RESEARCH ARTICLE

Mitochondrial Capacity and Muscle Endurance in Individuals with Parkinson's disease

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Abstract:
Introduction: Parkinson's disease (PD) is associated with loss of motor control and difficulty exercising. This study measured skeletal muscle mitochondrial capacity and endurance in individuals with and without PD using novel non-invasive methods. We hypothesized that individuals with PD will have decreased mitochondrial capacity, reduced oxygen recovery, and decreased endurance compared to controls. Methods: Eight participants with PD and nine healthy controls were tested. Mitochondrial capacity was measured as the rate of recovery of muscle metabolism after electrical stimulation using near-infrared spectroscopy (NIRS) and repeated short arterial occlusions. Oxygen recovery was measured as the half time of recovery of oxygen levels after 5 minutes of ischemia. Muscle endurance was determined from changes in twitch contraction acceleration during electrical stimulation at 2, 4, and 6 Hz. Results: Mitochondrial capacity was lower in individuals with PD compared to controls (1.5±0.1min-1 vs. 1.7±0.1min-1, p=0.02). Individuals with PD had slower oxygen recovery after ischemia compared to controls (8.9±2.3s vs. 5.4±0.8s, p=0.01). Endurance was not different between groups at 6 Hz (PD vs controls: 58±23% vs. 69±16%, p=0.34). The effect sizes for mitochondrial capacity and oxygen recovery were large (Cohen's d >0.8). The Cohen's d for endurance was 1.11. Conclusion: Individuals with PD had slight impairments in mitochondrial capacity and blood flow but did not have reduced muscle endurance. While our study suggests that muscle metabolic dysfunction may play a minor role in exercise intolerance in people with PD, it demonstrates the use of noninvasive technologies to evaluate muscle function in people with neurological disorders.

Key Words: Neurological disorders; skeletal muscle; near infrared spectroscopy; oxidative metabolism

1.0. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting more than 10 million people worldwide. The Centers for Disease Control ranks PD as the 14th leading cause of death in the United States.[1] PD is characterized pathologically by the loss of dopaminergic neurons in the substantia nigra. The disease has been hypothesized to have genetic and environmental interaction causes. This leads to the development of motor symptoms that impair ability to exercise and perform daily activities, such as muscle tremors at rest, bradykinesia, limb rigidity, and postural instability.[2]

Although exercise therapy is believed to be beneficial for people with PD,[3] the role of potential impairments to muscle energy capacity in exercise intolerance in people with PD remains unclear. Evidence of mitochondrial dysfunction in PD has been linked to 1-methyl-4-phenylpyridinium (MPP+), which inhibits respiratory activity at mitochondrial complex I leading to apoptosis. Mitochondrial respiratory chain impairment in dopaminergic neurons of the substantia nigra provides further evidence for mitochondrial dysfunction in PD.[4]

Functional measurements of muscle associated with mitochondrial capacity are difficult to perform in individuals with PD, because impaired motor control makes voluntary exercise tests difficult to perform and interpret. Recently, noninvasive tests of muscle mitochondrial capacity,[5] oxygen delivery,[6] and muscle specific endurance[7] have been developed that allows assessment of muscle function in people with poor motor control. However, these methods have yet to be utilized in individuals with PD. The aims of this study were to: 1) evaluate muscle mitochondrial capacity in patients with PD and healthy control subjects, 2) evaluate oxygen recovery after ischemia, and 3) evaluate skeletal muscle endurance using acceleration and twitch muscle stimulation. The hypotheses were that participants with PD will have reduced mitochondrial capacity, slower oxygen recovery, and reduced muscle endurance compared to healthy age-matched controls.

2. Materials and Methods

Eight participants with physician-diagnosed PD and nine healthy age-matched controls were recruited for this study. All participants did not have any other neuromuscular or mitochondrial diseases. The University of Georgia Institutional Review Board approved this study (STUDY00006817). All participants were informed of the procedure and potential risks and complications before consent.

This experiment was a cross-sectional evaluation between individuals with PD and age-matched controls. All participants completed medical history, mental state, and physical state questionnaires. Participants with PD completed the Barthel Index of Daily Activities and the Unified Parkinson's Disease Rating Scale (UPDRS) questionnaires.[8] Adipose tissue thickness over the forearm muscles was measured with B-mode ultrasound (GE Logic Q, GE Medial, Milwaukee, WI).

Each participant was tested on one occasion which consisted of three main components: near-infrared spectroscopy (NIRS) tests of mitochondrial capacity and oxygen recovery after ischemia, and a neuromuscular electrical stimulation (NMES) endurance test. The tests were performed on the flexor muscles of the dominant forearm: the flexor carpai radialis, flexor carpai ulnaris, and palmaris longis muscles.

2.1 Measuring Muscle Endurance

The nine-minute NMES muscle endurance test was conducted while the participant was in the supine position on a firm bed.[7] The shoulder was abducted to 90 degrees and the arm was strapped to a padded ledge attached to the bed to limit movement of the forearm. 6 x 5 cm electrodes were placed on the distal and proximal positions of the flexor muscles of the forearm. The muscles were electrically stimulated using medical grade electrical stimulation machines (Theratouch 4.7, Rich-Mar, USA). The magnitude of muscle contractions of the flexor muscles was measured using a tri-axial, wireless accelerometer (WAX3, Axivity, UK) placed on the medial upper one-third of the arm. Measurements for the endurance test followed a previously developed protocol with progressing stages of stimulation (2, 4, and 6 Hz). Stimulation was maintained at an intensity tolerable for the participant (35-110 mA).

2.2 Measuring Mitochondrial Capacity

The NIRS test of mitochondrial capacity was performed after the endurance test.[5] The NIRS probe (Oxymon MKIII, Artinis Medical Systems) was placed on the medial upper one-third of the forearm. Electrodes were placed above and below the NIRS probe and an inflatable blood pressure cuff was placed above the bend of the elbow. The cuff was inflated to approximately 100 mm Hg above systolic pressure. Measurements for the mitochondrial capacity test followed a previously developed protocol. To measure the rate of oxygen recovery, the cuff was inflated for a maximum of five minutes to deplete oxygen in the muscle to 0% O_2 saturation and released for seven minutes to allow oxygen levels to returned to 100% O_2 saturation. The time from the release of the cuff to 100% O_2 saturation was recorded as the time-to-half recovery of oxygen saturation and was used as an index of microvascular blood flow.[6] The mitochondrial capacity test protocol consisted of repeated cuff series performed after 30 seconds of electrical stimulation. The rate of recovery of muscle metabolism as measured by the slope of oxygen consumption with each cuff was used to allow oxygen levels to return to 100% O2.[5]

2.3 Data Analysis

Microsoft Excel and a MATLAB analysis program were used to calculate the endurance index by dividing the end fatigued muscle twitch acceleration by the beginning peak acceleration of each stimulation period. Endurance index values are presented as percentages to allow for comparison between groups. Oxymon software was used to collect NIRS test data, and data was analyzed using a MATLAB analysis program. To determine statistical significance between groups for muscle endurance, mitochondrial capacity, and muscle reperfusion, comparisons between groups were made with unpaired, two-tailed T tests. For analysis of muscle endurance, the difference in endurance at 6 Hz was analyzed. Statistical significance was accepted with a p value < 0.05.

3.0 Results

Participant characteristics are shown in Table 1. Nine participants with PD were recruited and tested. However, muscle tremors in the forearm resulted in excluding data from one subject for the endurance measurements and two subjects for the mitochondrial capacity analysis.

Table 1. Participant characteristics

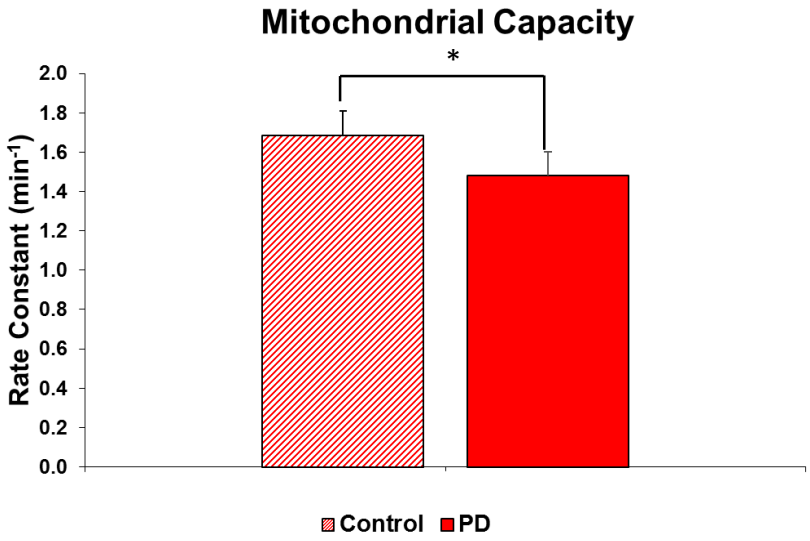
Characteristics	PD (n=8)	Control (n=7)
Gender (M/F)	5/3	5/2
Age (years)	72.8 ± 5.9	69.0 ± 4.5
Height (cm)	170.5 ± 7.5	175.4 ± 11.8
Weight (kg)	74.6 ± 17.2	78.6 ± 16.9
Forearm tested (R/L)	8/0	5/2
Adipose tissue thickness (cm)	0.32 ± 0.14	0.29 ± 0.09
UPDRS	20.6 ± 6.8	-
Barthel Index	18.8 ± 1.3	-

Values are means ± SD. Unified Parkinson's Disease Rating Scale (UPDRS) and Barthel Indices are not appropriate for controls without PD symptoms.

3.1 Mitochondrial Capacity and Oxygen Recovery

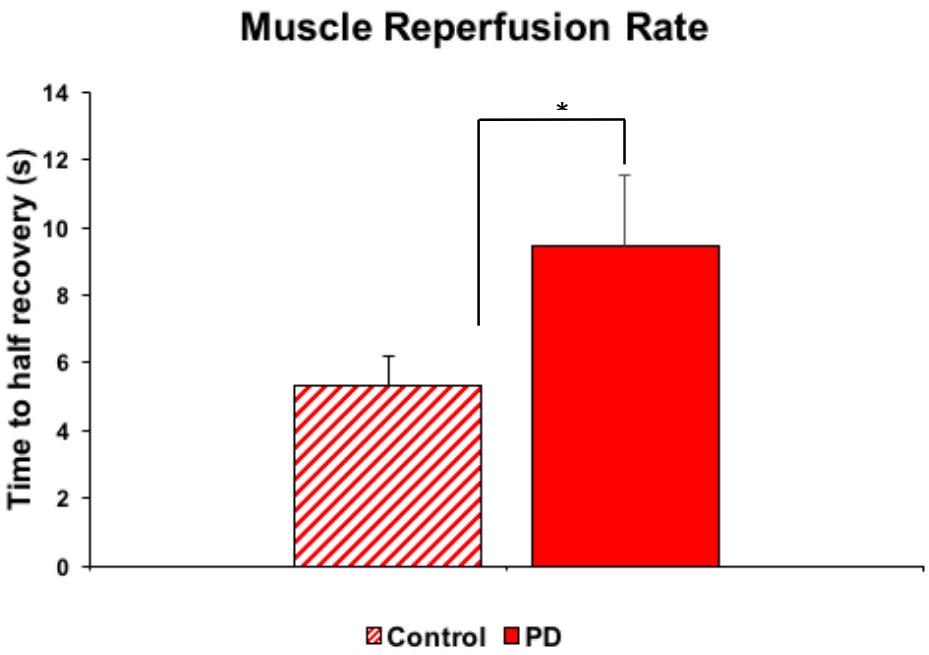
The difference in mitochondrial capacity rate constants between PD and controls was significant (p = 0.02) [Figure 1a]. The effect size (Cohen's d) of the rate constant was calculated to be 1.67. The time to half recovery after five minutes of ischemia was significantly slower in the PD group compared to the control group (p = 0.01) [Figure 1b]. The effect size (Cohen's d) of the time to half recoveries was calculated to be 2.55.

Figure 1a.



Mitochondrial capacity given in rate constant (min^{-1}) in individuals with PD versus controls. n=6 for PD and n=7 for controls. Values are means ± SD. Asterisks (*) indicate significance between all PD and all controls (p = 0.01).

Figure 1b.

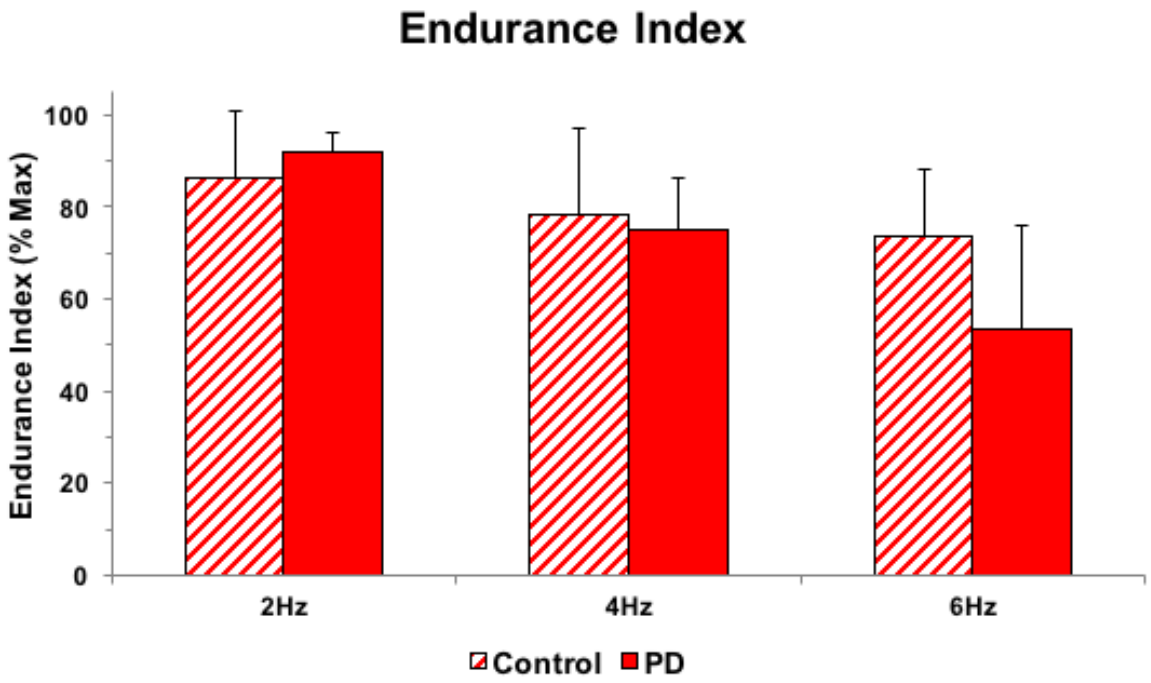


Muscle reperfusion rate measured in seconds in people with PD versus controls; n=17 both groups, n=6 for PD and n=7 for controls. Values are means ± SD. Asterisks (*) indicate significance between all PD and all controls (p = 0.005).

1.1. Muscle Endurance

There were no significant differences in the endurance index values at any frequency between the PD and control group (p = 0.53 for 2Hz, 0.88 for 4 Hz and 0.34 for 6 Hz) [Figure 2]. The differences in endurance indices at 2, 4, and 6 Hz between PD and controls were not significant. The effect size (Cohen's d) of the endurance index at 6 Hz was calculated to be 1.11.

Figure 2.



Muscle endurance measured in percentage in individuals with PD versus controls at 2, 4, and 6 Hz. n=6 for PD and n=7 for controls. Values are means ± SD ($p = 0.09$ at 6 Hz).

4. Discussion

This study found that individuals with PD have lower mitochondrial capacity relative to healthy age-matched controls, indicating impaired skeletal muscle metabolism. Previous studies that used invasive methods have shown impairments in the respiratory chain of neural and muscle mitochondria in individuals with PD.[4] Not all studies have found evidence of impaired muscle mitochondria in individuals with PD.[9] Our finding of a small (~12%) decrease in mitochondrial capacity is consistent with some studies finding decreases and some not. A strength of our study was the use of the forearm muscles rather than locomotory muscles in the leg, as this reduces the need to account for differences in physical activity levels between the PD and control groups. If decreases in mitochondrial capacity influence exercise capacity, this would have a clinical impact as exercise is considered beneficial in slowing the progression of the disease or optimizing function in the face of the disease.[3, 10, 11]

The NIRS approach to measuring mitochondrial capacity has been used to evaluate patients with multiple sclerosis (MS) and amyloid lateral sclerosis (ALS).[6] Mitochondrial capacity was considerably lower in those populations relative to controls than what was found for people with PD. Further investigation of disease severity and mitochondrial capacity is needed to determine if mitochondrial capacity is less affected in people with PD relative to their disease severity than other patient populations.

Muscle endurance was not reduced in people with PD relative to controls. While the results did not reach the accepted level of significance, the magnitude of difference between the groups was 17% at 6 Hz, with a large effect size (Cohen's $d = 1.11$). Thus it may be that muscle endurance might also be

reduced in people with PD. Previous studies on other patient populations have found agreement between decreased mitochondrial capacity and decreased muscle endurance.[12]

People with PD also had slower rates of recovery of oxygen after ischemia (49% longer to return to 50% O_2 saturation from completely depleted O_2 levels). Circulation problems specific to PD are not expected and have not been reported previously. The rates of recovery of oxygen levels in this study for both the PD and control groups were within reported values for healthy control groups in previous studies (mean values ~10 seconds). In contrast, reperfusion rates for patients with documented for peripheral arterial disease are much slower (30-120 seconds).[13] Thus, while further studies should example potential differences in oxygen recovery rates, the relatively healthy rates seen in this study most likely would not influence muscle metabolism or exercise capacity.

The most significant limitation of this study is small sample size. The COVID-19 pan epidemic precluded testing additional subjects. In addition, while nine participants with PD were recruited initially, data from one participant's endurance test and data from two participants' mitochondrial capacity tests could not be used. The use of electrical stimulation was an advantage as it did not require the subjects with PD to performed controlled voluntary exercise. However, the tests did require the subjects to be able to relax their forearms, and this was not possible for some participants with PD. It is possible that more tightly securing the forearm would have alleviated this problem for some subjects, but it might be an issue that studies using people with PD will continue to face. The small sample size was also a limitation of this study. For the mitochondrial capacity test, the study did have adequate power to detect a meaningful difference between groups (20%), such that the lack of statistical differences suggest that mitochondrial capacity was not different between PD and controls. However, for the endurance test the calculated power for a sample size of 7/8 per group was 0.29. The lack of statistical differences between groups with a 16% difference in the mean values suggests larger samples sizes will be needed to rigorously test the hypothesis that the endurance index is reduced in people with PD. For the time to half recovery measurements of microvascular blood flow, the power to detect a 20% difference in between groups was also low, 0.30. However, a 66% difference was seen between groups which was statistically significant. So while underpowered for the most part, this study can suggest that mitochondrial capacity was not different in the PD group compared to controls while the microvascular blood flow is reduced. No firm conclusions on the endurance index are warranted with this study.

Participants with PD in this study were diagnosed with stage 1 of PD (stages based on the Hoehn and Yahr rating scale). Thus the conclusions of our study are limited to less-affected individuals with PD. Future studies should evaluate patients with more severe disease, although as mentioned above, the complication of overcoming tremors will need to be addressed. Because of the small sample size of this study, it is not clear how well the subjects with PD represent the broader population of people with (less affected) PD. The volunteers in both the PD and control groups reported a history of engaging in physical activity, which may have led to slightly higher mitochondrial capacity than the less active older adults with or without PD. Physical activity is recommended for optimal health for both control and subjects with PD. We attempted to control for the influence of physical activity by evaluating muscles in the forearm, as most physical activity programs focus on exercising using the legs. Future studies

should recruit various physical activity levels. Finally, medication amount and dosage (Carbidopa-levodopa) was not controlled for in participants with PD; it is not clear how levels of exogenous dopamine in this sample could confound assessments of mitochondrial dysfunction[2] and impact the generalizability of these results.

5.0 Conclusions

Individuals affected by PD had reduced forearm mitochondrial capacity and with potentially reduced muscle endurance compared with age-matched healthy controls. A major strength of this study was the novel techniques used to study muscle metabolism in participants with PD. The techniques utilized were non-invasive and time-efficient. This research supports the need for further investigations into the role of skeletal muscle metabolism and endurance might play in exercise tolerance in people with PD.

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Declaration of Conflicting Interests

One of the authors; Kevin K. McCully is the President and Chief Science Officer of Infrared Rx, Inc, a company that develops analysis software related to NIRS measurements.

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