutes of Health, USA), respectively.

*Data analysis*

According to the sample size (n=4), a logarithmic transformation (Log10) was performed, and a paired two-factor analysis of variance (Two-Way ANOVA) was applied: time (rest and post-exercise) and condition (normoxia and hypoxia) as well as the interaction between both factors. Statistical significance was considered for $p<0.05$ and statistical trend was taken for $0.05<p<0.08$. Statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, Inc., San Diego, CA). For the effect size, partial eta squared ($\eta p^2$) was estimated using JASP (Version 0.95.3) [Computer software]. Following Cohen, the thresholds were defined as: very small (< 0.01), small (0.01–0.04), small-to-medium (0.04–0.06), medium (0.06–0.14), and large (≥ 0.14).

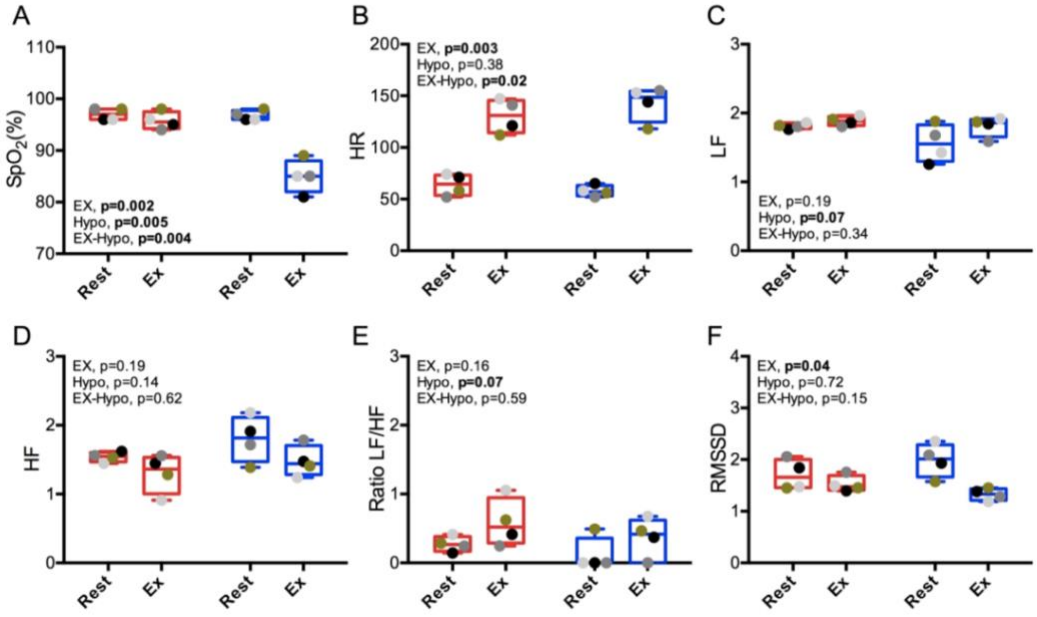
## Results

EX and NH decrease SpO2 (Figure 1A), and, as expected, NH conditions strengthen the effect of exercise in SpO2 (EX×NH; p=0.004, Figure 1A). HR increased with EX, but not under NH conditions (Figure 1B), and like for SpO2, NH intensified the effect of exercise in HR (EX×NH; p=0.02, Figure 1B). Regarding HRV, EX resulted in a decrease in RMSSD, with lower values under NH conditions (Figure 1F). NH also tended to decrease LF and LF/HF index (Figure 1 C, E) corroborating the combined effect of exercise and hypoxia on the cardiac autonomic balance[21]. However, no interactions between exercise (EX) and normobaric hypoxia (NH) were detected in the HRV parameters, potentially due to the limited number of participants (Figure 1, C-F).

Concerning oxidative phosphorylation, NH tended to decrease mitochondrial complex I (CI) (p=0.07; Figure 2 A, D), while EX increased CII (p=0.04; Figure 2 A, D), with no changes observed in the other mitochondrial complexes (Figure 2 A, D). For OCR, no differences were observed in EX nor NH (Figure 2 B). Regarding pro-mitophagy proteins, EX tended to increase parkin protein (p=0.07; Figure 2 A, C), while NH led to a decreased parkin protein abundance (p=0.01; Figure 2 A, C), with no significant interaction between conditions (Figure 2 A, C). Additionally, no changes were observed in the mass of BNIP3L or MFN2 proteins mass in none of the conditions (Figure 2 A, C).

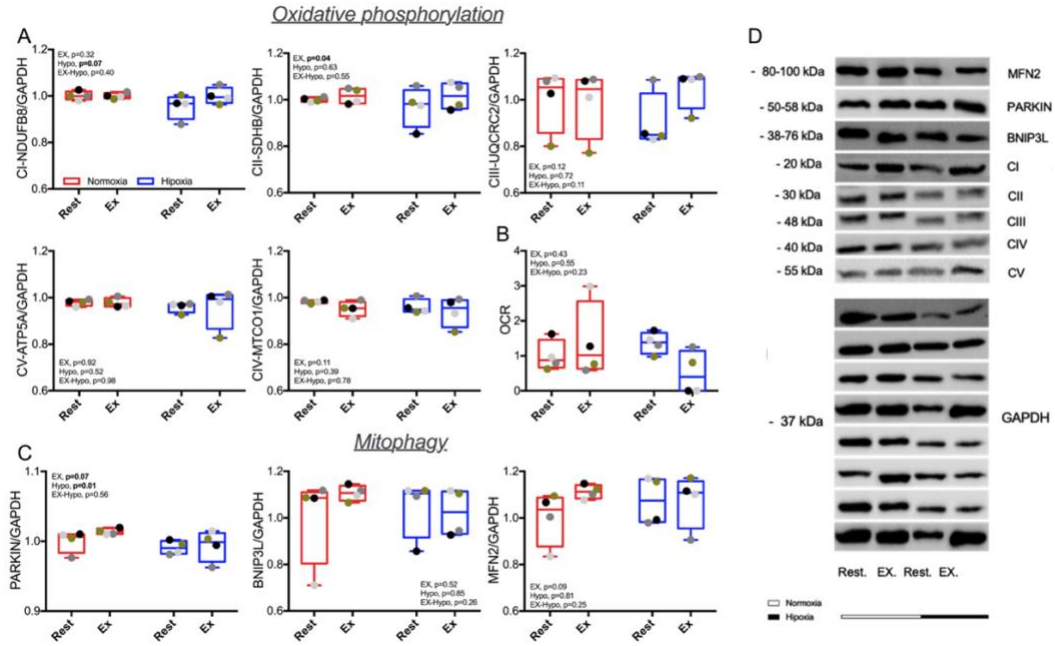
DOI: https://doi.org/10.5027/jmh-Vol23-Issue2(2026)art277

**Figure 1.** Pre- and post-endurance exercise cardiovascular response in normoxia or normobaric hypoxia.




Effects of acute endurance exercise (EX) and normobaric hypoxia (NH) on (A) SpO2, (B) heart rate (HR), and (C) heart rate variability (HRV). HRV parameters include Low Frequency (LF), Low Frequency/High Frequency (LF/HF) ratio and RMSSD. Each different colored dot represents the same volunteer in each graph. The red color represents the normoxic condition, and the blue color represents the normobaric hypoxic condition.

**Figure 2.** Oxidative phosphorylation and pro-mitophagy protein response to exercise and normobaric hypoxic conditions.




Oxidative phosphorylation and pro-mitophagy protein response to exercise and normobaric hypoxic conditions. Effects of acute endurance exercise (EX) and normobaric hypoxia (NH) on (A) mitochondrial complexes protein mass, (B) oxygen consumption rate (OCR), and (C) pro-mitophagy proteins mass. (D) Representative images of the measured proteins. Each different colored dot represents the same volunteer in each graph. The red color represents the normoxic condition, and the blue color represents the normobaric hypoxic condition.

DOI: https://doi.org/10.5027/jmh-Vol23-Issue2(2026)art277

Following the ANOVA, the effect size for the sample was evaluated using partial eta squared ($\eta p^2$)[22] for the OXPHOS complex (OXPHOS effect size in Table 2), as well as for the BNIP3L, parkin, and Mf2 proteins, and also for the OCR of PBMCs under the effects of NH x EX, NH, and EX (Table III Effect size of parkin , BNIP3L, MFN2 and OCR in Table 3).The $\eta p^2$ values exhibited greater variability among effects, with some interactions approaching small-to-moderate (OCR and parkin) but without significance (all $p > 0.05$). For the OXPHOS complex, for CI, CIII, and CV, $\eta p^2$ values observed could be interpreted as moderate or large, but should not be interpreted as significant effects, given that their p-values are greater than 0.05. In summary, with this sample size, the evidence of consistent changes in the proteins and OCR studied by EX x NH, EX, or by NH is limited, and the $\eta p^2$ estimates should be interpreted with caution due to low power.

**Table II.** Effect Size of OXPHOS

| OXPHOS Complex | Effect | $\eta p^2$ | p |
|---|---|---|---|
| CI | Interaction | 0.079 (moderate) | ns |
| | NH | 0.007 (very small) | ns |
| | EX | 0.035 (very small) | ns |
| CII | Interaction | 0.000–0.044 (very small to small) | ns |
| | NH | 0.001–0.044 (very small to small) | ns |
| | EX | 0.096 (moderate) | ns |
| CIII | Interaction | 0.228 (large) | ns |
| | NH | 0.083 (moderate) | ns |
| | EX | 0.300 (large) | ns |
| CIV | Interaction | 0.816 (large) | ns |
| | NH | 0.384 (small) | ns |
| | EX | 0.202 (moderate) | ns |
| CV | Interaction | 0.986 (large) | ns |

Notes: EX= Endurance Exercise; NH=Normobaric Hypoxia; ns= not significant ($p > 0.05$); $\eta p^2$ = partial eta squared.

**Table III.** Effect size of parkin, BNIP3L, MFN2 and OCR

| OXPHOS Complex | Effect | $\eta p^2$ | p |
|---|---|---|---|
| Parkin | Interaction | 0.044 (small-moderate) | ns |
| | NH | 0.245 (large) | ns |
| | EX | 0.096 (moderate) | ns |
| BNIP3L | Interaction | 0.079 (moderate) | ns |
| | NH | 0.007 (very small) | ns |
| | EX | 0.035 (small) | ns |
| MFN2 | Interaction | 0.100 (moderate) | ns |
| | NH | 0.001 (very small) | ns |
| | EX | 0.098 (moderate) | ns |
| OCR | Interaction | 0.164 (large) | ns |

Notes: EX= Endurance Exercise; NH=Normobaric Hypoxia; ns= not significant ($p > 0.05$); $\eta p^2$ = partial eta squared.

## Discussion

Our findings corroborate the effect of hypoxia on cardiac autonomic balance, consistent with prior investigations[21]. Despite the small sample size available for molecular assessments, these findings provide valuable preliminary insight into the physiological responses of PBMCs. For instance**,** the trend observed in our study towards a decrease in mitochondrial Complex I expression in PBMCs exposed to hypoxia aligns with certain previous research[23], although the significance of this observation within our limited cohort necessitates circumspect interpretation.

DOI: https://doi.org/10.5027/jmh-Vol23-Issue2(2026)art277

Regarding the pro-mitophagic protein parkin, we documented a trend towards increased protein abundance following acute exercise ($p = 0.07$; Figures 2 A, C) and a significant decrease induced by normobaric hypoxia ($p = 0.01$; Figures 2 A, C). Nevertheless, the absence of a statistically significant interaction between both conditions (Figures 2 A, C) suggests that these stimuli may exert independent effects on parkin levels in PBMCs under these acute conditions. It is crucial to note that a reduction in parkin protein levels does not per se constitute unequivocal evidence of increased mitophagic flux, necessitating the evaluation of a more extensive panel of markers to infer mitophagic activity, particularly in the absence of direct quantification of real-time flux. The absence of direct real-time flux quantification and key markers of autophagosome formation limits our current conclusions, as confirming complete lysosomal degradation requires a broader molecular assessment. Consequently, future investigations should integrate the assessment of the LC3-II/LC3-I ratio[24] and p62 levels[25]. This multiparametric approach is indispensable to provide a comprehensive and conclusive characterization of mitophagic dynamics under hypoxia and exercise conditions, even within short temporal intervals

The significant increase in mitochondrial Complex II subunit expression solely following acute exercise ($p = 0.04$) exhibited a seemingly modest magnitude and did not present an interaction with hypoxic exposure. Given the subtle nature of alterations in isolated mitochondrial protein subunits and the absence of concomitant modifications in basal oxygen consumption rate (OCR), the inference that these acute interventions induce a substantial alteration of the overall bioenergetic profile to maintain mitochondrial OXPHOS function is not robustly supported by our current data. Additionally, it is pertinent to consider the temporal framework of our evaluations (immediately post-exercise), as significant modifications in protein content may necessitate more protracted time periods involving gene regulation and protein synthesis. The absence of variations in BNIP3L and MFN2 protein mass under any experimental condition further suggests a limited acute impact of these stimuli on the broader molecular machinery involved in mitophagy and mitochondrial dynamics in PBMCs.

The observed divergence in parkin responses in PBMCs compared to other cell types, such as skeletal muscle [26, 9], underscores the potential for cell-type specificity in the regulation of mitochondrial quality control in response to these stressors. While in vivo studies in murine models subjected to extreme hypobaric hypoxia have revealed alterations in mitochondrial responses[27,28], the direct extrapolation of these findings to acute normobaric hypoxia in humans warrants cautious evaluation. Future human investigations should focus on elucidating the time-dependent effects of more pronounced hypoxic stimuli on oxygen-sensitive transcription factors, such as HIF-1 in PBMCs[28], and their subsequent impact on a broader spectrum of mitochondrial mechanisms, including biogenesis and dynamics, over extended temporal intervals (e.g., 4-6 hours) and at altitudes compatible with human habitation. These investigations should likewise prioritize the implementation of functional assays, such as the direct quantification of mitophagic flux and comprehensive characterization of the mitochondrial respiration profile, to obtain a more integrated understanding of the physiological consequences of the identified molecular alterations.

Finally, we acknowledge a methodological limitation of this study regarding the single temporal assessment point (immediately post-exercise). Mitochondrial protein synthesis dynamics and mitophagy regulation often exhibit a physiological latency that was not captured in our current design. While our assessment reflects the immediate acute cellular perturbation, more robust protein adaptations driven by gene regulation may occur subsequently. Consequently, the lack of temporal follow-up prevents the identification of such delayed responses. Future investigations should incorporate assessments at 4–6 hours, and ideally up to 24 hours post-stimulus, to better elucidate mitochondrial responses and identify clearer proteomic alterations.

DOI: https://doi.org/10.5027/jmh-Vol23-Issue2(2026)art277

## Conclusions

Our findings indicate that acute endurance exercise and normobaric hypoxia differentially modulate parkin protein abundance in PBMCs; however, no statistically significant condition-by-condition interaction was detected and the magnitude of change was modest. Neither intervention, alone or combined, produced detectable changes in basal oxygen consumption rate, and the limited alterations observed in OXPHOS proteins were not accompanied by immediate changes in basal cellular respiration. Given the very small sample size of this pilot study, these results should be interpreted with caution and should not be generalized to larger populations. Future studies with larger cohorts, a broader panel of mitophagy and mitochondrial function outcomes (including functional assays and temporal profiling), and a specific focus on normobaric hypoxia are warranted to clarify the physiological relevance of these responses.

## References


1. Bailey DM, Davies B, Young IS. Intermittent hypoxic training: implications for lipid peroxidation i by acute normoxic exercise in active men. Clin Sci. 2001;101(5):465-475. doi:10.1042/cs1010465
2. Ponsot E, Dufour SP, Zoll J, et al. Exercise training in normobaric hypoxia in endurance runr Improvement of mitochondrial properties in skeletal muscle. J Appl Physiol. 2006;100(4):124 doi:10.1152/japplphysiol.00361-2005
3. Picca A, Faitg J, Auwerx J, Ferrucci L, D'Amico D. Mitophagy in human health, ageing and disea Metab. 2023;5(12):2047-2061. doi:10.1038/s42255-023-00930-8
4. Bellot G, Garcia-Medina R, Gounon P, et al. Hypoxia-induced autophagy is mediated through hy inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. Mol Cell Biol. 2009;29(10 2581. doi:10.1128/MCB.00166-09
5. Liu L, Feng D, Chen G, et al. Mitophagy in human health, ageing and disease. Nat Cel 2012;14(2):177-185. doi:10.1038/ncb2422
6. Laker RC, Drake JC, Wilson RJ, et al. AMPK phosphorylation of ULK1 is required for targe mitochondria to lysosomes in exercise-induced mitophagy. Nat Commun. 2017;8( doi:10.1038/s41467-017-00520-9
7. Sliter DA, Martinez J, Hao L, et al. Parkin and PINK1 mitigate STING-induced inflammation. 2018;561(7722):258-262. doi:10.1038/s41586-018-0448-9
8. Palikaras K, Lionaki E, Tavernarakis N. Mechanisms of mitophagy in cellular homeostasis, physiolc pathology. Nat Cell Biol. 2018;20(9):1013-1022. doi:10.1038/s41556-018-0176-2
9. Díaz-Castro F, Tuñón-Suárez M, Rivera P, et al. A single bout of resistance exercise triggers mitc potentially involving the ejection of mitochondria in human skeletal muscle. Acta Physiol 2024;240(9):e14203. doi:10.1111/apha.14203
10. Millet GP, Debevec T, Brocherie F, Malatesta D. Therapeutic use of exercising in hypoxia: promi limitations. Front Physiol. 2016;7:224. doi:10.3389/fphys.2016.00224
11. Hermand E, Lhuissier FJ, Voituron N, Richalet JP. Ventilatory oscillations at exercise in hypc mathematical model. J Theor Biol. 2016;411:92-101. doi:10.1016/j.jtbi.2016.10.002
12. Buchheit M, Richard R, Doutreleau S, Lonsdorfer-Wolf E, Brandenberger G, Simon C. Effect o hypoxia on heart rate variability at rest and during exercise. Int J Sports Med. 2004;25(4):2( doi:10.1055/s-2004-819938
13. Pedersen BK, Steensberg A. Exercise and hypoxia: effects on leukocytes and interleukin-6 mechanisms? Med Sci Sports Exerc. 2002;34(12):2004-2013. doi:10.1097/00005768-200212000-0

DOI: https://doi.org/10.5027/jmh-Vol23-Issue2(2026)art277

14. Hoppel F, Garcia-Souza LF, Kantner-Rumplmair W, et al. Human platelet mitochondrial function systemic mitochondrial alterations: a protocol for application in field studies. Cells. 2021;10(8 doi:10.3390/cells10082088
15. Braganza A, Annarapu GK, Shiva S. Blood-based bioenergetics: An emerging translational and tool. Mol Aspects Med. 2020;71:100835. doi:10.1016/j.mam.2019.100835
16. Castro-Sepúlveda M, Morio B, Tuñón-Suárez M, et al. The fasting-feeding metabolic transition re mitochondrial dynamics. FASEB J. 2021;35(10):e21891. doi:10.1096/fj.202100929R
17. Brocherie F, Millet GP. Hypoxic exercise as an effective nonpharmacological therapeutic interventi Mol Med. 2020;52(3):529-530. doi:10.1038/s12276-020-0400-6
18. Heffernan KS, Fahs CA, Shinsako KK, Jae SY, Fernhall B. Heart rate recovery and heart rate com following resistance exercise training and detraining in young men. Am J Physiol Heart Circ F 2007;293(5):H3180-H3186. doi:10.1152/ajpheart.00648-2007
19. Álvarez-Herms J, Julià-Sánchez S, Gatterer H, Corbi F, Viscor G, Burtscher M. Effects of a Single Strength Training Session on Heart Rate Variability When Performed at Different Simulated Al High Alt Med Biol. 2020;21(3):292-296. doi:10.1089/ham.2020.0014
20. Pagani M, Lombardi F, Guzzetti S, et al. Power spectral analysis of heart rate and arterial p variabilities as a marker of sympatho-vagal interaction in man and conscious dog. Cir 1986;59(2):178-193. doi:10.1161/01.RES.59.2.178
21. Favret F, Richalet JP. Exercise and hypoxia: the role of the autonomic nervous system. Respir Neurobiol. 2007;158(2-3):280-286. doi:10.1016/j.resp.2007.04.001
22. Richardson J. Eta squared and partial eta squared as measures of effect size in educational researcl Res Rev. 2011;6(2):135-147. doi:10.1016/j.edurev.2010.12.001
23. Okoye CN, Koren SA, Wojtovich AP. Mitochondrial complex I ROS production and redox signa hypoxia. Redox Biol. 2023;67:102926. doi:10.1016/j.redox.2023.102926
24. Klionsky DJ, Abdelmohsen K, Abe A, et al. Guidelines for the use and interpretation of ass monitoring autophagy (3rd edition). Autophagy. 2016;12(1):1-222. doi:10.1080/15548627.2015.11
25. Komatsu M, Waguri S, Chiba T, et al. Loss of autophagy in the central nervous system neurodegeneration in mice. Nature. 2006;441(7095):880-884. doi:10.1038/nature04723
26. Chen CCW, Erlich AT, Hood DA. Role of Parkin and endurance training on mitochondrial turn skeletal muscle. Skeletal Muscle. 2018;8(1):10. doi:10.1186/s13395-018-0157-y
27. Chitra L, Boopathy R. Adaptability to hypobaric hypoxia is facilitated through mitochondrial bioene an in vivo study. Br J Pharmacol. 2013;169(5):1035-1047. doi:10.1111/bph.12179
28. Jain K, Prasad D, Singh SB, Kohli E. Hypobaric Hypoxia Imbalances Mitochondrial Dynamics in Ra Hippocampus. Neurology Res Int. 2015;2015:742059. doi:10.1155/2015/742059
29. Xin Y, Zhao L, Peng R. HIF-1 signaling: an emerging mechanism for mitochondrial dynamics. J Biochem. 2023;79(3):489-500. doi:10.1007/s13105-023-00966-0

**Affiliations**

[1] Department of Rehabilitation, Intervention, and Therapeutic Approach. University of Playa Ancha, Valparaíso, Chile.

[2] Center of Exercise Physiology and Metabolism, Department of Kinesiology, Finis Terrae University, Santiago, Chile.

[3] Physiology Section, Department of Cell Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona, Spain.

DOI: https://doi.org/10.5027/jmh-Vol23-Issue2(2026)art277

## Author Contributions

MC-S conceived the study. MC-S and JA-C designed the study. MC-S, JA-C, RV-F, JG, and AF executed the experiments and processed and analyzed the data. JA-C and MC-S drafted the manuscript; ML, GV, AF, JG and JA-C and MC-S revised the manuscript and contributed significantly with intellectual content. All authors approved the submitted version of the manuscript.

## Conflict of Interest

The authors declare no conflicts of interest.

## Acknowledgements and funding

Thanks to all participants. This study was funded by grants from the Agencia Nacional de Investigación y Desarrollo (ANID), Chile: Magíster Nacional para funcionarios del sector público 79210112 (JA-C) and FONDECYT 11230548 (MC-S).

## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author used Copilot to improve readability and check grammar.

## Data availability

Data will be made available on request.