Blood Biomarker and Physical Activity Correlation in Persons with Parkinson's Disease

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ACKNOWLEDGEMENTS

Parkinson's disease (PD) is a common age-related neurodegenerative disease encompassing many debilitating symptoms. Motor symptoms include tremors, bradykinesia, gait dysfunction, freezing, and postural instability (Hayes, 2019). However, nonmotor symptoms, such as dementia, depression, gastrointestinal issues, and sleep disturbances, are just as detrimental (Beitz, 2014). Overall, PD negatively reduces an individual's quality of life (QOL) and capacity for independence (Tuon et al., 2015).

Inflammation is a crucial factor leading to PD development. Inflammation removes harmful stimuli and initiates the healing cascade as part of the immune system response (Yang et al., 2012). Individuals with PD have elevated concentrations of inflammatory biomarkers, such as interleukin 1β (IL1β), tumor necrosis factor-alpha (TNFα), and interferon-gamma (IFNγ).

IL1β is an inflammatory cytokine that regulates the nervous system's inflammatory responses and T-/B-cells to increase nitric oxide production (Belfer et al., 2004). TNFα is another cytokine produced by macrophages that leads to cellular apoptosis and necrosis (Idriss & Naismith, 2000). Finally, T-cells secrete IFNγ to stimulate the release of other cytokines through positive feedback mechanisms (Castro et al., 2018).

Although PD is irreversible, numerous treatments exist to minimize symptoms and improve patients' QOL (Ascherio & Schwarzschild, 2016). Physical activity is a non-pharmacological treatment established to have positive outcomes. Exercise focusing on flexibility, strength, and balance, are most beneficial. Physical activity including treadmill running, dance, Tai Chi, and kickboxing improve motor, nonmotor, and cognitive functioning (Beitz, 2014; Raza et al., 2019). Therefore, regular exercise is beneficial for individuals with PD.

LITERATURE REVIEW

As the average age of Americans increases, understanding age-related health conditions is essential. Parkinson's disease (PD) is an increasingly common age-related neurodegenerative disease that includes debilitating motor and nonmotor symptoms. PD has a multifaceted pathophysiology, but researchers agree inflammation is a major contributing factor (Yang et al., 2012). Increases in inflammatory markers, such as tumor necrosis factor-alpha (TNFα), interferon-gamma (IFNγ), and interleukin 1-beta (IL1β), are useful to study PD pathologies. Although PD is irreversible, numerous treatments address PD symptoms. One non-pharmacological treatment established to have beneficial outcomes is physical activity (Ascherio & Schwarzschild, 2016). The following literature review will address the pathology and

symptoms of PD, the relationship between inflammation and PD, and how exercise benefits individuals diagnosed with PD.

Understanding Parkinson's Disease

Parkinson's disease is the second-most common neurodegenerative disease (Öberg et al., 2021). Currently, 0.3% of the population has PD, and between 8.7-9.3 million people will develop PD by 2030 (Raza et al., 2019). Gender and age are two factors influencing PD development. Men are likely to develop PD at least two years earlier than women, and twice as many men develop PD than women (Hayes, 2019). Age is another key determinant for developing PD. PD is uncommon for young people under 40 years old. However, between 1-3% of the population over 65 years old will develop PD (Raza et al., 2019). Specifically, people over 60 years old have a 1% estimated occurrence of PD, and people over 80 have a 4% estimated occurrence of PD (Zorina et al., 2020).

Hallmarks & Causes of Parkinson's Disease

PD has specific, identifiable hallmarks. Patients with PD lose pigmented dopamine neurons in the midbrain, and Lewy bodies and neurites replace this neuronal tissue (Tuon et al., 2015). Lewy bodies and neurites are α-synuclein aggregations of filamentous cytoplasmic inclusions. Lewy bodies and neurites undergo three major neurodegenerative phases leading to abnormal changes in the brain and neurotransmitter concentrations (Orr et al., 2002). Neurotransmitters, such as dopamine, acetylcholine, serotonin, γ-aminobutyric acid, and glutamate, also change in concentration in response to Lewy bodies and neurites (Lotankar et al., 2017). Lewy bodies and neurites affect all parts of the nervous system, including the basal ganglia, cerebellum, hypothalamus, limbic system, locus coeruleus, and glial cells (Lotankar et al., 2017).

Researchers are not sure what causes PD, yet recent literature suggests PD is a multifactorial disorder (Orr et al., 2002). The four leading hypotheses include changes in mitochondrial morphology, dopamine loss, genetic mutations, and protein-handling dysfunction. Patients with PD have two major mitochondrial metabolism abnormalities. First, the mitochondria have increased α-ketoglutarate and decreased electron transport chain complex I activity (Tuon et al., 2015). Additionally, mitochondria face oxidative stress, which forms free radicals and activates the immune system response (Real et al., 2017). Dopamine loss might also contribute to PD. Patients with PD have a chronic and progressive degeneration of dopaminergic neurons in the mesencephalon. Dopamine concentration decreases lead to

reduced muscle coordination, muscle activities, posture, fine motor skills, and gait dysfunction (Any & Mirela, 2020). Next, genetics is a significant factor. Approximately 5-10% of all PD cases are due to an autosomal dominant pattern of inheritance that follows classic Mendelian genetics (Antony et al., 2013). Twenty-eight different chromosomal mutations trigger PD development (Antony et al., 2013). Finally, changes in protein handling, such as protein aggregation, contribute to PD (Raza et al., 2019). These changes in protein handling directly relate to the pathways that cause impaired metabolism, oxidative stress, and dopamine malfunctions, which, as previously mentioned, contribute to PD.

Parkinson's Disease Symptoms

Individuals with PD have both motor and nonmotor symptoms. Initial PD symptoms include mild tremors, soft speed, focus loss, and general fatigue (Raza et al., 2019). At first, these symptoms do not appear to have a common origin. However, PD has characteristic secondary motor symptoms including bradykinesia, tremors, gait dysfunction, freezing, and postural instability (Hayes, 2019). Individuals with bradykinesia move slowly and have decreased spontaneous movements, blink rate, and facial movements. PD patients also have unilateral tremors in one thumb or finger occurring at approximately 4-6 Hz (Hayes, 2019). Next, changes in gait include an asymmetrical decrease in arm swing, a decrease in stride length, and the inability to pivot quickly (Hayes, 2019). Finally, individuals with PD have difficulty maintaining proper posture and balance because they have decreased postural support.

Until recently, many researchers and physicians did not recognize nonmotor PD symptoms. Physicians previously only considered PD a movement impairment, but they are gradually identifying several key nonmotor symptoms. PD is now a "multi-system neurodegenerative disease" (Zorina et al., 2020, p. 67). Recognizing nonmotor symptoms is critical because they are just as detrimental as motor symptoms (Hayes, 2019). Some nonmotor symptoms include dementia, hyposmia, gastrointestinal issues, depression, hyperhidrosis, and sleep disturbances (Beitz, 2014). Nonmotor PD symptoms greatly affect the individual's quality of life and capacity for independence (Tuon et al., 2015).

Treatments for Parkinson's Disease

There are many different treatments for PD. In the beginning, at-risk populations focus on neuroprotection to delay PD progression and minimize early symptoms (Raza et al., 2019). Mild neuroprotective drugs include monoamine oxidase B inhibitors, N-methyl-D-aspartate receptor blockers, and anticholinergic drugs (Raza et al., 2019). Additionally, protective factors,

such as tobacco, coffee, green and black tea, urate, NSAIDs, calcium channel blockers, statins, and flavonoids, slow PD progression (Ascherio & Schwarzschild, 2016). However, if symptoms continue developing, there are more aggressive treatment methods. The most common treatment for PD utilizes dopaminergic medications and deep brain stimulation. Specifically, many physicians prescribe levodopa and dopamine agonists to manage early motor symptoms (Tuon et al., 2015). Surgery is a viable option for high-risk individuals with significant motor impairments (Beitz, 2014; Raza et al., 2019). However, PD correcting surgery is risky, so it is the last resort to improve the patient's quality of life when other medications are no longer working.

Inflammation and Parkinson's Disease

Understanding Inflammation

As previously mentioned, inflammation is a characteristic molecular hallmark of PD. Inflammation is a biological process that removes harmful stimuli and initiates the healing cascade (Yang et al., 2012). The four cardinal signs of peripheral inflammation include fever, swelling, pain, and redness (Tansey, 2008). Both peripheral and central inflammation are relevant in PD. Peripheral inflammation increases the blood-brain barrier permeability, so inflammatory markers enter the central nervous system (Öberg et al., 2021). In the central nervous system, reactive astrocytes and activated microglia disrupt biological homeostasis, increase phagocytosis, and increase the production of cytokines, chemokines, and interferons (Öberg et al., 2021).

Currently, researchers are unsure if neuroinflammation is a symptom or cause of PD (Antony et al., 2013). However, recent studies illustrate that neuroinflammation is a causative factor due to positive feedback responses (Öberg et al., 2021). The aging process upregulates inflammatory biomarkers, which explains why they are common in older patients with PD (Yang et al., 2012). Specifically, cells release inflammatory markers, such as TNFα, IFNγ, and IL1β. These inflammatory mediate neuroinflammation and neurotoxicity, which initiates sustained low-grade, chronic inflammation (Öberg et al., 2021).

TNFα

Tumor necrosis factor α (TNFα) is an inflammatory cytokine produced by macrophages and monocytes during acute inflammatory responses (Idriss & Naismith, 2000). TNFα release leads to a variety of intracellular signaling pathways, including apoptotic or necrotic cell death. Although cell death universally causes inflammation, apoptosis and necrosis proceed through different biochemical mechanisms (Idriss & Naismith, 2000). Necrosis involves cell swelling,

organelle destruction, and cell lysis, while apoptosis includes cell shrinkage, apoptotic body formation, and DNA fragmentation (Idriss & Naismith, 2000). The binding of TNFα to receptors in the tumor necrosis factor (TNF) superfamily stimulates cell death (Idriss & Naismith, 2000). Once activated, TNF superfamily receptors stimulate pathways involved in nitric oxide production and transcription of nuclear factor-kappa B (NF-κB), which is a common inflammatory marker for neuroinflammation (Tuon et al., 2015). Nitric oxide and NF-κB upregulation subsequently increase inflammation and cellular death.

A recent study illustrates how TNFα levels correspond to inflammation using an in vivo mice model of PD (Wang et al., 2021). In the study, researchers exposed a group of mice to PM2.5 (particles smaller than 2.5 μm in aerodynamic diameter). PM2.5 destroys the blood-brain barrier and activates microglia to induce PD (Wang et al., 2021). Following PM2.5 exposure, TNFα mRNA levels in the substantia nigra drastically increased (Wang et al., 2021). The TNFα increase triggered autophagy and mitophagy, which induced oxidative stress and neuronal apoptosis (Wang et al., 2021). This study established a direct relationship between TNFα levels and PD severity in mice models.

IFNγ

Interferon γ (IFNγ) is a pleiotropic cytokine important in the inflammatory response (Castro et al., 2018). Activated lymphocytes, such as T-helper cells, T-cytotoxic cells, T-natural killer cells, and antigen-presenting cells, produce IFNγ (Castro et al., 2018). Once produced, inflammatory signaling molecules, cell cycle regulators, and transcriptional activators activate IFNγ. Increased IFNγ concentrations stimulate a positive feedback loop to promote increased cytokine and inflammatory signaling molecule production (Castro et al., 2018). IFNγ and PD are directly related. Studies illustrate that individuals with a PD often have a specific single nucleotide polymorphism (SNP). This SNP increases IFNγ concentrations in patients with PD compared to the general public (Tansey, 2008). IFNγ accumulation in the midbrain stimulates dopamine neuron degeneration, which is a classic physiological hallmark of PD (Gan et al., 2019; Tuon et al., 2015).

IL1β

Interleukin (IL) 1β is an inflammatory cytokine that regulates inflammatory responses (Belfer et al., 2004). Activated microglia and astrocytes produce IL1β and activate T- and B-cells. IL1β works with other cytokines, such as IL6 and TNFα, to stimulate the hypothalamic-pituitary-adrenal axis (HPA) (Belfer et al., 2004). The HPA responds to external and internal

stimuli and returns the body to homeostasis. Several genetic polymorphisms in IL1A, IL1B, and IL1 receptor antagonist protein (IL1BRN) genes encode for IL1β increases (Belfer et al., 2004). Specifically, a C/T polymorphism in the IL1B promoter (position 511) directly increases IL1β secretion during inflammation (Belfer et al., 2004).

According to several recent studies, patients with PD have high concentrations of pro-inflammatory cytokines, such as IL1β (Tuon et al., 2015). Additionally, individuals with high baseline levels of plasma IL1β are at a greater risk of developing PD (Koprich et al., 2008). Elevated levels of IL1β increase nitric oxide production in the hippocampus (Tuon et al., 2015). Activated microglia produce nitric oxide. Therefore, physicians should measure IL1β concentrations to properly diagnose and identify PD severity.

Exercise and Parkinson's Disease

Exercise is a leading strategy to manage PD symptoms. An inverse relationship exists between an individual's physical activity level and PD symptom severity (Ascherio & Schwarzschild, 2016). Specifically, exercises that improve flexibility, strength, and balance are most beneficial (Beitz, 2014). Treadmill running, dance, and Tai Chi are frontrunners in exercise-based PD management (Beitz, 2014; Raza et al., 2019). These exercises improve motor, nonmotor, and cognitive functioning in PD patients. Therefore, regular exercise and physical therapy are important treatments to improve a patient's quality of life.

Exercise and physical activity do not cure PD; instead, they reduce symptom severity and delay disease progression. Specifically, physical activity improves motor and nonmotor symptoms. Following exercise, individuals with PD have decreased joint torque and increased limb strength, trunk muscle strength, and muscle flexibility (Zorina et al., 2020). These changes improve one's posture and gait control (Raza et al., 2019). Motor improvements preserve the physical independence of individuals diagnosed with PD. Additionally, physical activity improves neuroplasticity. Exercise increases concentrations of serum urate, brain-derived neurotrophic factor (BDNF), peroxisome proliferator-activated receptor gamma coactivator 1-alpha, and dopamine (Ascherio & Schwarzschild, 2016). An increase in all four biomolecules decreases PD severity.

Individuals with PD also have various nonmotor changes. Physical activity stimulates the area of the brain associated with learning, which improves the individual's quality of life by lowering the risk of depression and anxiety (Zorina et al., 2020). Individuals who exercise have a positive impact on their physical appearance, mood, and social interactions (Zorina et al., 2020). Overall, exercise positively influences both motor and nonmotor symptoms of PD to

increase the individual's quality of life.

Studying the Relationship Between Exercise and Parkinson's Disease

Multiple mice studies support these claims. A research team in Brazil investigated the effects of physical activity on inflammation in mice with 6-OHDA lesions (Tuon et al., 2015). Mice with 6-OHDA lesions are frequent models of PD that interfere with dopamine production pathways (Tuon et al., 2015). In this study, mice were divided into treadmill training, strength training, and control groups. The mice completed their assigned exercise protocol for eight weeks. After eight weeks, the study measured levels of different inflammation biomarkers using Western blots and enzyme-linked immunosorbent assay (Tuon et al., 2015). Mice in the treadmill and strength training programs had lower levels of IL1β compared to the control group (Tuon et al., 2015). Therefore, physical activity prevented dopaminergic cell loss and increased microglial activation (Tuon et al., 2015).

Another study investigated the preventative exercise-induced changes on neuroinflammatory processes in 6-OHDA mice (Real et al., 2017). They divided the mice into a control group and an exercise group. Following the intervention, researchers biopsied the mice's brains to examine dopaminergic and neuroinflammatory markers. The mice in the control group increased astrocyte, microglial, and free radical concentrations and performed worse on behavioral testing (Real et al., 2017). Following this study, the researchers repeated the study using different treadmill intensities (Real et al., 2017). The mice with a higher exercise intensity exhibited a greater increase in BDNF concentrations. They concluded that exercise intensity is crucial to prevent PD development (Real et al., 2017). Overall, dopaminergic neurons in the mice in the sedentary control group decreased significantly more than the decrease in any of the treadmill exercise groups with varying intensities. An enhanced inflammatory response also accompanied the dopaminergic neuron decreases (Real et al., 2017). Therefore, exercising at intense levels is effective to prevent PD (Real et al., 2017).

Another study, using human subjects, also supports the claim that exercise is beneficial for patients with PD. In the study, patients were divided into a control group and an experimental group that exercised two to three times a week (Any & Mirela, 2020). The one-hour-long dance movement therapy sessions were balance-focused and emphasized simple movements such as flexion, extension, and rotation (Any & Mirela, 2020). The study measured the individual's quality of life using questionnaires and psychological analyses. After six months, individuals who completed the physical activity program had significant improvements in their thinking and memory (Any & Mirela, 2020). Individuals had positive changes in their mood,

self-confidence, and social interactions. Additionally, according to the Unified Parkinson's Disease Rating Scale, patients in the control group had higher scores than patients in the exercise group (Any & Mirela, 2020). Overall, physical activity helped mitigate the severity of the nonmotor and motor PD progression.

Summary

PD is one of the fastest-growing neurodegenerative diseases and affects individuals in multiple motor and nonmotor ways. Researchers must understand PD's pathophysiology to properly diagnose and treat patients. Neuroinflammation is a leading hypothesis of PD development, and researchers are studying various inflammatory markers. As shown by the literature review, TNFα, IFNγ, and IL1β indicate the severity of PD, and exercise benefits individuals with PD.

Many studies examine PD causes and treatments, but there are three major gaps in the literature. First, is inflammation a cause or symptom of PD? This is almost a paradoxical question. Many studies offer conflicting explanations due to the positive feedback and cyclic nature of inflammation. Next, do individuals exercise because they have low inflammation levels, or does exercise lead to having low inflammatory marker concentrations? Again, this is another paradoxical question that researchers have not agreed upon. Finally, do decreases in inflammatory blood markers lead to QOL improvements following physical activity? The literature shows that inflammation decreases an individual's QOL and that exercise helps QOL. However, are these QOL changes correlated to decreases in inflammation? This is the question this study attempted to answer.

The above information was used to design an observational, cross-sectional study to analyze differences in quality of life and serum TNFα, IFNγ, and IL1β concentrations between different physical activity levels of patients with PD.

Research Aims and Hypotheses

Aim 1 will examine the correlation between inflammatory blood marker concentrations and quality of life. We hypothesized there was an indirect, negative relationship between blood biomarker concentrations and quality of life. As inflammatory blood marker concentrations increased, quality of life would decrease. Aim 2 will examine the correlation between quality of life and physical activity level. We hypothesized there was a direct, positive relationship between quality of life and physical activity level. As physical activity levels increased, quality of life would increase. Aim 3 will examine the correlation between inflammatory blood marker

concentrations and physical activity level. We hypothesized that there was an indirect relationship between blood biomarker concentrations and physical activity level. As physical activity levels increased, inflammatory blood marker concentrations increase would decrease.

METHODS

Participants

Ten participants (9 males, 1 female) between 46-83 years old (70.2 ± 10.8) who were diagnosed with PD participated in our study. Inclusion criteria for participants were as follows: a diagnosis (by a neurologist) of PD at age 45 or older with no indication of cognitive impairment or dementia. Participants were excluded if they had another neurological disorder besides PD. Community dwelling people with PD and individuals over the age of 45 years old were recruited from existing volunteer databases, regional support groups, and snowball sampling methods. Email, flyer information, and social media postings were also incorporated into recruitment procedures at local physical therapists' and physicians' offices.

Protocol

Upon arrival for the laboratory appointment, written informed consent was obtained from each participant. Participants had all procedural information read and explained in detail by the researcher before signing consent documents. The participant was assigned a subject code to store their data that did not include any identifying information. Participants were reminded that this study is completely voluntary, and participants could withdraw from the study at any time.
Ample opportunities were extended to the participants to have any questions about the study answered.

Participants reported to the Applied Metabolic and Physiology Laboratory in the Kinesiology department for an individual one-hour visit. The assessment included reviewing and signing the informed consent, completion of questionnaires, a fingerstick blood draw, and a venipuncture blood draw.

Questionnaires

Following consenting procedures, each participant completed four questionnaires related to patient-reported symptoms and outcomes. They included the following:

- Parkinson's Disease Questionnaire (PDQ-39)- This self-administered survey is a

report of how PD affects the participant's behavior and quality of life (Appendix A).

- International Physical Activity Questionnaire -Short (IPAQ)- This self-administered survey evaluates the level of physical activity that people do as part of their everyday lives over the past seven days (Appendix B).

- Saltin-Grimby Physical Activity Level Scale (SGPALS)- This self-administered survey evaluates the level of physical activity pattern over the previous 12 months (Appendix C).

- Mini Mental State Examination (MMSE)- This is a widely used test of cognitive function among the elderly; it includes tests of orientation, attention, memory, language (Appendix D).

Blood Draw

Blood was collected with the participant in a 12-hour fasted state. All samples were collected using sterile techniques and only by qualified investigators. Blood was sampled using two different techniques (fingerstick and venipuncture). A fingerstick blood sample was collected and immediately analyzed. The fingerstick technique consisted of collecting a few drops of blood in tubes specific to the blood analyzer required for specific outcome measurements. The venipuncture sample was collected using a traditional blood draw needle to extract blood from a vein in the antecubital region of the arm. Two 7 mL vials of blood were collected, centrifuged to separate the plasma and serum, and stored in a -80°C in the Exercise Physiology Laboratory (Rickel, 259). All blood collection vials and plasma/serum storing containers were labeled using participant numbers (deidentified) for participant privacy. These procedures were approved by the TCU Institutional Biosafety Committee and Institutional Review Boards.

TNFα, IFNγ, and IL1β concentrations were analyzed using an MSD Multispot V-Plex Assay. The V-Plex Assay is a rapid and convenient method for measuring cytokines with a single, small sample. The MSD machine uses an electrochemiluminescent assay to measure light. The voltage of the emitted light is proportional to the cytokine concentration.

Statistical Analysis

Data was presented with means and standard deviation. Statistical examination of the data was conducted to determine if there is a correlation (Pearson r) between self-reported physical activity levels and each blood marker of interest. R Studio 1.3.1073 (Boston, MA) was used for statistical analysis, and alpha was set at 0.05.

RESULTS

With an alpha level of 0.05, this project only yielded two significant results: the relationship between IL1β and physical activity level (measured by SGPALS) and IL1β and quality of life. Additionally, although they were not significant, several results trended in the expected directions.

Blood Biomarker Concentrations versus Quality of Life

The average cytokine concentrations for TNFα, IFNγ, and IL1b were 12.3, 58.5, and IL1β pg/mL, respectively. Table 1 illustrates the correlation between quality of life and each cytokine of interest. There was a significant negative correlation between quality of life and IL1β (p = 0.038) as shown in Figure 1, which was expected. There were two moderate positive trends between quality of life and TNFα concentration (p = 0.301) and between quality of life and IFNγ concentration (p = 0.528) that were unexpected.

Variable	*M*	*SD*	1	2	3	4
1. Quality of Life	181	10.9	—			
2. [TNFα] (pg/mL)	12.3	2.1	0.301	—		
3. [IFNγ] (pg/mL)	58.5	30.2	0.528	-0.502	—	
4. [IL1β] (pg/mL)	2.9	0.7	-0.038	-0.050	0.185	—

Table 1

Correlation Table between Blood Biomarker Concentrations and Quality of Life
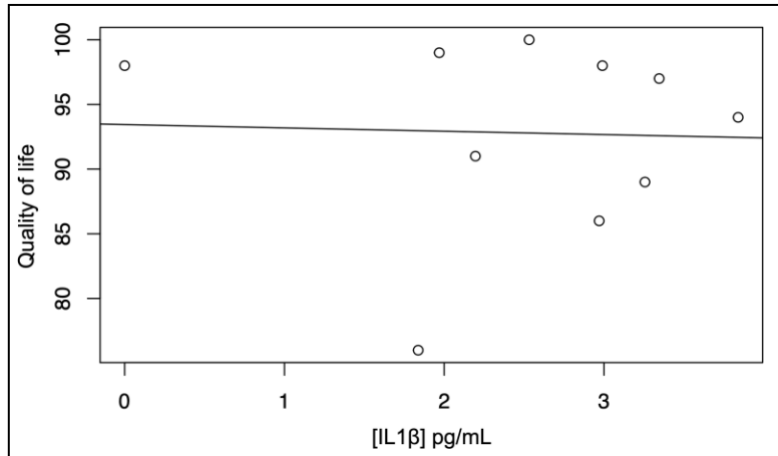
Figure 1

Correlation between [IL1β] and quality of life (p =0.038)

Physical Activity (measured by SGPALS) versus Quality of Life

Using the SGPALS questionnaire, participants were divided into two groups: individuals participating in light physical activity (n = 3) and individuals participating in regular exercise (n = 7). There was a weak positive trend between physical activity levels and quality of life (p = 0.253) as shown in Figure 2, which was expected.
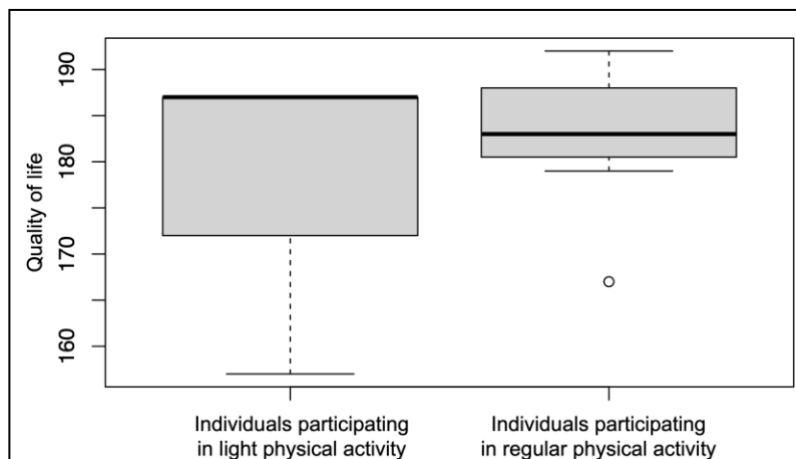

Figure 2

Correlation between quality of life and physical activity (SGPALS) (p = 0.253)

Blood Biomarker Concentrations versus Physical Activity (measured by SGPALS)

Again, using the SGPALS questionnaire, participants were divided into two groups: individuals participating in light physical activity (n = 3) and individuals participating in regular exercise (n = 7). Figure 3 shows there was a significant positive correlation between IL1β concentrations and physical activity level, which was unexpected (p = 0.026). Figure 4 shows there was a weak positive correlation between IFNγ concentrations and physical activity level that was also unexpected (p = 0.254). Finally, Figure 5 shows there was a weak negative correlation between TNFα concentrations and physical activity that was expected and aligned with current literature (p = 0.166).
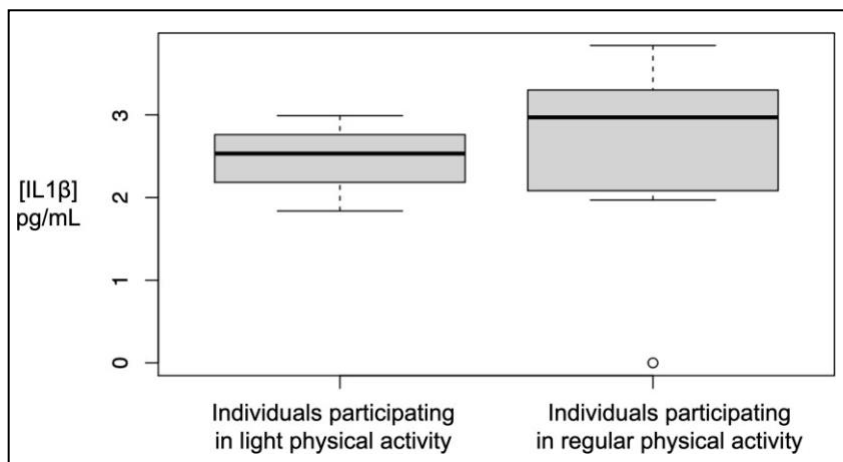
Figure 3

Correlation between [IL1β] and physical activity (p =0.026)
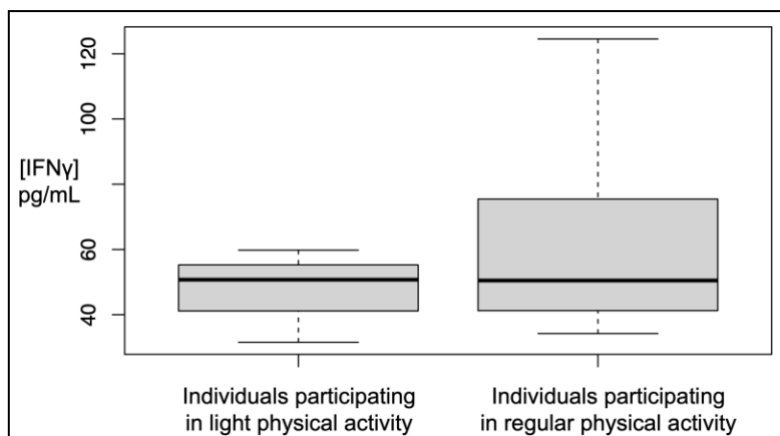
Figure 4

Correlation between [IFNγ] and physical activity (p =0.254)
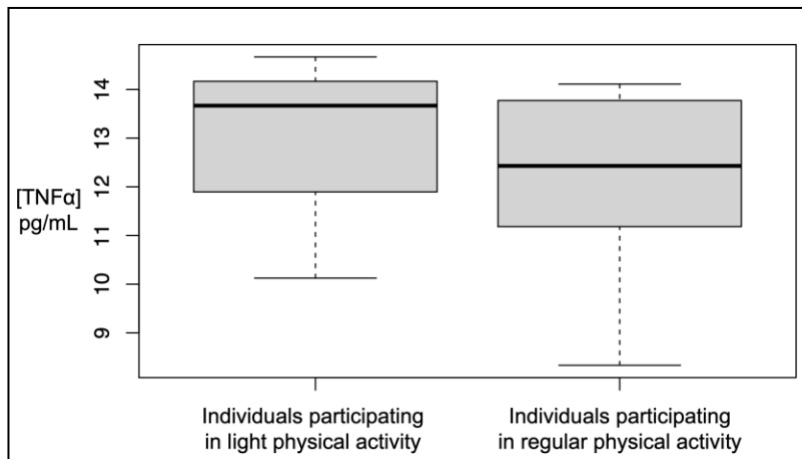
Figure 5

*Correlation between [*TNFα*] and physical activity (p =0.166)*

Blood Biomarker Concentrations versus Physical Activity (measured by IPAQ)

Table 2 displays self-reported physical activity data from the IPAQ questionnaire. The participants had high levels of physical activity compared to the average older person. Most individuals participated in regular physical activity. On average, they exercised 1.60 days per week at a vigorous intensity and 3.20 days per week at a moderate intensity.

	Days of Vigorous Exercise per Week	Hours of Vigorous Walking per Day	Days of Moderate Exercise per Week	Hours of Moderate Exercise per Day	Days of Walking 10+ minutes per week	Hours of Walking per Day	Hours of Sedentary Behavior per Day
N	10	6	10	9	10	10	9
M	1.60	1.44	3.20	1.67	4.80	1.46	4.56
SD	1.58	0.996	1.93	0.850	2.44	1.21	2.19

Table 2

Descriptive Table from IPAQ Questionnaire

Each cytokine will be examined individually below. While not statistically significant, the data from the IPAQ and blood analysis illustrated that increasing physical activity decreases inflammatory blood biomarker levels, and increasing sedentary behavior increases inflammatory blood biomarker levels, which is aligned with current literature.

TNFα Concentrations and Physical Activity

There were three expected TNFα trends that were not statistically significant. Figure 6 illustrates frequent walking decreases TNFα concentration levels ($p = 0.116$). Figure 7 shows that increasing vigorous physical activity strongly decreased TNFα concentration levels ($p = 0.085$). Figure 8 displays a moderate positive direct correlation between TNFα concentration levels and sedentary activity ($p = 0.180$).
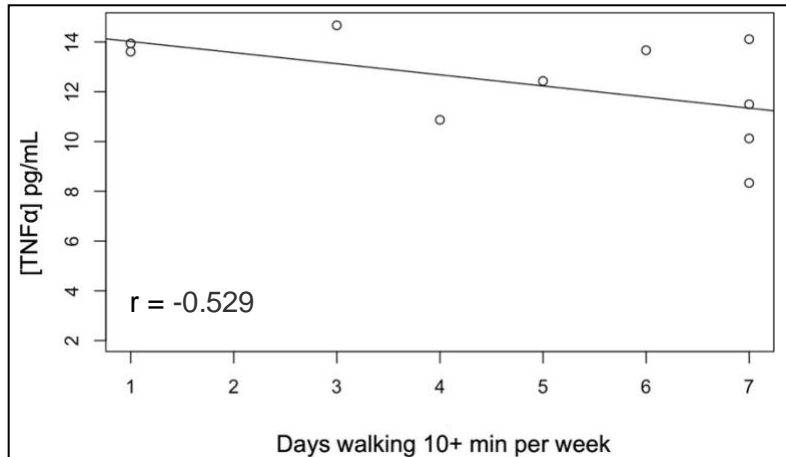

Figure 6

Correlation between [TNFα] and walking (p = 0.116)
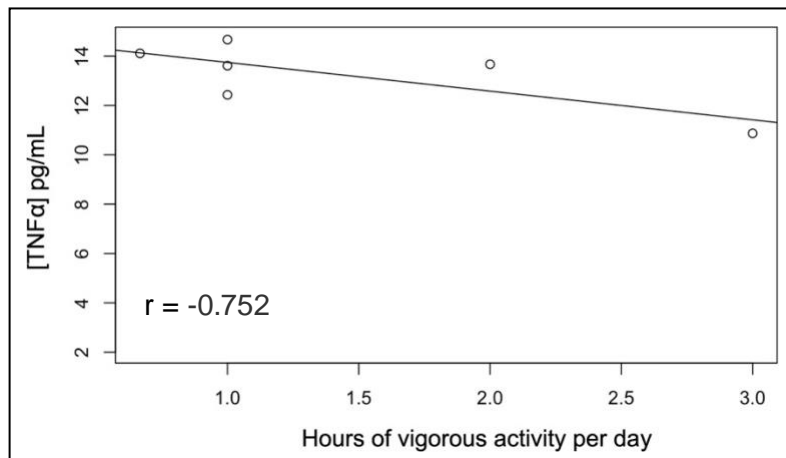
Figure 7

Correlation between [TNFα] and vigorous exercise (p = 0.085)
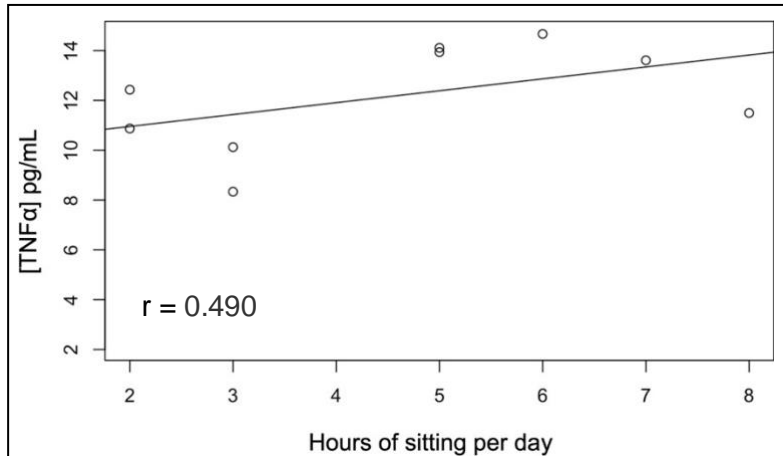
Figure 8

Correlation between [TNFα] and sitting (p = 0.180)

IFNγ Concentrations and Physical Activity

IFNγ only had one moderately weak negative indirect relationship between IFNγ concentration levels and moderate physical activity (p = 0.195) as illustrated in Figure 9, which was expected.

Figure 9
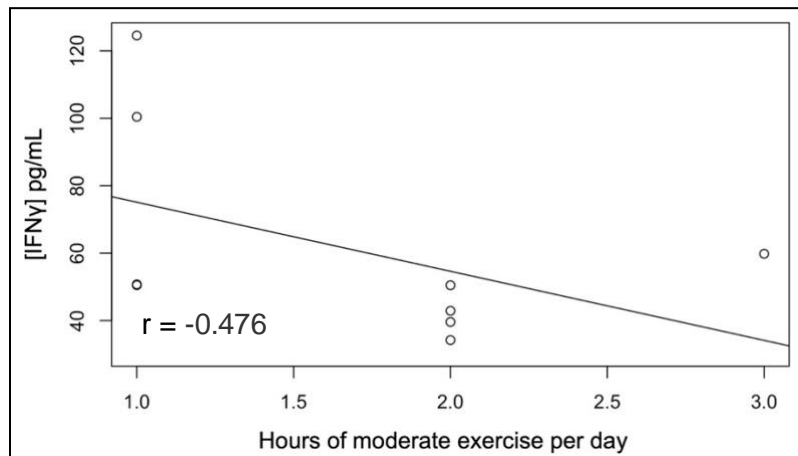
Correlation between [IFNγ] and moderate physical activity (p = 0.195)

IL1β Concentrations and Physical Activity

Finally, Figure 10 illustrates that frequent walking decreases IL1β concentration levels. Figure 11 shows another weak negative correlation between IL1β concentration levels and moderate exercise. Figure 12 illustrates a moderate positive correlation between IL1β
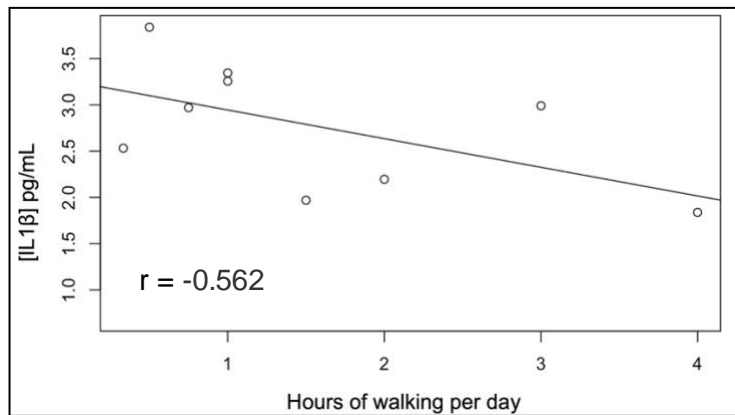
concentration levels and sedentary activity.

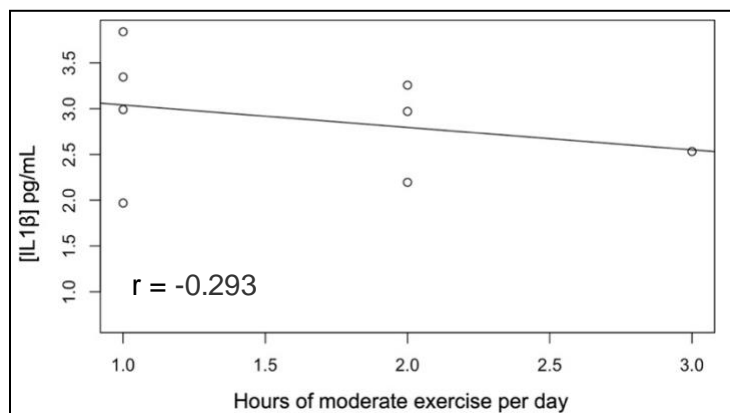Figure 10

Correlation between [IL1β] and walking (p =0.116)

Figure 11

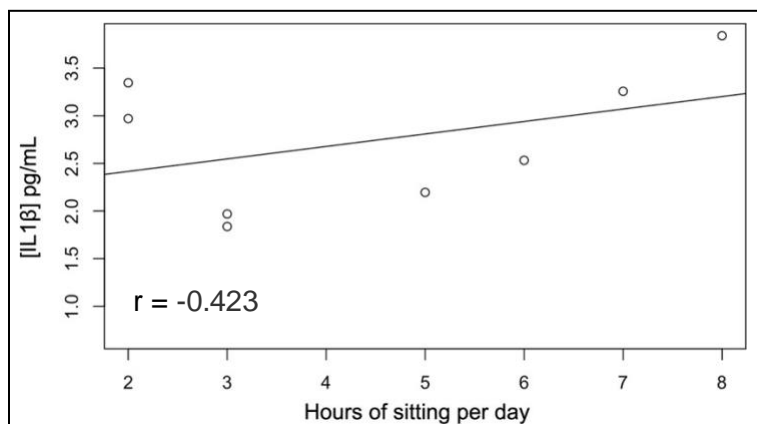*Correlation between [IL1β] and moderate exercise (p =0.481)*

Figure 12

Correlation between [IL1β] and sitting (p =0.296)

DISCUSSION AND CONCLUSION

Overall, some of the results supported while others refuted the three original hypotheses. Starting with Hypothesis 1, there was a significant negative correlation with IL1β versus quality and positive trends between TNFα and IFNγ compared to quality of life. Ultimately, only IL1β strongly supported the first hypothesis. Next, the SGPALS physical activity levels and self-reported quality of life had positive trends, which supported the second hypothesis. Lastly, there was a significant positive correlation with IL1β and a weak positive trend with IFNγ and PA level. However, only the weak negative trend with TNFα and PA level supported the third hypothesis.

Several of our results matched the existing literature. First, the demographics of our participant population fit the general demographics of people with PD. Men are more common than women to develop PD, and we had 9 males and 1 female participate in our study (Hayes, 2019). Additionally, the average age of our participants was 70.2 ± 10.8 years old, and according to the literature, PD is most common in individuals older than 65 years old (Raza et al., 2019). As shown by the PDQ-39 questionnaire, PD negatively affects one's quality of life.

Several of our participants reported having difficulties walking half a mile, performing activities of daily living such as dressing themselves and writing clearly, maintaining personal relationships, and feeling anxious. These results from the questionnaires illustrate how PD motor and nonmotor symptoms affect one's quality of life. Many of these deficient can be attributed to PD's characteristic symptoms. For example, individuals with PD have unilateral tremors in their hands at approximately 4-6 Hz (Hayes, 2019). These tremors might have contributed to difficulties writing clearly. Additionally, patients with PD walk very slowly and have dressed arms swing and stride length (Hayes, 2019). Not having a biomechanically efficient gait pattern might have contributed to our participants having difficulties walking about half a mile. Finally, our patients felt anxious and had trouble maintaining personal relationships, which illustrates the negative impact of PD's nonmotor symptoms on social interactions.

Additionally, our participants with PD had notable concentrations of TNFα, IFNγ, and IL1β biomarkers. Inflammation is a highly individualized process, so researchers have not established norm values to compare inflammatory blood marker concentrations. However, our participants had an average TNFα concentration of 12.3 pg/mL. According to a study by Wang et al. (2021), TNFα mRNA levels increase in mice models with PD. This increase in TNFα mRNA leads to oxidative stress and cellular death to provoke different PD symptoms. Our participants also have an IFNγ concentration of 58.5 pg/mL, which matches previous studies. Castro et al. (2018) explained that patients with PD have a single nucleotide polymorphism (SNP) in their

genetic code which increases IFNγ concentrations. It would be reasonable to assume that many of our participants had this SNP to increase their IFNγ production. Finally, our participants had an average IL1β concentration of 2.9 pg/mL. Patients with PD have increased IL1β concentration due to changes in the genetic code, specifically a C/T polymorphism in the IL1B promoter region, which increased nitric oxide production (Belfer et al., 2004).

Finally, some of the results supported literature examining the relationship between physical activity and inflammation levels with patients with PD. Studies illustrate there is a negative relationship between an individual's physical activity level and inflammatory marker levels. Specifically, a study completed by Tuon et al. (2015) showed that mice who ran on a treadmill and participated in strength training programs had lower IL1β concentrations than those in the control group. However, as previously mentioned, in our study, participants completing regular physical activity surprisingly had higher IL1β concentrations than those completing light

physical activity. Another study by Any & Mirela (2020) claimed that patients completing regular physical activity had a higher quality of life than sedentary individuals. In our study, we did not have any sedentary participants. However, in our study, there was not a noticeable difference between quality of life with individuals completing regular or light physical activity.

LIMITATIONS

As with a majority of studies, the design of the current research project is subject to several limitations. First, the MSD Multispot V-Plex Assay could not analyze certain cytokines relevant to PD. As mentioned in the above literature review, nuclear factor-kappa B (NF-κB) is a common inflammatory marker for neuroinflammation in patients with PD. NF-κB concentration increases are a hallmark of PD development. Examining changes in NF-κB would have been relevant to the study's aims; however, we could not assess that biomarker due to technology limitations.

Additionally, several factors limited our recruitment efforts. Due to time constraints, we only recruited 10 participants. Originally, we aimed to have 100 individuals participate. Because we had a smaller sample size, there was high variability which decreased the study's reliability. Next, several local physicians and physical therapists helped us recruit most of our participants, which contributed to selection bias. Most of our participants were under the care of a physical therapist or physician. Because they were receiving services from a medical professional, they might be healthier or exercising more regularly than the general public. Our participants were

not representative of the entire population of people with PD. Therefore, we cannot generalize the study's results to everybody with PD.

Finally, as previously mentioned, time was a significant constraint. This project was initially designed to be a longitudinal study. We wanted to take a sedentary population of individuals and measure their inflammatory blood marker concentrations before and after completing an exercise intervention program. However, due to delays with IBC and IRB approval, we chose to complete a cross-sectional analysis instead. Although cross-sectional analyses are quicker, the conclusions are more limited than longitudinal studies. Because this was an observational study, we could not determine a causal relationship between physical activity, blood marker concentrations, and quality of life. Our data suggested an inverse relationship between physical activity and inflammatory biomarker concentrations. However, we could not determine if exercise instructed to lower inflammatory marker concentrations or if lower inflammatory marker concentrations increased an individual's likelihood to exercise. Ultimately, this study's results must be interpreted with caution, and several limitations should be kept in mind.

FUTURE STUDIES

Future studies further analyzing the relationship between preventive medicine and PD would be beneficial. Future studies could investigate the relationship between inflammation and exercise in patients with PD in a longitudinal study. Researchers could design an experimental study using a sedentary population with PD and measure inflammatory blood biomarker concentrations before and after implementing an exercise program. In addition, future research can analyze if exercise helps prevent other comorbidities associated with PD, such as hypertension and diabetes. Using a similar cross-sectional study as described here, researchers could investigate if exercise decreases biomarkers such as aldosterone and hemoglobin A1C. Finally, using exercise as preventative medicine in other inflammatory disorders, besides PD, is an interesting topic for further studies. Rheumatoid arthritis and lupus are common debilitating inflammatory diseases, so examining if exercise also decreases inflammation and increases quality of life in these disorders would be helpful. Overall, this study is a good starting point for discussion, and further research can continue exploring the benefits of preventive medicine, especially with PD patients.

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Appendix A- Parkinson's Disease Questionnaire (PDQ-39)

PDQ-39 QUESTIONNAIRE

Please complete the following

Please tick __one__ box for each question

Due to having Parkinson's disease, how often __during the last month__ have you....

		Never	Occasionally	Sometimes	Often	Always or cannot do at all
1	Had difficulty doing the leisure activities which you would like to do?	☐	☐	☐	☐	☐
2	Had difficulty looking after your home, e.g. DIY, housework, cooking?	☐	☐	☐	☐	☐
3	Had difficulty carrying bags of shopping?	☐	☐	☐	☐	☐
4	Had problems walking half a mile?	☐	☐	☐	☐	☐
5	Had problems walking 100 yards?	☐	☐	☐	☐	☐
6	Had problems getting around the house as easily as you would like?	☐	☐	☐	☐	☐
7	Had difficulty getting around in public?	☐	☐	☐	☐	☐
8	Needed someone else to accompany you when you went out?	☐	☐	☐	☐	☐
9	Felt frightened or worried about falling over in public?	☐	☐	☐	☐	☐
10	Been confined to the house more than you would like?	☐	☐	☐	☐	☐
11	Had difficulty washing yourself?	☐	☐	☐	☐	☐
12	Had difficulty dressing yourself?	☐	☐	☐	☐	☐
13	Had problems doing up your shoe laces?	☐	☐	☐	☐	☐

Please check that you have ticked __one box for each question__ before going on to the next page

Page 3 of 12   Questionnaires for patient completion

Due to having Parkinson's disease, how often <u>during the last month</u> have you....

Please tick <u>one</u> box for each question

		Never	Occasionally	Sometimes	Often	Always or cannot do at all
14	Had problems writing clearly?	☐	☐	☐	☐	☐
15	Had difficulty cutting up your food?	☐	☐	☐	☐	☐
16	Had difficulty holding a drink without spilling it?	☐	☐	☐	☐	☐
17	Felt depressed?	☐	☐	☐	☐	☐
18	Felt isolated and lonely?	☐	☐	☐	☐	☐
19	Felt weepy or tearful?	☐	☐	☐	☐	☐
20	Felt angry or bitter?	☐	☐	☐	☐	☐
21	Felt anxious?	☐	☐	☐	☐	☐
22	Felt worried about your future?	☐	☐	☐	☐	☐
23	Felt you had to conceal your Parkinson's from people?	☐	☐	☐	☐	☐
24	Avoided situations which involve eating or drinking in public?	☐	☐	☐	☐	☐
25	Felt embarrassed in public due to having Parkinson's disease?	☐	☐	☐	☐	☐
26	Felt worried by other people's reaction to you?	☐	☐	☐	☐	☐
27	Had problems with your close personal relationships?	☐	☐	☐	☐	☐
28	Lacked support in the ways you need from your spouse or partner?	☐	☐	☐	☐	☐
	If you do not have a spouse or partner tick here		☐			
29	Lacked support in the ways you need from your family or close friends?	☐	☐	☐	☐	☐

*Please check that you have ticked **one box for each question** before going on to the next page*

Due to having Parkinson's disease, how often *during the last month* have you....

Please tick <u>one</u> box for each question

		Never	Occasionally	Sometimes	Often	Always
30	Unexpectedly fallen asleep during the day?	☐	☐	☐	☐	☐
31	Had problems with your concentration, e.g. when reading or watching TV?	☐	☐	☐	☐	☐
32	Felt your memory was bad?	☐	☐	☐	☐	☐
33	Had distressing dreams or hallucinations?	☐	☐	☐	☐	☐
34	Had difficulty with your speech?	☐	☐	☐	☐	☐
35	Felt unable to communicate with people properly?	☐	☐	☐	☐	☐
36	Felt ignored by people?	☐	☐	☐	☐	☐
37	Had painful muscle cramps or spasms?	☐	☐	☐	☐	☐
38	Had aches and pains in your joints or body?	☐	☐	☐	☐	☐
39	Felt unpleasantly hot or cold?	☐	☐	☐	☐	☐

*Please check that you have ticked **one box for each question** before going on to the next page*

Thank you for completing the PDQ 39 questionnaire

Appendix B- International Physical Activity Questionnaire -Short (IPAQ)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE
(August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ
The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ
Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation
Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ
International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information
More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.
INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of

their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1.   During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ days per week

☐   No vigorous physical activities   ➡️   ***Skip to question 3***

2.   How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ hours per day
_____ **minutes per day**

☐   Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3.   During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ days per week

☐   No moderate physical activities   ➡️   ***Skip to question 5***

4.   How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ hours per day
_____ **minutes per day**

☐   Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home,

walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5.   During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ days per week

☐   No walking   ➡️   ***Skip to question 7***

6.   How much time did you usually spend **walking** on one of those days?

_____ hours per day
_____ **minutes per day**

☐   Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7.   During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ hours per day
_____ **minutes per day**

☐   Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

Appendix C- Saltin-Grimby Physical Activity Level Scale (SGPALS)

Saltin-Grimby Physical Activity Level Scale (SGPALS)

PHYSICAL ACTIVITY AND EXERCISE

Mark only one option**!**

How much do you move and exert yourself physically during your leisure time? If your activity varies greatly between, for example, summer and winter, try to estimate anaverage. The question refers to the past year.

1. **Physically inactive**
Almost completely inactive, reading, watching television, watching movies, using computers or doing other sedentary activities, during leisure time........................ ☐

2. **Some light physical activity**
Physically active for at least 4 hours/week, such as riding a bicycle or walking to work, walking with the family, gardening, fishing, table tennis, bowling etc....... ☐

3. **Regular physical activity and training**
Spending time doing heavy gardening, running, swimming, playing tennis, badminton, calisthenics and similar activities, for at least 2-3 hours/week..................................... ☐

4. **Regular hard physical training for competitive sports**
Spending time running, orienteering, skiing, swimming, playing football, handball etc. several times per week.. ☐

Grimby G, Borjesson M, Jonsdottir IH, Schnohr P, Thelle DS, Saltin B. The "Saltin-Grimby PhysicalActivity Level Scale" and its application to health research. Scand J Med Sci Sports. 2015;25 Suppl4:119-25.

Originally published in:
Saltin B, Grimby G. Physiological analysis of middle-aged and old former athletes. Comparison withstill active athletes of the same ages. Circulation. 1968: 38: 1104-1115.

Appendix D- Mini Mental State Examination (MMSE)

Mini-Mental State Examination (MMSE)

Patient's Name: _____   Date: _____

Instructions: Score one point for each correct response within each question or activity.

Maximum Score	Patient's Score	Questions
5		"What is the year? Season? Date? Day? Month?"
5		"Where are we now? State? County? Town/city? Hospital? Floor?"
3		The examiner names three unrelated objects clearly and slowly, then the instructor asks the patient to name all three of them. The patient's response is used for scoring. The examiner repeats them until patient learns all of them, if possible.
5		"I would like you to count backward from 100 by sevens." (93, 86, 79, 72, 65, …) Alternative: "Spell WORLD backwards." (D-L-R-O-W)
3		"Earlier I told you the names of three things. Can you tell me what those were?"
2		Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.
1		"Repeat the phrase: 'No ifs, ands, or buts.'"
3		"Take the paper in your right hand, fold it in half, and put it on the floor." (The examiner gives the patient a piece of blank paper.)
1		"Please read this and do what it says." (Written instruction is "Close your eyes.")
1		"Make up and write a sentence about anything." (This sentence must contain a noun and a verb.)
1		"Please copy this picture." (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.)
30		TOTAL

Interpretation of the MMSE:

Method	Score	Interpretation
Single Cutoff	<24	Abnormal
Range	<21	Increased odds of dementia
	>25	Decreased odds of dementia
Education	21	Abnormal for 8th grade education
	<23	Abnormal for high school education
	<24	Abnormal for college education
Severity	24-30	No cognitive impairment
	18-23	Mild cognitive impairment
	0-17	Severe cognitive impairment

Interpretation of MMSE Scores:

Score	Degree of Impairment	Formal Psychometric Assessment	Day-to-Day Functioning
25-30	Questionably significant	If clinical signs of cognitive impairment are present, formal assessment of cognition may be valuable.	May have clinically significant but mild deficits. Likely to affect only most demanding activities of daily living.
20-25	Mild	Formal assessment may be helpful to better determine pattern and extent of deficits.	Significant effect. May require some supervision, support and assistance.
10-20	Moderate	Formal assessment may be helpful if there are specific clinical indications.	Clear impairment. May require 24-hour supervision.
0-10	Severe	Patient not likely to be testable.	Marked impairment. Likely to require 24-hour supervision and assistance with ADL.

Source:

- Folstein MF, Folstein SE, McHugh PR: "Mini-mental state: A practical method for grading the cognitive state of patients for the clinician." *J Psychiatr Res* 1975;12:189-198.