PROTOCOL TITLE:

Mild Cognitive Impairment and Endurance Exercise in Parkinson's Disease

PRINCIPAL INVESTIGATORS:

Daniel Corcos, PhD
Department of Physical Therapy & Human Movement Sciences
(312) 908-6792
Daniel.corcos@northwestern.edu

Jennifer G. Goldman, MD, MS
Department of Neurological Sciences
Rush University
(312) 563-2900
Jennifer_Goldman@rush.edu

VERSION NUMBER:

Version 12

VERSION DATE:

2/6/2019

NCT03542474

PROTOCOL TITLE: Mild Cognitive Impairment and Endurance Exercise in Parkinson's Disease

Table of Contents

PROTOCOL TITLE: Mild Cognitive Impairment and Endurance Exercise in Parkinson's Disease

1.0 Objectives

1.1 The overall purpose and objective of this project is to test the hypothesis that high intensity endurance exercise causes beneficial brain adaptation in patients with mild cognitive impairment in Parkinson's disease (PD-MCI). Aim 1 will determine the effect of high intensity endurance exercise on cognitive function and other clinical symptoms in PD-MCI. Aim 2 will determine the effect of high intensity endurance exercise on brain structure and function in PD-MCI. Aim 3 will determine the effect of high intensity endurance exercise on cortisol in PD-MCI. Aim 4 will determine the effect of high intensity endurance exercise on inflammation-related biomarkers found in blood in PD-MCI. Aim 5 will determine the effect of high intensity endurance exercise on peripheral levels of neurotrophic factors found in blood in PD-MCI.

1.2 Hypothesis 1 is that high intensity endurance exercise will improve cognitive function, specifically attention, executive function and memory in PD-MCI patients. In addition, high intensity endurance exercise will improve other PD-related symptoms such as sleep, fatigue, mood, health-related quality of life, and motor function.

Hypothesis 2 is that high intensity endurance exercise will modify brain structure and function as measured by structural MRI, diffusion tensor imaging (DTI), and resting state functional MRI (rsfMRI), specifically by: a) increasing hippocampal volume, b) increasing microstructural integrity of white matter pathways connecting different brain regions (e.g.,fronto-striatal, fronto-parietal, and hippocampal regions, and c) enhancing functional connectivity among brain networks (e.g., default mode and central executive networks).

Hypothesis 3 is that high intensity endurance exercise will lead to decreased levels of cortisol and an increase in the magnitude of the cortisol awakening response (CAR).

Hypothesis 4 is that high intensity endurance exercise will lead to decreased levels of inflammation suggested by a decrease in levels of inflammation-related biomarkers found in blood.

Hypothesis 5 is that high intensity endurance exercise will lead to increased levels of peripheral neurotrophic factors.

2.0 Background

2.1 There is a growing interest in physical exercise as a treatment for PD symptoms and ultimately as a potential disease modification strategy. Most PD exercise studies have focused on motor function (Salgado, Williams et al. 2013), though our group also found improved executive function after balance/strengthening and progressive resistance training. (Corcos, Robichaud et al. 2013, David, Robichaud et al. 2015) The knowledge gap is that no study has specifically targeted PD-MCI patients to determine how exercise affects cognitive functions or the underlying neurobiology of PD-

MCI. Thus, the central hypothesis is that high intensity endurance exercise not only will improve the cognitive function of patients with PD-MCI, but also will produce neurobiological changes in their brain and body.

We have just finished collecting data on the effect of high intensity endurance exercise regimens on parkinsonian motor signs in 129 never medicated patients with PD. Patients exercised 4 days a week at either 65% of their maximal heart rate or at 80% of their maximal heart rate. As such, we have considerable experience in using endurance exercise protocols.

2.2 We have no preliminary data for this study. The purpose of this IRB is to collect pilot data for a subsequent R01.

2.3 Parkinson's disease (PD) is a neurodegenerative disorder that affects over 4 million people older than age 50.(Dorsey, Constantinescu et al. 2007) While the motor features of PD are well-recognized and well-treated with dopaminergic medications, PD is also complicated by non-motor symptoms, which cause significant disability, lack effective therapies, and typically do not respond to dopamine replacement therapy. One of the most disabling non-motor symptoms in PD is cognitive impairment, which ranges from mild deficits to severe dementia.(Emre, Aarsland et al. 2007, Hely, Reid et al. 2008, Litvan, Goldman et al. 2012) In recent years, mild cognitive impairment (PD-MCI) has been identified as a frequent occurrence in PD and a precursor to PD dementia, which can develop in 80% of PD patients over time.(Hely, Reid et al. 2008, Litvan, Aarsland et al. 2011, Litvan, Goldman et al. 2012) Recent research has characterized the cognitive deficits of PD-MCI, developed diagnostic criteria, and identified neuroimaging markers of PD-MCI.(Goldman, Stebbins et al. 2012, Goldman, Holden et al. 2013, Goldman, Holden et al. 2015) However, there are currently no therapeutic interventions that can effectively improve the symptoms of PD-MCI or slow down or halt cognitive decline in PD.(Goldman and Weintraub 2015)

While PD-MCI is a relatively new concept within the PD field, the idea of MCI as a state between normal cognitive aging and Alzheimer's disease has been recognized for many years and has led to treatment trials with pharmacological and non-pharmacological interventions, including physical exercise training. For example, Baker and colleagues (2010) have shown improved executive function in women with MCI and improved performance on the Trail B in men as a result of endurance exercise at between 75% and 85% heart rate reserve. Indeed, studies of exercise training, with a variety of techniques (e.g., endurance [also called aerobic], resistance, balance and tone training) demonstrate improvement in cognitive functions of MCI (non-PD) patients, as well as in some studies, other symptoms such as mood, sleep, fatigue and health-related quality of life.(Wang, Yu et al. 2014) Exercise also exerts neurobiological effects, as seen in animal models of exercise,(Silverman and Deuster 2014, Petzinger, Holschneider et al. 2015, Duzel, van Praag et al. 2016)as well as human studies of aging and MCI utilizing neuroimaging and other peripheral

biomarkers.(Voss, Prakash et al. 2010) Endurance exercise, in particular, has been linked to brain plasticity with magnetic resonance imaging (MRI) scans revealing structural changes (e.g., increased volume in the hippocampus, a key brain region for memory) and functional changes (e.g., increased connections among brain networks involved in cognitive processes) in aging adults and MCI patients.(Voss, Prakash et al. 2010, Erickson, Voss et al. 2011, ten Brinke, Bolandzadeh et al. 2015) Endurance exercise at between 65% and 75% maximal heart rate reserve can enhance the mental aspect of both health-related quality of life (HRQOL) and also global quality of life (global HQL).(Awick, Wojcicki et al. 2015)

Exercise can affect neurobiological markers of stress (e.g., cortisol), inflammation (e.g., cytokines) and growth factors (e.g., brain-derived neurotrophic factor [BDNF], insulin-like growth factor [IGF-1]), and these peripheral markers may mediate exercise effects on brain structure and function. (Foster, Rosenblatt et al. 2011, Voss, Vivar et al. 2013). Cortisol is released during exercise as a contributory factor to both muscular and mitochondrial function. Aside from the influential effects of cortisol on the muscular system, the release of cortisol during exercise also modulates different neurotransmitters and influences both cognition and neuroplasticity. (Diamond, Bennett et al. 1992, Piazza, Rouge-Pont et al. 1996, Joels and Krugers 2007, Sousa, Cerqueira et al. 2008, Mora, Segovia et al. 2012) Cortisol receptors can be found in brain regions involved in cognitive processing, such as the neocortex, cerebellum, and the hippocampus (Heffelfinger and Newcomer 2001) and it is widely recognized that glucocorticoids easily enter the brain and exert effects on cognition (de Quervain, Aerni et al. 2009). Cortisol levels in healthy elderly adults studied over a period of five years revealed that individuals with high basal cortisol levels and increasing cortisol levels over five years had hippocampal-dependent memory impairments and a 14% reduction in hippocampus volume when compared with individuals who either had moderate basal cortisol levels or decreasing cortisol levels over the five-year period.(Lupien, Fiocco et al. 2005) Flatter diurnal cortisol slopes have been associated with a decline over 4 years in visuospatial performance and visual memory in men, and in verbal fluency for women.(Beluche, Carriere et al. 2010) In the extreme, no morning cortisol response has been observed a 6 male patients with amnesia,(Wolf, Fujiwara et al. 2005) and also in patients with hippocampal damage.(Buchanan, Kern et al. 2004) In older adults diagnosed with MCI, the magnitude of the CAR and the extent of the subsequent drop in cortisol levels are both associated with level of fitness.(Tortosa-Martinez, Clow et al. 2015) It was shown that a three month exercise program improved fitness, increased the fall in cortisol concentration from the morning peak to midday, and enhanced indices of executive function in older adults with MCI. (Tortosa-Martinez, Clow et al. 2015). Earlier peaking and greater magnitude of the CAR has also been associated with superior performance on executive function related tasks in older adults.(Evans, Hucklebridge et al. 2012) To the best of our knowledge,

the CAR has not been measured in individuals with Parkinson's disease. Because the cortisol awakening response is distinct from circadian components of cortisol control, assessment of the CAR in individuals with PD may provide additional insight into additional neuroendocrine processes that other cortisol measures may not.(Stalder, Kirschbaum et al. 2016)

There is increasing evidence that inflammation is a contributing factor to both the pathogenesis and progression of PD (Beal, 2003; Chung et al., 2010; Sawada, Imamura, & Nagatsu, 2006). Chronic inflammation mediated by microglial cells is a fundamental process contributing to the death of dopamine-producing neurons in the brain, and it is suggested that physical stress may facilitate the progression of neurodegenerative disorders such as PD (Sugama, 2009). Regular physical activity tends to result in lower serum concentrations of inflammatory markers (Kasapis & Thompson, 2005). Chronic exercise training is thought to reduce levels of inflammatory markers both directly by attenuating cytokine production by adipose tissue, skeletal muscle, and mononuclear cells, and indirectly through improved insulin sensitivity and endothelial function, decreased body weight, and by upregulating antioxidant enzymes (Kasapis & Thompson, 2005).

Neurotrophic factors (NFs) are a family of proteins that play a significant role in the growth, survival, and maintenance of neurons (Rangasamy, Soderstrom, Bakay, & Kordower, 2010). Different proteins such as brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), insulin-like growth factor (IGF), and vascular endothelial growth factor (VEGF) have been classified as NFs, due to their ability to effect neuronal survival, differentiation, and maturation, and synaptogenesis (da Silva, Domingues, de Carvalho, Allodi, & Correa, 2016). Many studies have demonstrated the influence of trophic factors on neuroprotection, neurorestoration, and functional improvement in both PD animal models and some human clinical trials (Rangasamy et al., 2010). It has been suggested that NFs influence cell survival and restoration of dopaminergic cells in the nigrostriatial pathway (Petzinger et al., 2007; Tajiri et al., 2010; Tillerson, Caudle, Reveron, & Miller, 2003; Yoon et al., 2007).
The exercise-induced benefits of improved brain plasticity, function, and health can be at least partially attributed to growth factors, primarily BDNF, IGF-1, VEGF, and GDNF (Cotman et al., 2007; Knaepen, Goekint, Heyman, & Meeusen, 2012).

3.0 Inclusion and Exclusion Criteria

3.1 Individuals will be screened for eligibility as follows. Participants will meet the criteria outlined below. Participants will be assessed for eligibility criteria by the PI and study team via telephone screening after referral by the Neurologist. The screening will last approximately 30 minutes. A telephone scipt will be used to screen participants.

3.2 Inclusion criterion:

- Male or female, ages 40-85
- A diagnosis of idiopathic PD based on the UK PD brain bank criteria.(Hughes, Ben-Shlomo et al. 1992, Hughes, Daniel et al. 1992, Hughes, Ben-Shlomo et al. 2001)
- A diagnosis of PD-MCI as determined by the Movement Disorder Society (MDS) Task Force PD-MCI diagnostic criteria (Litvan, Goldman et al. 2012) and a SCOPA-Cog score of 17-24 (Marinus, Visser et al. 2003, Isella, Mapelli et al. 2013, Marras, Armstrong et al. 2013)
- Hoehn and Yahr stage < or equal to 3
- Stable medication regimen for > or equal to 30 days before entering the study
- Living with a carepartner

Exclusion criterion:

- PD subjects meeting MDS criteria for PD dementia (Emre, Aarsland et al. 2007) or having a SCOPA-Cog score less than or equal to 16, or PD subjects having normal cognition defined as a SCOPA-Cog Score of greater or equal to 25.
- Atypical or secondary parkinsonism as determined by referring Neurologist
- Other disorders that might interfere with ability to perform high intensity endurance exercise (e.g. history of stroke, respiratory problems, traumatic brain injury, known advanced osteoarthritis, or neuromuscular disease).
- Individuals with known injury, disease, or condition that would affect the ability to perform endurance exercise.
- Any other clinically significant medical condition, psychiatric condition, drug or alcohol abuse that would, in the judgment of the investigator, interfere with the subject's ability to participate in the study.
- "Vigorous athletes" participating in any exercise program 2X/week or more will be excluded.
- Individuals with any type of implanted electrical device (such as a cardiac pacemaker or a neurostimulator), or a certain type of metallic clip

(i.e., an aneurysm clip in the brain) are not eligible for participation in the MRI study.

- Persons who have had a long history of working in metal shops will be excluded unless they have had a previous orbital x-ray scan clearing them for participation in MRI studies.
- Females who are pregnant or might be pregnant will be excluded from participation.
- Individuals who are claustrophobic and unable to tolerate a brain MRI scan will be excluded.
- Treatment with cholinesterase inhibitors or memantine (i.e., medications approved for treatment of dementia) or medications affecting cognition (i.e., anticholinergics)
- Use of medications that might interfere with neuromuscular junction function such as D-penicillamine and aminoglycoside antibiotics.
- Use of medications that might interfere with the BOLD contrast or heart response to exercise (e.g. β blockers).
- Individuals at high risk for cardiovascular disease as defined in Table 2.2 by the new ACSM guidelines.(Medicine 2014)
- Younger than 40 and older than 85. We exclude participants younger than 40 because these young onset patients may not be typical of the majority of patients who get the disease later in life. We exclude participants older than 85 because of the limited evidence they can successfully complete a high-intensity endurance exercise program. We also exclude patients whose disease was diagnosed before the age of 40.
- Individuals with known endocrine abnormalities or steroid use that could affect cortisol levels (e.g., Cushing's syndrome, pituitary or adrenal gland disease or adrenalectomy, use of steroid-based medications.
- Individuals with strong history of chronic inflammatory or autoimmune diseases or history of chronic use of NSAIDs, which could cause abnormal levels of inflammatory markers in the plasma.

3.3 We will exclude adults unable to consent, individuals who are not yet adults, pregnant women, prisoners and children from this study.

4.0 Study-Wide Number of Subjects: NA (not a multicenter study)

5.0 Study-Wide Recruitment Methods: NA

6.0 Multi-Site Research: NA

7.0 Study Timelines

7.1 The duration of an individual subject's participation in the study will be 6 months. Achieving full participant accrual will require enrolling up to 16 participants over 18 months. The estimated date for the investigators to complete this pilot study is January 1, 2017.

8.0 Study Endpoints

8.1 Primary endpoints:

The primary endpoint will be the mean change in cognitive function from baseline to 6-months as measured by global statistical test (GST) for attention/working memory, executive function, memory, and processing speed. The GSTs will be calculated from the following tests reflecting specific cognitive domains and administered in the cognitive test battery (Attention/working memory, Executive function, Memory, Processing Speed) including:

Symbol Digit Modality Test,(Smith 1973)

Trail Making Test-Parts A and B,(Reitan and Wolfson 1993)

Raven's Progressive Matrices,(Raven, Raven et al. 2003)

Clock Drawing,(Goodglass and Kaplan 1983)

Hopkin Verbal Learning Test-Revised (Word list learning and delayed recall),(Brandt and Benedict 2001)

Free and Cued Selective Reminding Test,(Grober and Buschke 1987)

Figural memory test,(Wilson, Gilley et al. 2000)

Secondary endpoints:

The secondary endpoints will include the following:

a) Change from baseline to 6-months for clinical, behavioral, and additional cognitive measures including the following:

1) Sleep (Pittsburgh Sleep Quality Index)(Buysse, Reynolds et al. 1989), (Epworth Sleepiness Scale),(Johns 1991)
2) Fatigue (Fatigue Severity Scale),(Krupp, LaRocca et al. 1989)
3) Mood (Hamilton Depression Scale),(Hamilton 1967)
4) PD Neurol QOL (NINDS 2015) and Health-related quality of life (PDQ-39),(Hagell and Nilsson 2009)
5) additional cognitive tasks
Language:
Boston Naming Test,(Kaplan 1983)
Verbal fluency [semantic - animals, phonemic - letters];{{Morris, 1989 #47
Visuospatial:
Judgment of Line Orientation,{Benton, 1978 #35
Intersecting pentagons;{Bourke, 1995 #36}.

b) Change from baseline to 6-months for motor function including the following:

1) Movement Disorder Society Unified PD Rating Scale [MDS-UPDRS] part III motor score,(Goetz, Tilley et al. 2008)

2) Mini Balance Evaluation Systems Test (mini-BESTest) (Franchignoni, Horak et al. 2010)

3) Timed up and go (TUG) (Morris, Morris et al. 2001, Huang, Hsieh et al. 2011)

4) The six-minute walk test (6MWT) (Schenkman, Ellis et al. 2011)

5) The Activities-Specific Balance Confidence Scale (ABC) (Dal Bello-Haas, Klassen et al. 2011)

6) The New Freezing of Gait Questionnaire (NFOG-Q)(Nieuwboer, Rochester et al. 2009)

Participants will wear an activity monitor for one week per month. This will allow us to collect average physical activity across one week time periods which will be used to analyze the impact of the intervention outside of the structured exercise sessions of the endurance exercise group. This will allow us to determine if the endurance exercise group alters its overall activity level over the 6 month time period.

c) Change from baseline to 6-months for brain imaging measures including the following:

1) Hippocampal volumes on structural MRI,

2) Microstructural integrity of white matter pathways on diffusion tensor imaging (DTI),

3) Functional connectivity among brain networks on resting state fMRI,
4) Responses to task-based fMRI Digit Symbol Substitution Test.

d) Change from baseline to 6-months for biospecimen measures including the following:

1) Salivary cortisol measurements; in addition, changes in salivary cortisol measures collected over 8 time points on two consecutive days will be explored at the pre-test and the post-test. We will measure cortisol at time points 0 minutes, 15 minutes, 30 minutes, 45 minutes after the person wakes up.(Stalder, Kirschbaum et al. 2016) In addition, we will measure cortisol at 3 hours, 6 hours, 9 hours and 12 hours after awakening.(Edwards, Clow et al. 2001)

2) Soluable inflammatory biomarkers in plasma

3) Peripheral levels of neurotrophic factors in plasma

e) We will collect the NINDS common data elements (CDE) for sex, ethnicity, date-of-birth, education level, family history, and employment status.

8.2. Safety Endpoints: There are no safety endpoints, but all participants will be monitored for safety and for adverse events.

Adverse Events (AEs) are defined as exercise-related discomforts (e.g., muscle and joint soreness/pain), minor injuries (e.g., strains, sprains), and non-injurious falls; and attrition is defined as the number of participants who withdraw from the study. Serious Adverse Events (SAEs) are defined as hospitalization, surgery, death, or permanent disability. We will keep detailed logs of all the training sessions so that we can monitor their progress in the endurance exercise program and assess compliance, which we will take to be completion of 80% of the prescribed exercise sessions.

All participants will be prescreened for MRI scans as per Radiology guidelines to ensure MRI safety including screening for cardiac pacemakers, metal implants of any kind in the body, pregnancy, among other conditions.

9.0 Procedures Involved

9.1 We will conduct a single coordination site, blinded-evaluator, open-label, intervention study of the effects of 6 months of high intensity endurance exercise on cognitive and other clinical/behavioral measures, brain imaging, and cortisol and blood specimens. These measures will be collected at baseline and then again at 6-months or at an early termination visit, should that occur. Cortisol will be collected twice at baseline on consecutive days and twice at 6 months on consecutive days. Blood will be drawn both at baseline and again at 6 months.

9.2 The research procedures and when they are performed are explained below.

Telephone Screening

Telephone screening will occur after referral by the Neurologist. The subject will learn about the study. The screen will rule out any medical conditions that preclude participation in endurance exercise training, brain MRI imaging, or behavioral testing. The screening will last approximately 30 minutes. None of the information attained from the screening will be recorded or utilized as research data. The screening will take place prior to scheduling the subject for any study related data collection and prior to consenting to take part in the research study. If the subject passes the telephone screening, they will be scheduled to come in for testing.

Baseline Visit

Day 1 Afternoon

Obtain written consent and written HIPAA authorization. We will collect NINDS common data elements for sex, ethnicity, date-of-birth, education level, PD disease history, medication history, family history, and employment status from all subjects. In addition, Movement Disorder Society-Unified Parkinson Disease Rating Scale (MDS-UPDRS) Part I, II, and IV of the MDS-UPDRS, and non-motor rating scales will be administered.

1. Informed consent and HIPAA Authorization – 20 minutes to complete
2. SCOPA-Cog – 15 minutes
3. NINDS common data elements – 20 minutes
4. MDS-UPDRS
    a. Part IA completed by rater – 10 minutes to administer
    b. Part IB questionnaire completed by patient – 5 minutes to complete
    c. Part II questionnaire completed by patient – 5 minutes to complete
    d. Part IV completed by rater – 5 minutes to administer
5. Montreal Cognitive Assessment – 10 minutes to complete
6. Pittsburgh Sleep Quality Index – 7 minutes to complete
7. Epworth Sleepiness Scale – 7 minutes to complete
8. Fatigue Severity Scale – 7 minutes to complete
9. Hamilton Depression Scale – 10 minutes to complete
10. PD Neurol QOL – 10 minutes to complete
11. PDQ-39 Scale– 10 minutes to complete
12. Maximal graded exercise test – 1 hour to complete
    - A maximal graded exercise test (GXT) will be performed during intake day to determine HRmax. A resting 12-lead ECG will be recorded in the recumbent and upright positions immediately prior to the exercise test, as well as during the test. If the findings on the resting ECG do not contraindicate exercise, the exercise test will be performed.
    - Contraindicators include the following: (a) ST-segment depression of more than 0.2 mV that is either horizontal, downsloping, or slowly upsloping (less than 1 mV/sec) and lasts for 0.08 sec, or ST-segment elevation greater than 0.1 mV; (b) chest pain or discomfort; (c) serious arrhythmias, including multifocal PVCs, ventricular tachycardia, frequent (>10/min) PVCs or couplets, or sustained atrial tachyarrhythmias; (d) A-V block or other conduction defects; (e) a fall of systolic blood

pressure of 10 mmHg or greater from the peak level with increasing exercise intensity; (f) diastolic blood pressure above 110 mmHg or systolic above 220 mmHg; (g) dizziness; (h) ataxic gait; and (i) pallor or cyanosis. Subjects who have a positive GXT will be ineligible for participation in the study.

- Maximal aerobic power (VO2max) will be measured by indirect calorimetry (TruMax 2400, ParvoMedics, Sandy, UT) during the GXT. A warm-up period on the treadmill will be used to identify the walking speed that generates a HR that is 65-70% of the age-predicted HRmax; for fit individuals, this may require increasing the elevation of the treadmill. After a brief rest interval to initiate the indirect calorimetry, the test will start at the designated walking speed (and grade). The treadmill grade will be increased by 2% every 2 minutes to volitional exhaustion or until the proctor stops the test because of abnormal responses to exercise.

Night Before Testing:

Last dose of antiparkinsonian medications no later than 8.30pm.

Last coffee and alcohol no later than 8.30 pm

Day 2:
Imaging 8:30 to 10:00 AM

MRI Data Acquisition
MRI data will be acquired using a state-of-the-art Prisma 3.0T whole-body clinical MRI system equipped with a high performance gradient system capable of on-axis (x, y and z) peak gradient of >=80 mT/m and 200 mT/m/ms slew rate, and a 64-channel head coil. The scanner is capable of implementing the Human Connectome Protocol (HCP) like sequences. The imaging protocols are designed for one-hour sessions, which in our extensive experience has been well tolerated by all types of PD patients. A headphone, which is a component of the dedicated task delivery system (Avotec system for the standard coil and earbuds from Sensimetrics for 64 channel coils), will be placed over the participant's ears for operator-participant communication. Video stimuli will be delivered through a rear projection system at Northwestern. The fMRI task is described below.

Scan#	Preferred Order NU	Scan Time (min:sec)	Total Time	Total Scan Time
0	Setup	10:00:00	10 min	0 min
1	Scout	0:14:00	10 min 14 sec	0 min 14 sec
2	T 1	4:22:00	14 min 36 sec	4 min 36 sec
3	T 2	3:52:00	18 min 28 sec	8 min 28 sec

4	Resting fMRI – MB8 A>>P	8:08:00	26 min 36 sec	16 min 36 sec
5	Resting fMRI – MB8 P>>A	0:14:00	26 min 50 sec	16 min 50 sec
6	BO field mapping	2:15:00	29 min 05 sec	19 min 05 sec
7	Diffusion (NODDI) AP	10:52:00	39 min 57 sec	29 min 57 sec
8	Diffusion (NODDI) PA	0:33:00	40 min 30 sec	30 min 30 sec

MRI Imaging Session

Scan #1. Auto-Align Scout (Scout, 12 seconds): A single low resolution structural scout scan is collected in the sagittal orientation (TR/TE/flip angle = 3.15 ms/1.37 ms/8^{o}, GRAPPA factor = 3, 1.6 mm isotropic voxels, 128 contiguous slices). The imaging parameters are chosen to obtain a low resolution image which can be used for planning purposes.

Scan #2. T1w structural MRI (MPRAGE, 4 minutes 22 seconds): Volumetric/structural images are obtained using a high resolution three-dimensional (3D) T1-weighted (T1WI) fast gradient echo sequence (TR/TE/flip angle = 2000ms/2.99ms/8^{o}, TI=900 ms, GRAPPA = 2, Partial Fourier = 6/80.8 mm isotropic voxels, 208 contiguous slices). The imaging parameters are chosen to obtain excellent contrast among gray matter, white matter, and cerebral spine fluid (CSF).

Scan #3. T2w structural MRI (3 minutes 52 seconds): Volumetric/structural images are obtained using a high resolution three-dimensional (3D) T2-weighted (T2WI) fast gradient echo sequence (TR/TE = 2500ms/370ms, GRAPPA = 3, 0.8mm isotropic voxels, 208 contiguous slices). The purpose of this scan is to delineate T2 lesions and assist in coregistration and alignment.

Scan #4, and #5. Resting state BOLD fMRI (2 separate resting state scans, 8 minutes and 8 seconds and 14 seconds): Whole brain activation fMRI is obtained with a single-shot gradient echo EPI sequence sensitized to BOLD effect (TR/TE/flip angle = 613ms/22ms/47^{o}, 2mm isotropic voxels, 72 interleaved slices, zero gap). Multiband technology is utilized to speed up data acquisition (MB = 8). The participant is instructed to focus his/her vision on the fixation point (a cross) on the screen, and try to keep their minds free of thought.

Scan #6. B0 field mapping (2 minutes 15 seconds): A dual-echo fast 3D gradient echo sequence is run to map the B0 field (TR/TE1/TE2/flip angle = 733ms/4.92ms/7.38ms/50^{o}, 2mm isotropic voxels, 72 contiguous slices).

Scan #7. and #8 dMRI (NODDI) scan (2 separate scans, 10 minutes 52 seconds and 33 seconds): Whole brain diffusion MRI is obtained with a single-shot spin echo EPI sequence sensitized to diffusion effect (TR/TE/flip angle = 3200ms/70ms/90^{o}/180^{o}, 2.0 mm isotropic voxels, 69 interleaved

slices, zero gap. 64 directions are collected for b=1000, b=2000 and b=3000 s/mm^2), including 5 b = 0 (1 at the beginning and 4 at the end of the scan). Multiband technology is utilized to speed up data acquisition (MB = 3) along with GRAPPA = 2.

Blood draw – 15 minutes to complete

- 30 mL of blood will be taken via antecubital blood draw by a trained nurse from the Clinical Research Unit. Blood will be collected into 10mL K2EDTA tubes (3 per subject) to collect plasma. Blood will be centrifuged at 100-1300RCF for 10min before plasma is collected and transferred to 500ul aluqouts. Plasma will be stored at -80 degrees until it is shipped on dry ice to Dr. Malu Tansey at Emory University. Half of the samples from each participant will be shipped to Emory University, while the other half will stay stored at the Clinical Research Unit as a back up, in case for any reason something happens to the shipment. Both the samples shipped to Emory University and stored in the Clinical Research Unit will be coded using subject study code numbers and will not contain any identifiable subject information. At Emory University, plasma will be subjected to multiplexed immunoassay for measurement of soluble inflammatory markers and neurotrophic factors. Emory University will destroy the samples following analysis. The Clinical Research Unit will destroy the back up samples left behind once they have received written authorization to do so from our research staff, which will happen as soon as the shipped samples are analyzed.

Behavioral testing session 1: (10:30 to 11:30)

Pending subject consent, some of the following behavioral tests may be video or audio recorded to aid in data analysis.

1) MDS-UPDRS, PART III, Motor Section: to measure PD disease severity – 15 minutes to administer
2) Mini Balance Evaluation Systems Test – 10 minutes to administer
3) Timed up and Go (3 trials) - 3 minutes to administer
4) Six-minute walk test – 7 minutes to administer
5) Activities-Specific Balance Confidence Scale – 15 minutes to administer
6) New Freezing of Gait Questionnaire – 10 minutes to administer

Testing is over for the day.

The last dose of antiparkinsonian medications is taken no later than 8.30pm.

Last coffee and alcohol no later than 8.30 pm.

Days 3:

Behavioral testing session 2: (8:30 to 10:25)

Pending subject consent, some of the following behavioral tests may be video or audio recorded to aid in data analysis.

Cognitive testing:

1. Montreal Cognitive Assessment, alternate version – 10 minutes to complete
2. Symbol Digit Modality Test – 5 min to complete
3. Trail Making Test-Parts A and B – 10 min to complete
4. Raven's Progressive Matrices — 10 min to complete
5. Clock Drawing/copying – 10 min to complete
6. Hopkins HVLT-R Word list learning, delayed recall and recognition – 10 min to complete
7. Free and Cued Selective Reminding Test, delayed recall and recognition – 15 min to complete
8. Figural memory test - to measure visual memory – 10 min to complete
9. Boston Naming Test (15 item) - 5 min to complete
10. Verbal fluency [semantic - animals, phonemic - letters] – 5 min to complete
11. Judgment of Line Orientation (15 item) - 10 min to complete
12. Intersecting pentagons – 5 min to complete

Day 4 and Day 5 (These will be 2 typical week days):
Cortisol Assessment

We will collect saliva samples during the post-awakening period (0, 15, 30, and 45 minutes post-awakening) to measure the cortisol awakening response. We will also collect samples at approximately (3, 6, 9 and 12 hours post-awakening) on two consecutive weekdays. The participant will be required to record and report their awakening and saliva sampling times. There will be objective measurement of awakening and sampling times in the 45 minutes post awakening.

Exercise Intervention

Intervention, Administration and Duration

Subjects will exercise on a treadmill using procedures that have been used over the past two decades.(Kohrt, Malley et al. 1991, Kohrt, Spina et al.

1993, Kohrt, Spina et al. 1998) The regimen will include 5-10 min of warm up, 30 min of exercise at target HR and 5-10 min of cool down. They will exercise 3 days per week. The intervention group will be given a HR range to achieve during exercise sessions: 80.0%-85.0% HRmax. During the first 8 weeks of training, exercise duration and intensity will be gradually increased to the target levels. Subjects will be instructed on monitoring HR and adjusting the exercise to remain in the target HR range (i.e., by changing treadmill speed and/or incline). During exercise, subjects will wear a heart rate monitor that captures and stores HR throughout the exercise bout. The exercise Research Assistant (RA) will electronically transfer the HR file from each exercise session (supervised and unsupervised) into the study database.

During the first 2 weeks, subjects must exercise at a main study site under supervision of an Exercise training RA. All 'on-site' exercise sessions will be supervised by an exercise RA. After 2 weeks, the RA will determine whether the subjects may exercise off-site at a community fitness facility or at home to maximize the likelihood of compliance. If the subjects are exercising in their target HR range for the prescribed duration and have demonstrated to the exercise RA that they are able to operate the HR monitor, the subjects will exercise 2X/wk at the main site for 2 weeks and then 1X/wk for 2 weeks. Thereafter, if cleared by the RA, subjects will be expected to exercise on-site at least 1 x/month at the main site. Other exercise sessions will take place at the approved off-site facility. HR monitors must be worn for all sessions. HR data will be downloaded once weekly by an approved person at the facility or brought to a main site to be downloaded by the RA. In addition, the subject will be given an exercise diary log to document his/her wear time sessions on a weekly basis. The RA will check adherence to their prescription each week and will work with the participant as needed to assure appropriate adherence to the protocol.

The intent of allowing exercise off site is to enhance recruitment, retention, and long-term adherence to exercise. Safety with respect to treadmill exercise will be established for each subject during the first two weeks of exercise at the main study site and also has been established in our previous work for 129 subjects with PD, Stages 1.5-3 of H&Y who exercised for up to 6 months without incident (Schenkman and Corcos, unpublished data). Additionally, many of the designated health clubs we work with are affiliated with hospitals and specialize in exercise programs for people with PD.

The exercise training RA will assure exercise fidelity for each subject by comparing exercise sessions, duration and mean HR to the subject's target on a weekly basis and by working with the subject to make necessary adjustments.

An optional online survey administered via email through REDCAP will be sent to participants following completion of the study. The purpose of the online survey is to better understand each subject's experience and satisfaction with the study procedures.

9.3 Procedures performed to lessen the probability or magnitude of risk and source records are described below:

- Minimizing physical, psychological, and social risks: We see no psychological, social, or legal risks beyond those of participation in health-related research in general.

- Minimizing risks to confidentiality: These risks will be minimized by not including personal identifying information on the forms, and by conducting interviews and collection of personal information in a private setting.

- Minimizing risks associated with testing protocol:
  - Clinical assessments: Possible risks associated with clinical evaluations are minimal but may include fatigue, emotional distress, or embarrasment related to some questions on thinking and mood; to minimize these, we will provide breaks in the testing schedule and ask questions in a sensitive, non-judgmental manner.
  - MRI Imaging: All potential participants will be screened for eligilibity for a MRI scan as per Radiology guidelines for safety precautions including cardiac pacemaker, metallic implants of any kind in the body, pregnancy, among others. Prior to entering the MRI scan, subjects will be asked again about these safety precautions to determine eligibility for MRI scan. Other possible risks include claustrophobia, boredom, noise, or discomfort from lying supine which will be minimized by talking with the subject periodically during the scanning, providing ear plugs, and providing cushioning. The movement tests are associated with minimal risks, such as stiffness or pain from performing maximal voluntary contractions. This risk will be minimized by providing rest breaks between trials, excluding participants who experience hand pain or other adverse events, and monitoring discomfort. If an individual requires emergency medical care, 911 will be called immediately. This has never happened previously and we have tested more than 100 individuals.
  - Exercise testing: We are using an endurance exercise protocol that we have extensive experience with. Participants are very closely supervised over the first 2 weeks of training to make sure that they are safe and can perform the exercise protocol.
  - Medication withdrawal: All subjects are offered hotel

accomodations near the testing facility on the nights prior to testing days during which they are asked to withdrawal from anti-Parkinsonian medication in order to avoid the need to commute to the testing facility while off medication. A research assistant will meet the subjects each morning at the hotel ad will accompany the subject via wheelchair, if needed, to the testing facility.

- The risk associated with blood draws are minimal and are reduced in this study because trained personnel who have extensive venipuncture experience, and are trained in aseptic technique, will implement the blood drawing procedures in a clean room.

- No drugs and devices will be used in the research - NA.

- Data collection forms will be used to record the study data and are attached. Forms for test measures are attached for the following tests:

1. SCOPA-Cog
2. NINDS Demographics, CDE
3. MDS UPDRS parts 1,2, 3 (motor) and 4
4. PD Neurol QOL,
5. MOCA
6. Pittsburgh Sleep Quality Index,
7. Epworth Sleepiness Scale,
8. Fatigue Severity Scale,
9. Hamilton Depression Scale,
10. Health-related quality of life (PDQ-39),
11. Mini Balance Evaluation Systems Test
12. Timed up and Go (3 trials)
13. Six-minute walk test
14. Activities-Specific Balance Confidence Scale
15. New Freezing of Gait Questionnaire
16. MoCA Alternate version
17. Symbol Digit Modality Test,
18. Trail Making Test-Parts A and B,
19. Raven's Progressive Matrices,
20. Clock Drawing,
21. Hopkins HVLT-R,
22. Free and Cued Selective Reminding Test,
23. Figural memory test, ,
24. Boston Naming Test,
25. Verbal fluency [semantic - animals, phonemic - letters],
26. Judgment of Line Orientation,
27. Intersecting pentagons,

9.4 The data that will be collected is outlined in Table 1.

Table 1 **Dependent Measures**	**Baseline**	**Each ex session**	**Each mo**	**6 mo**
Primary Measures				
Cognitive Measures: Symbol Digit Modality Test, Trail Making Test-Parts A and B, Raven's Progressive Matrices, Clock Drawing, Word list learning and delayed recall, Free and Cued Selective Reminding Test, Figural memory test,	X			X
Secondary Measures				
National Institute of Neurological Disorders and Stroke common data elements	X			
MDS-UPDRS I, II, IV	X			X
Sleep (Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale	X			X
Fatigue (Fatigue Severity Scale)	X			X
Mood (Hamilton Depression Scale)	X			X
PDQ-39	X			X
Cognitive Measures: Boston Naming Test, Verbal Fluency, Judgment of Line Orientation, Intersection pentagons, Spatial working memory	X			X
MDS-UPDRS III	X			X
Neuro-QOL	X			X
Mini Balance Evaluation Systems Test (Mini-BESTest)	X			X
Timed Up and Go	X			X
6 minute walk test	X			X
Activities-Specific Balance Confidence Scale (ABC scale)	X			X
New Freezing of Gait Questionnaire (NFOG-Q)	X			X
Activity Monitor			X	
MRI: Hippocampal volumes	X			X
MRI: DTI measures	X			X
MRI: Functional connectivity	X			X
Serious Adverse Events (SAEs) and Adverse Events (AEs)			X	
Attrition			X	
EE training log		X		
Levodopa Equivalent	X			X
Cortisol (2 consecutive time points)	X (8)			X (8)
Blood Draw	X			X

10.0 Data and Specimen Banking:

10.1 The primary investigator and co-investigators will have access to the raw data. Research staff at Northwestern University will be restricted assess to the online database for data entry and report generation. Any data viewed by others/collaborators will be de-identified. Hard copies of data collection forms will be stored in a locked office or laboratory. Data entered into spreadsheets will be kept on password protected computer drives.

10.2 Participants will be requested to conduct the saliva sampling protocol in their domestic setting on two weekdays. Saliva sampling will commence in the post-awakening period and across the day. Saliva will be collected by salivettes. Prior to saliva sampling (30 min) participants will be instructed to take nil by mouth bar (except water). During the post-awakening period they will be asked to refrain from brushing their teeth to avoid abrasion and vascular leakage. SMS text messages will be used to send reminders to participants to complete saliva samples. Electronic devices will be used to record awakening and saliva sampling times (actigraph & trackcaps respectively). Participants will received detailed verbal and written instructions on the study protocol. Saliva samples will be stored in the freezer at -80°C in a special purpose labatory on the 7th floor of 645 N. Michigan Avenue. For salivary cortisol assay, the Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit will be used. This assay captures the full range of salivary cortisol levels (0.003 to 3.0µg/dL) requiring only 25 uL of saliva per test. A built-in pH indicator detects acidic or basic samples.

10.3 The data will be shared with Rush University Medical Center. The data will not be identifiable when shared. Cortisol data will be shared with Dr. Angela Clow and Nina Smyth at the University of Westminster. Data shared with the University of Westminster will be de-identified. Data that can be used for descriptive statistics including but not limited to gender, age, disease duration, MDS-UPDRS scores, and cognitive data will also be shared with the University of Westminster. No data shared with the University of Westminster will be identifiable data.

11.0 Data and Specimen Management

11.1 We are collecting pilot data primarily for feasibility, though statistical analyses may be conducted to provide descriptive data of the subject cohort, baseline and 6-month test scores, and potentially exploratory analyses of change in outcome measures or correlations of variables.

11.2 Since we are collecting pilot data, there is no power analysis.

11.3 The research will be conducted in compliance with state and federal laws, including the Health Insurance Portability and Accountability Act (HIPAA that require researchers to protect and maintain confidentiality of an individual's health information. All subjects will be asked to sign an "Authorization To Use and Disclose (Release) Health Information For a Research Study". Access to all study datasets will be password protected. All research staff will be trained in proper research procedures. We have extensive experience in data capture, data flow and data management based on our SPARX Phase II clinical trial.(Moore, Schenkman et al. 2013) We will use REDCap (Research Electronic Data Capture) to define and manage our own data collection forms. This service is provided through the Northwestern University Clinical and Translational Institute.

11.4 Quality control is addressed at the point of data collection, data entry, and data analysis.

- Imaging – the imaging center conducts tests for quality control of the imaging data. A quality control scan is run every day before data acquisition begins.
- All of our data is double checked for accuracy by a member of our research team. Periodically we have our data checked by a person who is not directly involved in the laboratory.

11.5 Study-wide handling of data and specimens is described below:

- Our data consists of analog data and text.
- Our data is stored on hard disks and backed up regularly.
- Our data is stored indefinitely.
- The clinical coordinator, statistician and students and post- doctoral fellows will have access to the data.
- The clinical coordinator is responsible for receipt of the data.
- The data will not be transported.
- The cortisol data will initially stored in a freezer at -80°C before being shipped out for analysis.

12.0 Provisions to Monitor the Data to Ensure the Safety of Subjects:

This study does not involve more than minimal risk.

13.0 Withdrawal of Subjects*

13.1 Participation in this study will be stopped by the investigator without subject consent if circumstances arise which warrant doing so. This would include injury that would limit participation in the exercise portion of the study or if the person became ill during the research, they may have to drop out, even if they would like to continue. The investigators, Daniel Corcos, PhD and Jennifer G. Goldman, M.D. will make the decision and let the person know if it is not possible

for them to continue. The decision may be made to protect the health and safety of the participant.

13.2 Subjects may withdraw for the research at any time point. Subjects will be asked if they would be willing to take part in the post-test.

14.0 Risks to Subjects*

14.1 Overall, potential foreseeable risks associated with participation in the study are very unlikely and of low risk.

- The potential risks of endurance exercise includes injury to tendons, ligaments, joints, and muscles. There is little likelihood of any physical risk as a result of participation in this research project. The exercise and testing procedures have been well established for people with Parkinson's disease. The use of a well-trained personal trainer further reduces any possible risks.
- During exercise testing there is a chance that subjects could fall, feel pain in their muscles or joints, feel dizzy or faint, have irregular heartbeats, have a stroke or heart attack, or die. The risk of death from an exercise stress test has been estimated to be 1 in 100,000. The risk of a heart attack is about 4 in 10,000 and the risk of a problem that would require hospitalization (for example, chest pain) is about 2 in 1,000. To minimize this risk, only subjects at low to moderate risk will be allowed to particiate in exercise testing. Subjects can request to terminate the exercise test at any point during testing. ACSM subject screening and testing guidelines will be followed. The exercise test will be administered by an exercise physiologist who is Advanced Cardiac Life Support (ACLS) certified and has automated external defibrillator (AED) experience. The exercise physiologist administering the test will also have knowledge of appropriate contraindications, risk, and risk assessment of testing, the absolute and relative indications for termination of the exercise test, knowledge of cardiac arrhythmias, and the ability to recognize and treat serious arrhythmias if they arise.
- There are no known hazards to diffusion magnetic resonance imaging, functional magnetic resonance imaging, other than the discomfort associated with confinement and remaining stationary for the duration of the testing procedure. We have found that training in the MR simulation scanner helps to reduce the stress associated with the noise and confinement of the actual MR scanner environment. Testing of all participants will be terminated immediately should they become distressed. We will carefully screen out individuals who have an implanted electrical device (such as a cardiac pacemaker or a neurostimulator), or a certain type of metallic clip (i.e., an aneurysm clip

in the brain). These devices can malfunction and cause harm to the patient in the magnet environment. We will also carefully screen individuals who are claustrophobic to reduce any adverse events. We anticipate that most patients will come by limousine to the testing facility when off medication. Under no circumstance will we allow a patient to drive when off medication.

- Risk associated with blood draws: pain, a bruise at the point where blood is taken, redness and swelling of the vein and infection, and a rare risk of fainting. These risks associated with blood draws are minimal, and are reduced in this study because trained personnel who have extensive venipuncture experience, and are trained in aseptic technique, will implement the blood drawing procedures in a clean room.

14.2 N/A
14.3 N/A
14.4 N/A

15.0 Potential Benefits to Subjects

15.1 Individuals who participate in this research may get in better physical shape. They may be able to more easily perform daily activities, and may experience improvements in the symptoms associated with Parkinson's disease. There is also the possibility of improved cognition. The duration is the time course of the study (6 months) plus a few extra months.

15.2 N/A

16.0 Vulnerable Populations:

Participants in this study may have mild cognitive impairment but will not have dementia as determined by the referring Neurologist and screening criteria. Cognitive impairment will be assessed by the clinicians and study personnel through interview of the patient (and their caregiver) and cognitive testing to determine the presence of cognitive impairment, clinical diagnosis of dementia, and capacity to consent. This information will be used to determine if the person is able to understand the information relevant to making a decision about the study and appreciate what it means to participate or not participate in the study. Patients who are considered for study participation will have a caregiver or legal representative present as part of the inclusion criteria. If the subject does not have the capacity to consent, they will be exluded from the study. Because the criteria for participation in the study requires only a mild cognitive impairment, eligible participants will have the capacity to consent.

17.0 Community-Based Participatory Research: NA

18.0 Sharing of Results with Subjects

18.1 No specific results are shared with subjects. It is possible that we may observe what might be considered abnormalities on brain scans. We might also observe scores on some of the tests that we administer that are considered outliers. We share these results with the neurologist of the subject and let the neurologist decide whether or not to share the result with the patient.

19.0 Setting

19.1 The locations where our research team will conduct the research are described below:

- Participants with PD who are currently taking medication for PD will be recruited from the outpatient Neurology Movement Disorder practices at Northwestern University and Rush University Medical Center in Chicago, IL. All potential participants will be invited to the study by their primary neurologist.
- All research procedures will be performed at Northwestern University Department of Physical Therapy & Human Movement Sciences using one of two dedicated research rooms. All of the brain scanning will be conducted at Olson Pavilion, 710 N Fairbanks – Lower Concourse.
- There will be no community advisory board.
- There will be no research conducted outside of the institution.
- Participants will initially exercise at Northwestern University or a local gym or fitness facility of their choosing until they are comfortable exercising independently, after which they will exercise either at home or in community exercise facilities of their own choosing.

20.0 Resources Available

20.1 NIH has funded Dr. Corcos, one of the PIs of this study, continuously since 1986. He is currently the co-PI on the SPARX NIH sponsored Phase II Clinical Trial on the effects of endurance exercise in Parkinson's disease. He has published more than 170 research articles. He has chaired 2 different study sections at NIH. The other PI is Dr. Jennifer G. Goldman who is a Movement Disorders specialist at Rush University Medical Center. She is an expert on cognitive impairment in Parkinson's disease, in particular PD-MCI. They have a very experienced research team (Dr. David and Ms. Skender) that has helped him conduct one clinical trial and who are currently working on another clinical trial. Dr. Corcos has performed all aspects of this research study on this patient population in Chicago. Dr. Goldman has treated hundreds of patients with Parkinson's disease.

20.2 It is feasible for us to recruit up to 16 participants. The sections of Movement Disorders at Northwestern University and Rush University Medical Center see over 180 patients with Parkinson's disease every week, and 2,900 patients every year. Approximately 25% of these patients have MCI.

The PIs will each be devoting at least 10% effort to conducting and completing this research. This is ample for the protection of patients.

The facilities include:

- One room that is dedicated to collection of functional data, cognitive data and other questionnaire data. This room has a couch and is very quiet should patients need to rest at any time (room number 1124).
- The imaging center at Northwestern University is where the brain imaging will occur and is very well equipped for all of the proposed studies.

We have previously conducted a very similar endurance exercise program and had 77 people with PD enroll in the program. We have conducted all of the imaging experiments on more than 100 patients. We do not expect to need any medical or psychological resources. We do have access to all medical and psychological resources of the Department of Neurology should such a need arise.

Our staff is very well trained. They have extensive experience in conducting similar studies. They have all read the IRB. We have weekly meetings to make sure everyone knows their specific duties.

21.0 Prior Approvals

21.1 We will be using the exercise facilities at NU or community fitness facilities to train participants in the endurance exercise protocol. No community fitness facilities will be engaged in the research. We will attain premission from community fitness facilities to have an RA accompany the research subject for the first few weeks of the exercise protocol to ensure that the subject is familiar with the protocol and equipment.

22.0 Recruitment Methods

22.1 We will commence enrolling subjects and collecting data as soon as we receive IRB approval. Men and women with PD who are currently taking medication for PD will be recruited from the outpatient neurology practices at Northwestern University and Rush University Medical Center. They are located 4 miles apart in Chicago. All potential participants will be invited to the study by their primary neurologist. Nothing beyond recruitment will occur at Rush University Medical Center.

22.2 Subjects will come from outpatient neurology practices at Northwestern University and Rush University Medical Center in Chicago, IL.

22.3 Neurologists at Northwestern University or Rush University Medical Center will invite individuals to participate in the study if they have idiopathic PD, age 40-85, H&Y stage < or equal to 3, and able and willing to sign informed consent. They will be excluded if they have any condition that precludes taking part in an exercise intervention or undergoing MRI, have no cognitive impairment as assessed by the SCOPA Cog scores greater than or equal to 25, PD dementia, or if they are unwilling to comply with the study procedures.

22.4 Participants with PD will be recruited through Neurologists at Northwestern University and Rush University Medical Center. Flyers will be posted at Northwestern University.

22.5 There are no payments made to subjects for participation in the study. To avoid subjects having to pay for meals out of pocket, VISA gift cards pre-loaded with $320 will be given on each testing day to cover meals for both the subject and his or her caregiver who may accompany the subject. They will each be allowed $40 per person per meal for breakfasts, and $60 per person per meal for lunches and dinners. The cards will be reloaded daily and receipts will be collected for any expenses charged to the card. The subject will turn in the card to the RA at the end of the final day of testing. However, if the subjects for any reason do utilize their own money for subject related expenses such as meals, transportation, or parking, they will be required to turn in receipts in order to be reimbursed. Within a week, these receipts will be turned in to the accounting department for reimbursement. The accounting department will mail it to the subject within 40 business days from the last day of the subject's visit.

23.0 Local Number of Subjects

23.1 The total number of subjects to be accrued locally is up to 16.

23.2 We will study up to 16 patients in a pilot study.

23.3 Allowing for the possibility of screen failures or study drop-outs, we may need to recruit 16 participants.

24.0 Confidentiality: NA

25.0 Provisions to Protect the Privacy Interests of Subjects

25.1 The only people who will know that a participant is a research subject are members of the research team. No information about participants or information provided by the participants during the research will be disclosed to others without their written permission,

except: 1) if necessary to protect participant's rights or welfare (for example, if they are injured and need emergency care or when the Institutional Review Board monitors the research or consent process); or 2) if required by law.

25.2 Subjects who choose to be in this study will be informed that they have the right to be treated with respect, including respect for the decision whether or not to continue or stop participating in the study. All examinations and procedures will be thoroughly explained to each subject to ensure subjects feel at ease with the questions, examinations, and procedures involved. Subjects will be informed that if at any point they feel uncomfortable about answering any questions on any questionnaires or surveys, they will not be required to answer that question. Subjects will be informed that they are free to choose to stop being in the study at any time, and that choosing not to be in this study or to stop being in this study will not result in any penalty or loss of benefit to which they are entitled. Specifically, the choice not to be in this study will not negatively affect the right to any present or future medical treatment.

25.3 The NU research staff will obtain a release from the patient to access medical records from Rush University Medical Center.

26.0 Compensation for Research-Related Injury

26.1 Minimal risk is when the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater, in and of themselves, than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests. The proposed research does not exceed minimal risk. In the event of research-related injury, subjects will not be compensated for medical care required because of an untoward outcome resulting from participation in the research study. Subjects should seek medical treatment through his or her doctor or treatment center of choice.

26.2 If a participant becomes ill or get san injury or illness as a result of study (medications, devices or procedures), they should seek medical treatment through their doctor or treatment center of choice. They should promptly tell the study doctor about any illness or injury. The hospital [university, researchers] will not pay for medical care required because of a bad outcome resulting from their participation in this research study. This does not keep them from seeking to be paid back for care required because of a bad outcome.

27.0 Economic Burden to Subjects

27.1 The tests and procedures completed for this study will be at no cost to the subjects. Subjects will be given reimbursement for expenses related to hotel stay, food and parking. Every subject will be offered hotel accomodations during the nights that they are asked to start withdrawl from medication (the night of baseline visit and the night of Day 1 testing). To avoid subjects having to pay for meals out of pocket, VISA gift cards pre-loaded with $320 will be given on each testing day to cover meals for both the subject and his or her caregiver who may accompany the subject. They will each be allowed $40 per person per meal for breakfasts, and $60 per person per meal for lunches and dinners. The cards will be reloaded daily and receipts will be collected for any expenses charged to the card. The subject will turn in the card to the RA at the end of the final day of testing. If subjects become ill or injured as a result of this study, they will be advised to seek medical treatment through their medical doctor or treatment center of choice, which will be at his or her own expense and may lead to added costs for the subject or the subject's insurance company.

28.0 Consent Process

28.1 "SOP: Informed Consent Process for Research (HRP-090)" will be followed in order to obtain consent from each individual subject. On arrival to Northwestern University Department of Physical Therapy and Human Movement Science for data collection, subjects will be re-oriented on the study protocol, will be given an opportunity to ask any questions, and will sign an informed consent. If there is a significant change to the research protocol or if there is new information that may alter an individual's willingness to participate in the research, subjects will be provided an updated informed consent document and consent will be re-obtained.

29.0 Process to Document Consent in Writing

29.1 We will be following "SOP: Written Documentation of Consent (HRP-091)" to document consent in writing from all subjects. On arrival to Northwestern University Department of Physical Therapy and Human Movement Science for data collection, subjects will be re-oriented on the study protocol by a study team member, will be given an opportunity to ask any questions, will be walked through all sections on all pages of the informed consent, and will sign the informed consent.

29.2 A copy of the consent document is attached.

30.0 Drugs or Devices: NA

Relevant Literature:

Awick, E. A., T. R. Wojcicki, E. A. Olson, J. Fanning, H. D. Chung, K. Zuniga, M. Mackenzie, A. F. Kramer and E. McAuley (2015). "Differential exercise effects on quality of life and health-related quality of life in older adults: a randomized controlled trial." Qual Life Res **24**(2): 455-462.

Baker, L. D., L. L. Frank, K. Foster-Schubert, P. S. Green, C. W. Wilkinson, A. McTiernan, S. R. Plymate, M. A. Fishel, G. S. Watson, B. A. Cholerton, G. E. Duncan, P. D. Mehta and S. Craft (2010). "Effects of aerobic exercise on mild cognitive impairment: a controlled trial." Arch Neurol **67**(1): 71-79.

Beluche, I., I. Carriere, K. Ritchie and M. L. Ancelin (2010). "A prospective study of diurnal cortisol and cognitive function in community-dwelling elderly people." Psychol Med **40**(6): 1039-1049.

Brandt, J. and R. Benedict (2001). Hopkins Verbal Learning Test-Revised: Professional Manual. . Florida, PAR.

Buchanan, T. W., S. Kern, J. S. Allen, D. Tranel and C. Kirschbaum (2004). "Circadian regulation of cortisol after hippocampal damage in humans." Biol Psychiatry **56**(9): 651-656.

Buysse, D. J., C. F. Reynolds, 3rd, T. H. Monk, S. R. Berman and D. J. Kupfer (1989). "The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research." Psychiatry Res **28**(2): 193-213.

Corcos, D. M., J. A. Robichaud, F. J. David, S. E. Leurgans, D. E. Vaillancourt, C. Poon, M. R. Rafferty, W. M. Kohrt and C. L. Comella (2013). "A two-year randomized controlled trial of progressive resistance exercise for Parkinson's disease." Mov Disord **28**(9): 1230-1240.

Dal Bello-Haas, V., L. Klassen, M. S. Sheppard and A. Metcalfe (2011). "Psychometric Properties of Activity, Self-Efficacy, and Quality-of-Life Measures in Individuals with Parkinson Disease." Physiother Can **63**(1): 47-57.

David, F. J., J. A. Robichaud, S. E. Leurgans, C. Poon, W. M. Kohrt, J. G. Goldman, C. L. Comella, D. E. Vaillancourt and D. M. Corcos (2015). "Exercise Improves Cognition in Parkinson's Disease: The PRET-PD Randomized, Clinical Trial." Mov Disord.

de Quervain, D. J., A. Aerni, G. Schelling and B. Roozendaal (2009). "Glucocorticoids and the regulation of memory in health and disease." Front Neuroendocrinol **30**(3): 358-370.

Diamond, D. M., M. C. Bennett, M. Fleshner and G. M. Rose (1992). "Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation." Hippocampus **2**(4): 421-430.

Dorsey, E. R., R. Constantinescu, J. P. Thompson, K. M. Biglan, R. G. Holloway, K. Kieburtz, F. J. Marshall, B. M. Ravina, G. Schifitto, A. Siderowf and C. M. Tanner (2007). "Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030." Neurology **68**(5): 384-386.

Duzel, E., H. van Praag and M. Sendtner (2016). "Can physical exercise in old age improve memory and hippocampal function?" Brain **139**(Pt 3): 662-673.

Edwards, S., A. Clow, P. Evans and F. Hucklebridge (2001). "Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity." Life Sci **68**(18): 2093-2103.

Emre, M., D. Aarsland, R. Brown, D. J. Burn, C. Duyckaerts, Y. Mizuno, G. A. Broe, J. Cummings, D. W. Dickson, S. Gauthier, J. Goldman, C. Goetz, A. Korczyn, A. Lees,

R. Levy, I. Litvan, I. McKeith, W. Olanow, W. Poewe, N. Quinn, C. Sampaio, E. Tolosa and B. Dubois (2007). "Clinical diagnostic criteria for dementia associated with Parkinson's disease." Mov Disord **22**(12): 1689-1707; quiz 1837.

Erickson, K. I., M. W. Voss, R. S. Prakash, C. Basak, A. Szabo, L. Chaddock, J. S. Kim, S. Heo, H. Alves, S. M. White, T. R. Wojcicki, E. Mailey, V. J. Vieira, S. A. Martin, B. D. Pence, J. A. Woods, E. McAuley and A. F. Kramer (2011). "Exercise training increases size of hippocampus and improves memory." Proc Natl Acad Sci U S A **108**(7): 3017-3022.

Evans, P., F. Hucklebridge, C. Loveday and A. Clow (2012). "The cortisol awakening response is related to executive function in older age." Int J Psychophysiol **84**(2): 201-204.

Foster, P. P., K. P. Rosenblatt and R. O. Kuljis (2011). "Exercise-induced cognitive plasticity, implications for mild cognitive impairment and Alzheimer's disease." Front Neurol **2**: 28.

Franchignoni, F., F. Horak, M. Godi, A. Nardone and A. Giordano (2010). "Using psychometric techniques to improve the Balance Evaluation Systems Test: the mini-BESTest." J Rehabil Med **42**(4): 323-331.

Goetz, C. G., B. C. Tilley, S. R. Shaftman, G. T. Stebbins, S. Fahn, P. Martinez-Martin, W. Poewe, C. Sampaio, M. B. Stern, R. Dodel, B. Dubois, R. Holloway, J. Jankovic, J. Kulisevsky, A. E. Lang, A. Lees, S. Leurgans, P. A. LeWitt, D. Nyenhuis, C. W. Olanow, O. Rascol, A. Schrag, J. A. Teresi, J. J. van Hilten and N. LaPelle (2008). "Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results." Mov Disord **23**(15): 2129-2170.

Goldman, J. G., S. Holden, B. Bernard, B. Ouyang, C. G. Goetz and G. T. Stebbins (2013). "Defining optimal cutoff scores for cognitive impairment using Movement Disorder Society Task Force criteria for mild cognitive impairment in Parkinson's disease." Mov Disord **28**(14): 1972-1979.

Goldman, J. G., S. Holden, B. Ouyang, B. Bernard, C. G. Goetz and G. T. Stebbins (2015). "Diagnosing PD-MCI by MDS Task Force criteria: how many and which neuropsychological tests?" Mov Disord **30**(3): 402-406.

Goldman, J. G., G. T. Stebbins, B. Bernard, T. R. Stoub, C. G. Goetz and L. deToledo-Morrell (2012). "Entorhinal cortex atrophy differentiates Parkinson's disease patients with and without dementia." Mov Disord **27**(6): 727-734.

Goldman, J. G. and D. Weintraub (2015). "Advances in the treatment of cognitive impairment in Parkinson's disease." Mov Disord **30**(11): 1471-1489.

Goodglass, H. and E. Kaplan (1983). Clock Drawing Test. Assessment of aphasia and related disorders. Philadelphia, Lea and Febiger.

Grober, E. and H. Buschke (1987). "Genuine memory deficits in dementia." Dev Neuropsychol **3**: 13-36.

Hagell, P. and M. H. Nilsson (2009). "The 39-Item Parkinson's Disease Questionnaire (PDQ-39): Is it a Unidimensional Construct?" Ther Adv Neurol Disord **2**(4): 205-214.

Hamilton, M. (1967). "Development of a rating scale for primary depressive illness." Brit Jrl Soc Clin Psych **6**: 278-297.

Heffelfinger, A. K. and J. W. Newcomer (2001). "Glucocorticoid effects on memory function over the human life span." Dev Psychopathol **13**(3): 491-513.

Hely, M. A., W. G. Reid, M. A. Adena, G. M. Halliday and J. G. Morris (2008). "The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years." Mov Disord **23**(6): 837-844.

Huang, S. L., C. L. Hsieh, R. M. Wu, C. H. Tai, C. H. Lin and W. S. Lu (2011). "Minimal detectable change of the timed "up & go" test and the dynamic gait index in people with Parkinson disease." Phys Ther **91**(1): 114-121.

Hughes, A. J., Y. Ben-Shlomo, S. E. Daniel and A. J. Lees (1992). "What features improve the accuracy of clinical diagnosis in Parkinson's disease: a clinicopathologic study." Neurology **42**(6): 1142-1146.

Hughes, A. J., Y. Ben-Shlomo, S. E. Daniel and A. J. Lees (2001). "What features improve the accuracy of clinical diagnosis in Parkinson's disease: a clinicopathologic study. 1992." Neurology **57**(10 Suppl 3): S34-38.

Hughes, A. J., S. E. Daniel, L. Kilford and A. J. Lees (1992). "Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases." J Neurol Neurosurg Psychiatry **55**(3): 181-184.

Isella, V., C. Mapelli, N. Morielli, C. Siri, D. De Gaspari, G. Pezzoli, A. Antonini, M. Poletti, U. Bonuccelli, L. Picchi, A. Napolitano, M. Vista and I. M. Appollonio (2013). "Diagnosis of possible mild cognitive impairment in Parkinson's disease: validity of the SCOPA-Cog." Parkinsonism Relat Disord **19**(12): 1160-1163.

Joels, M. and H. J. Krugers (2007). "LTP after stress: up or down?" Neural Plast **2007**: 93202.

Johns, M. W. (1991). "A new method for measuring daytime sleepiness: the Epworth sleepiness scale." Sleep **14**(6): 540-545.

Kaplan, E., Goodglass, H. and Weintraub, S. (1983). Boston Naming Test Philadelphia Lea & Febiger.

Kohrt, W. M., M. T. Malley, A. R. Coggan, R. J. Spina, T. Ogawa, A. A. Ehsani, R. E. Bourey, W. H. Martin, 3rd and J. O. Holloszy (1991). "Effects of gender, age, and fitness level on response of VO2max to training in 60-71 yr olds." J Appl Physiol (1985) **71**(5): 2004-2011.

Kohrt, W. M., R. J. Spina, A. A. Ehsani, P. E. Cryer and J. O. Holloszy (1993). "Effects of age, adiposity, and fitness level on plasma catecholamine responses to standing and exercise." J Appl Physiol (1985) **75**(4): 1828-1835.

Kohrt, W. M., R. J. Spina, J. O. Holloszy and A. A. Ehsani (1998). "Prescribing exercise intensity for older women." J Am Geriatr Soc **46**(2): 129-133.

Krupp, L. B., N. G. LaRocca, J. Muir-Nash and A. D. Steinberg (1989). "The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus." Arch Neurol **46**(10): 1121-1123.

Litvan, I., D. Aarsland, C. H. Adler, J. G. Goldman, J. Kulisevsky, B. Mollenhauer, M. C. Rodriguez-Oroz, A. I. Troster and D. Weintraub (2011). "MDS task force on mild cognitive impairment in Parkinson's disease: Critical review of PD-MCI." Mov Disord **26**(10): 1814-1824.

Litvan, I., J. G. Goldman, A. I. Troster, B. A. Schmand, D. Weintraub, R. C. Petersen, B. Mollenhauer, C. H. Adler, K. Marder, C. H. Williams-Gray, D. Aarsland, J. Kulisevsky, M. C. Rodriguez-Oroz, D. J. Burn, R. A. Barker and M. Emre (2012).

"Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines." Mov Disord **27**(3): 349-356.

Lupien, S. J., A. Fiocco, N. Wan, F. Maheu, C. Lord, T. Schramek and M. T. Tu (2005). "Stress hormones and human memory function across the lifespan." Psychoneuroendocrinology **30**(3): 225-242.

Marinus, J., M. Visser, N. A. Verwey, F. R. Verhey, H. A. Middelkoop, A. M. Stiggelbout and J. J. van Hilten (2003). "Assessment of cognition in Parkinson's disease." Neurology **61**(9): 1222-1228.

Marras, C., M. J. Armstrong, C. A. Meaney, S. Fox, B. Rothberg, W. Reginold, D. F. Tang-Wai, D. Gill, P. J. Eslinger, C. Zadikoff, N. Kennedy, F. J. Marshall, M. Mapstone, K. L. Chou, C. Persad, I. Litvan, B. T. Mast, A. T. Gerstenecker, S. Weintraub and S. Duff-Canning (2013). "Measuring mild cognitive impairment in patients with Parkinson's disease." Mov Disord **28**(5): 626-633.

Medicine, A. C. o. S. (2014). ACSM's Guidelines for Exercise Testing and Prescription, Lippincott Williams & Wilkins.

Moore, C. G., M. Schenkman, W. M. Kohrt, A. Delitto, D. A. Hall and D. Corcos (2013). "Study in Parkinson disease of exercise (SPARX): translating high-intensity exercise from animals to humans." Contemp Clin Trials **36**(1): 90-98.

Mora, F., G. Segovia, A. Del Arco, M. de Blas and P. Garrido (2012). "Stress, neurotransmitters, corticosterone and body-brain integration." Brain Res **1476**: 71-85.

Morris, S., M. E. Morris and R. Iansek (2001). "Reliability of measurements obtained with the Timed "Up & Go" test in people with Parkinson disease." Phys Ther **81**(2): 810-818.

Nieuwboer, A., L. Rochester, T. Herman, W. Vandenberghe, G. E. Emil, T. Thomaes and N. Giladi (2009). "Reliability of the new freezing of gait questionnaire: agreement between patients with Parkinson's disease and their carers." Gait Posture **30**(4): 459-463.

NINDS (2015). " User Manual for the Quality of Life in Neurological Disorders (Neuro-QoL) Measures." (Version 2.0, March 2015.).

Petzinger, G. M., D. P. Holschneider, B. E. Fisher, S. McEwen, N. Kintz, M. Halliday, W. Toy, J. W. Walsh, J. Beeler and M. W. Jakowec (2015). "The Effects of Exercise on Dopamine Neurotransmission in Parkinson's Disease: Targeting Neuroplasticity to Modulate Basal Ganglia Circuitry." Brain Plast **1**(1): 29-39.

Piazza, P. V., F. Rouge-Pont, V. Deroche, S. Maccari, H. Simon and M. Le Moal (1996). "Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission." Proc Natl Acad Sci U S A **93**(16): 8716-8720.

Raven, J., J. C. Raven and J. H. Court (2003). Manual for Raven's Progressive Matrices and Vocabulary Scales. San Antonio, TX, Harcourt Assessment.

Reitan, R. M. and D. Wolfson (1993). The Trail-making Test. Tucson, AZ, Neuropsychology Press.

Salgado, S., N. Williams, R. Kotian and M. Salgado (2013). "An evidence-based exercise regimen for patients with mild to moderate Parkinson's disease." Brain Sci **3**(1): 87-100.

Schenkman, M., T. Ellis, C. Christiansen, A. E. Baron, L. Tickle-Degnen, D. A. Hall and R. Wagenaar (2011). "Profile of functional limitations and task performance among people with early- and middle-stage Parkinson disease." Phys Ther **91**(9): 1339-1354.

Silverman, M. N. and P. A. Deuster (2014). "Biological mechanisms underlying the role of physical fitness in health and resilience." Interface Focus **4**(5): 20140040.

Smith, A. (1968). "The Symbol Digit Modalities Test: a neuropsychologic test for economic screening of learning and other cerebral disorders." Learning Disorders **3**: 83-91

.

Smith, A. (1973). Symbol Digits Modality Test. Los Angeles, Western Psychological Services.

Sousa, N., J. J. Cerqueira and O. F. Almeida (2008). "Corticosteroid receptors and neuroplasticity." Brain Res Rev **57**(2): 561-570.

Stalder, T., C. Kirschbaum, B. M. Kudielka, E. K. Adam, J. C. Pruessner, S. Wust, S. Dockray, N. Smyth, P. Evans, D. H. Hellhammer, R. Miller, M. A. Wetherell, S. J. Lupien and A. Clow (2016). "Assessment of the cortisol awakening response: Expert consensus guidelines." Psychoneuroendocrinology **63**: 414-432.

ten Brinke, L. F., N. Bolandzadeh, L. S. Nagamatsu, C. L. Hsu, J. C. Davis, K. Miran-Khan and T. Liu-Ambrose (2015). "Aerobic exercise increases hippocampal volume in older women with probable mild cognitive impairment: a 6-month randomised controlled trial." Br J Sports Med **49**(4): 248-254.

Tortosa-Martinez, J., A. Clow, N. Caus-Pertegaz, G. Gonzalez-Caballero, I. Abellan-Miralles and M. J. Saenz (2015). "Exercise Increases the Dynamics of Diurnal Cortisol Secretion and Executive Function in People With Amnestic Mild Cognitive Impairment." J Aging Phys Act **23**(4): 550-558.

Voss, M. W., R. S. Prakash, K. I. Erickson, C. Basak, L. Chaddock, J. S. Kim, H. Alves, S. Heo, A. N. Szabo, S. M. White, T. R. Wojcicki, E. L. Mailey, N. Gothe, E. A. Olson, E. McAuley and A. F. Kramer (2010). "Plasticity of brain networks in a randomized intervention trial of exercise training in older adults." Front Aging Neurosci **2**.

Voss, M. W., C. Vivar, A. F. Kramer and H. van Praag (2013). "Bridging animal and human models of exercise-induced brain plasticity." Trends Cogn Sci **17**(10): 525-544.

Wang, C., J. T. Yu, H. F. Wang, C. C. Tan, X. F. Meng and L. Tan (2014). "Non-pharmacological interventions for patients with mild cognitive impairment: a meta-analysis of randomized controlled trials of cognition-based and exercise interventions." J Alzheimers Dis **42**(2): 663-678.

Wilson, R. S., D. W. Gilley, D. A. Bennett, L. A. Beckett and D. A. Evans (2000). "Person-specific paths of cognitive decline in Alzheimer's disease and their relation to age." Psychol Aging **15**(1): 18-28.

Wolf, O. T., E. Fujiwara, G. Luwinski, C. Kirschbaum and H. J. Markowitsch (2005). "No morning cortisol response in patients with severe global amnesia." Psychoneuroendocrinology **30**(1): 101-105.

Zampieri, C., A. Salarian, P. Carlson-Kuhta, K. Aminian, J. G. Nutt and F. B. Horak (2010). "The instrumented timed up and go test: potential outcome measure for

disease modifying therapies in Parkinson's disease." J Neurol Neurosurg Psychiatry **81**(2): 171-176.