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# Platelet Mitochondrial Activity and Pesticide Exposure in Early Parkinson's Disease

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## Abstract

**Background—**Mitochondrial dysfunction has been implicated in the pathogenesis of Parkinson's disease (PD) but the cause of this dysfunction is unclear.

**Methods—**Platelet mitochondrial complex I and I/III (NADH cytochrome c reductase, NCCR) activities were measured in early PD patients and matched controls enrolled in a population based case-control study. Ambient agricultural pesticide exposures were assessed with a geographic information system and California Pesticide Use Registry.

**Results—**In contrast to some previous reports, we found no differences in complex I and I/III activities in subjects with PD and controls. We did find that NCCR activity correlated with subjects' exposure to pesticides known to inhibit mitochondrial activity regardless of their diagnosis.

**Conclusions—**ETC activity is not altered in PD in this well-characterized cohort when compared to community-matched controls but appears to be affected by environmental toxins, such as mitochondria-inhibiting pesticides.

### Keywords

Electron transport chain; Complex I

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**Author Contributions:**

JMB and BR drafted/revised the manuscript, designed study, analyzed/interpreted data. KP and LY drafted/revised the manuscript, and analyzed/interpreted data. RHH designed study, analyzed ETC activity, revised manuscript TH analyzed ETC activity. CWS designed study and analyzed/interpreted data. He is now deceased.

## Introduction

The role of mitochondrial dysfunction in the pathophysiology of Parkinson's disease (PD) was first suggested by the discovery that MPTP, a neurotoxin selective for dopaminergic neurons, acts by inhibiting complex I of the electron transport chain (ETC) (1). This discovery stimulated the evaluation of mitochondrial function from tissues in PD patients. A number of studies reported reduced complex I activity in platelets of PD patients varying from 16 to 71% although this has not been a consistent finding (Supplementary Table 1) (2–11).

It is unknown whether the reported lower ETC activity associated with PD was due to genetic or environmental causes. Pesticides have been suspected as a possible etiological factor in PD for over 30 years (12). Several studies have reported an association of PD with pesticide exposure including rotenone, a known complex I inhibitor (12, 13). We utilized the California Pesticide Registry and subjects' addresses to estimate pesticide exposure in a case control study we conducted in California (14, 15). The recruitment of incident PD patients and matched controls from the same counties with detailed demographic, medical and exposure histories offered an excellent opportunity to study potential associations of PD with ETC activity and pesticides.

## Methods

### Subjects

A total of 23 PD patients and 23 controls were enrolled in this sub-study. Incident PD patients and controls from the parent Parkinson Environment-Gene (PEG) study were identified and recruited from three rural California counties (Fresno, Kern and Tulare) as previously described (14, 16). The controls were matched by sex, age, and race, and the diagnosis of PD was confirmed by examination by a specialist in Movement Disorders (JMB). For the sub-study reported here, 25 patients were initially randomly selected and recruited for the study however two subjects were excluded in our analysis. One subject was determined later not to have PD and the other was excluded due to poor mitochondria yield. The UCLA Institution Review Board approved the use of humans in this study.

### Mitochondrial Assays

Blood was drawn and shipped overnight in the identical manner as described for the Coenzyme $Q_{10}$ Evaluation-2 (QE2) Study (17, 18). All PD patients we enrolled were off PD meds for at least 12 hrs. prior to the blood draw. ETC activities were determined using methods previously described (19, 20). Samples were excluded if the mitochondrial yield was low based on citrate synthase activity and analyzed in a blinded manner.

### Pesticide Exposure Assessment

Pesticide exposures were assessed for both controls and PD patients using California state mandated pesticide use report (CA-PUR) data, land use maps, and subject residential and work place histories as previously described (14, 15, 21–26). We estimated the ambient pesticide exposure per year in an area within 500 m radius of each work and residential

address. We classified each participant as exposed to different pesticide groups (1) CA-PUR reported pesticide; (2) organophosphates (OP); (3) organochlorines (OC); (4) dithiocarbamates (DTC), and (5) mitochondrial complex I inhibiting pesticides (Supplementary Table 2). Pesticide chemical class information was based on the pesticide action network (PAN) and the pesticide database and California department of pesticide regulation (CDPR). The mitochondrial complex I inhibiting pesticide group classification was based on a previous study by Tanner et al (13). Since mitochondria ETC activity is likely affected by recent pesticide exposures, we limited exposure assessments to the previous five years in sensitivity analyses.

### Statistical Analysis

The study was powered to detect differences in ETC between PD subjects and controls as previously reported[6]. We used student's two-tailed t-test or chi-square tests to investigate between group differences in mitochondrial function. We then employed multiple linear regression to assess whether pesticide exposures predict mitochondrial function, adjusting for sex, age and minority status. We also used spearman correlation coefficients to measure the relationship between pesticide group exposures during the five years prior to the time of blood draw.

## Results

### ETC Activity in PD Patients and Controls

A total of 23 PD patients and 23 matched controls were enrolled in this study and their characteristics are listed in Table 1. Complex I and I/III activities were normalized to citrate synthase since it is a mitochondrial matrix enzyme that is unaffected by ETC activity and is relatively stable. The ratio of complex I and I/III activity to citrate synthase thus provides a more accurate measurement of mitochondrial function (27). ETC activities in all subjects' samples were very similar to those previously reported but in this current study, we found no significant differences in normalized complex I (CI/CS) or complex I and III (NCCR/CS) activities between the PD patients and matched controls (Table 1).

### Correlation of ETC Activity and Pesticide Exposure

The finding that Complex I activity was not lower in our PD cohort was unexpected given past reports. This finding that PD patients and controls have similar ETC activities was not likely due to technical issues since the diagnostic classification was carefully controlled for and the assays were performed using the same methods by the same group that had reported lower activity in PD (5, 19). Since the subjects in our study live and work in commercial farming communities, we hypothesized that ETC activities could be influenced by recent exposure to certain pesticides regardless of the subjects' diagnosis. We therefore estimated pesticide exposure for each pesticide for all subjects and tested for associations between pesticide exposure and ETC activities (Table 2). There were 134 exposure events in the 46 subjects during the five years prior to collection of the blood samples (Supplementary Table 2). Thus, most subjects were exposed to more than one pesticide. There was no correlation between CI/CS activity and pesticide exposure in general but we did find that pesticides proposed to be mitochondrial complex I inhibitors and DTCs were significantly inversely

correlated with NCCR/CS activity (Table 2). This was not observed for other pesticides. Those subjects exposed to mitochondrial inhibitors and DTCs did not differ from unexposed subjects except in their normalized NCCR activity (Supplementary Table 3). These data suggest an alteration in complex III and/or coenzyme Q since complex I was unchanged and NCCR activity reflects both complex I and III activities along with endogenous coenzyme Q levels.

## Discussion

Several studies have reported low ETC activities in PD tissues however, these defects have not been reported in all studies and methodological differences were considered the most likely factor to explain inconsistent findings. For this reason, we utilized methods and the same investigators who have consistently found that PD was associated with low complex I activity in purified normalized platelet mitochondria.

The disparate findings reported here compared to previous studies can best be explained by the different subject populations studied. In the study by Haas and colleagues in which they reported a significant reduction in ETC activities in PD, subjects were recruited from a tertiary referral care clinic in San Diego and control subjects likely did not reside in the same communities and cases and controls might have been exposed to different environmental factors affecting mitochondrial function. In support of this argument, the significant differences in this study were lost when the researchers compared the patients to spousal controls (5). The later finding may have been due to the fact that spousal controls come from a more similar environment than the other convenient sample controls. In the current study, PD patients and controls all were living within the same 3 counties in rural central California. For this reason, we investigated potential environmental influences on ETC activities with an emphasis on pesticides since exposure has been associated with PD and some pesticides are known mitochondrial inhibitors. Indeed, we found that NCCR activity correlated with exposure to pesticides reported to inhibit complex I and DTC pesticides. This association was not found for all pesticides. Interestingly, DTCs have also been found to inhibit complex I and III with preferential inhibition of complex III (28).

These findings are intriguing but need to be interpreted with caution. First, the sample size in our study is relatively small for performing exposure specific analysis. We also estimate exposures based on residential and work addresses but do not know if subjects were actually at home or work on the days of application. Furthermore, NCCR activity represents both complex I and III activity but surprisingly, we did not find an alteration in complex I activity in pesticide-exposed subjects even though this group of pesticides purportedly inhibits complex I (13).

In summary, we did not find alterations in ETC activity in PD subjects compared to controls in a well-matched community-based study as others have reported. We did find a correlation between NCCR activity and recent exposure to mitochondrial-inhibiting pesticides. Previous reports of lower complex I activity in PD may have been due to the use of controls from different communities and with different environmental exposure profiles and ETC activity acted as a surrogate marker for the PD patients' exposure to mitochondrial toxins.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Table 1

Characteristics and ETC Activities of PD and Control Subjects

| Variable | PD Patients (N=23) | Controls (N=23) | p-value* |
|---|---|---|---|
| ***Demographics*** | | | |
| Age (mean ± SD) | 70.5 (11.8) | 71.0 (9.9) | 0.88 |
| Duration of PD (mean ± SD) | 2.09 (1.2) | - | - |
| Female (%) | 56.5 | 52.2 | 0.76 |
| Never Smokers (%) | 34.8 | 47.8 | 0.47 |
| Non-Caucasian (%) | 17.4 | 13 | - |
| ***Health Indicators*** | | | |
| UPDRS motor score (mean ± SD) | 19.4 (8.4) | - | - |
| MMSE score (mean ± SD) | 27.0 (4.1) | 28.9 (1.1) | 0.03 |
| ***Mitochondrial Function:*** | | | |
| Complex I (CI) (mean ± SD) | 16.6 (12.6) | 14.3 (8.1) | 0.37 |
| Citrate synthase (CS) (mean ± SD) | 410.5 (216.0) | 361.8 (160.6) | 0.39 |
| CI/CS (mean ± SD) | 0.044 (0.02) | 0.040 (0.01) | 0.46 |
| NCCR (mean ± SD)† | 34.4 (23.2) | 29.5 (20.6) | 0.46 |
| NCCR/CS (mean ± SD)† | 0.078 (0.03) | 0.076 (0.03) | 0.82 |

*P-value based on ttest or chi-square, PD patients vs Controls; not calculated in some instances due to small numbers.

†1 additional PD case and 1 control failed NCCR experiment (n=44)

## Table 2

Parameter Estimates for Different Linear Regression Models Predicting NCCR/CS activity with Different Pesticide Group Exposures.

| Pesticide Exposure Group* | Exposed (%) | Unexposed (%) | *β (S.E.)* | *P-value* |
|---|---|---|---|---|
| Any Pesticide | 23 (0.53) | 21 (0.47) | 0.0045 (0.01) | 0.64 |
| Organophosphates | 17 (0.39) | 27 (0.61) | −0.0096 (0.01) | 0.32 |
| Organochlorines | 13 (0.30) | 31 (0.70) | −0.012 (0.01) | 0.25 |
| Dithiocarbamates | 10 (0.23) | 34 (0.77) | −0.022 (0.01) | 0.05 |
| Mitochondrial Complex I Inhibitors | 8 (0.18) | 36 (0.82) | −0.024 (0.01) | 0.04 |

* All models control for sex, PD, age (continuous), and minority status. (N = 46).