“A cross-sectional study of serum vitamin B6 levels in people living with Parkinson's disease”

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1. PURPOSE OF STUDY

In this single-site, one-year, cross-sectional study, we will estimate the prevalence of abnormal serum levels of vitamin B6 in a randomly selected sample of 144 adult people living with Parkinson's disease (PwPD). We will explore associations of those B6 levels with relevant demographic and clinical variables, compared to historical controls. The results of this study will inform future longitudinal studies to elucidate the natural history and clinical importance of abnormal vitamin B6 levels in PwPD, eventually followed by a clinical trial evaluating the safety and efficacy of vitamin B6 therapy in PwPD.

2. BACKGROUND AND RATIONALE

In older adults, the prevalence of polyneuropathy is approximately 10% and the prevalence of epilepsy is approximately 1%. For unclear reasons, the prevalence of neuropathy increases to 30-40% in PwPD [Rajabally 2011], and the prevalence of epilepsy increases to 8% in institutionalized PwPD [Birnbaum 2017].

Vitamin B6 (pyridoxine) is an essential cofactor for several enzymes involved in the synthesis and catabolism of essential neurotransmitters and amino acids. Low serum levels of vitamin B6 have been associated with polyneuropathy, epilepsy (including status epileptics), cognitive dysfunction and microcytic anemia [Romagnolo 2019, Tardy 2020]. High levels of vitamin B6 levels have been associated with polyneuropathy [Ghavanini 2014]. A growing body of evidence suggests that PwPD are at higher risk of having low B6 levels, which may in turn lead to potentially preventable and treatable complications, such as polyneuropathy and epilepsy [Modica 2020 and 2023, Canissario 2021]. Potential contributors to abnormal B6 levels in PwPD include age, dietary habits, vitamin supplement misuse, gastrointestinal dysfunction and complex interactions with levodopa.

The prevalence of abnormal B6 levels in PwPD remains unknown, as no prior study has specifically investigated this question. We recently completed a systematic review in which we estimated a 41% relative frequency of abnormal serum levels of vitamin B6 in PwPD (<20nmol/L or >125 nmol/L) [Modica 2023]. However, the clinical importance of abnormal B6 levels in PwPD is unclear. Also, no prior research has studied the potential associations between B6 levels and relevant demographic or clinical variables in PwPD.

Prior to the advent of aromatic-L-amino-acid decarboxylase inhibitors (DDCIs), such as carbidopa and benserazide, observations of reduced levodopa efficacy when taken with oral B6 supplementation prompted recommendations to avoid dietary B6 in PwPD [Duvoisin 1969]. Additional observations reported improvement of levodopa-associated motor complications when

taking B6 supplementation [Jameson 1970]. More recently, levodopa-carbidopa intestinal gel infusion (LCIG) and relatively high dosages of oral levodopa-carbidopa have been associated with low B6 levels, polyneuropathy and epilepsy in PwPD [Romagnolo 2019, Modica 2020, Wise 2022]. In a recent cross-sectional study of 99 consecutive PwPD comprehensively evaluated for polyneuropathy, 40 of them were found to have unexplained polyneuropathy. None of them had low B6 levels and six of them had high B6 levels despite reportedly not taking vitamin supplements [Corra 2023, Sardoeira 2022]. Dietary B6 avoidance is potentially harmful for PwPD (and unnecessary in the era of DDCIs) and B6 supplementation may benefit some PwPD but it might harm others by causing abnormally high B6 levels [Lizarraga 2022].

There is a need for rigorous studies that control for relevant variables to determine the prevalence, natural history and clinical importance of abnormal vitamin B6 levels in PwPD. This will facilitate the design of future studies to determine the extent to which vitamin B6 administration is safe and effective to prevent or treat polyneuropathy and epilepsy associated with abnormal vitamin B6 levels, as well as motor complications associated with levodopa in PwPD.

3. ADMINISTRATIVE ORGANIZATION

This study will take place entirely at the Movement Disorders Clinic, Department of Neurology, University of Rochester Medical Center (4901 Lac De Ville Boulevard, Bldg. D, Suite 120, Rochester, NY 14618).

4. STUDY DESIGN

In this single-center, cross-sectional study, we will estimate the prevalence of abnormal serum levels of vitamin B6 and we will explore associations of those levels with relevant demographic and clinical variables in a randomly selected sample of 144 adult PwPD, compared to historical controls. The results of this study will inform the design of future longitudinal or interventional studies. Subjects will be recruited at the Movement Disorders Clinic, Department of Neurology, University of Rochester Medical Center. We will pursue three ***specific aims***:

Aim 1. Estimate the prevalence of abnormal serum levels of vitamin B6 in PwPD.

Between July 2024 and June 2025, we will conduct a single-center, cross-sectional study to estimate the prevalence of abnormal serum levels of B6 in a randomly selected sample of 144 PwPD recruited from the Movement Disorders Clinic at the University of Rochester. We have recently completed a systematic review of the literature in which we estimated that the relative frequency of abnormal B6 levels in PwPD is ~41% [Modica 2023]. Given this information, the sample size of 144 PwPD is necessary to estimate the prevalence of abnormal B6 levels with 95% confidence and 8% precision (1/5 of the estimated prevalence, based on feasibility). To enroll 144 PwPD in one year, we will need to enroll at least 3 PwPD per week. Every week, we will use a computer program to randomly select 3 of the ~75 PwPD scheduled for an appointment at our clinic (See below section 6. Recruitment methods for details). After consent, blood samples will be obtained at the Movement Disorders Clinic or at the University of Rochester laboratory. The samples will then be analyzed by the University of Rochester laboratory to measure fasting serum levels of vitamin B6. For biochemical confirmation and exploration of potential interactions [Canissario

2021], we will also obtain serum levels of vitamin B12, homocysteine, methylmalonic acid and folic acid **(Figure 1)**. Once we receive results from the laboratory as per standard procedures, we will enter these results into the REDCap study database. Normal values are 20-125 nmol/L B6, 232-1245 pg/mL for B12, ≤15 μmol/L for homocysteine, ≤0.4 μmol/L for methylmalonic acid, and ≥4.6 ng/mL for folic acid levels. We will report abnormal values to subjects and providers for further workup and/or treatment as needed.

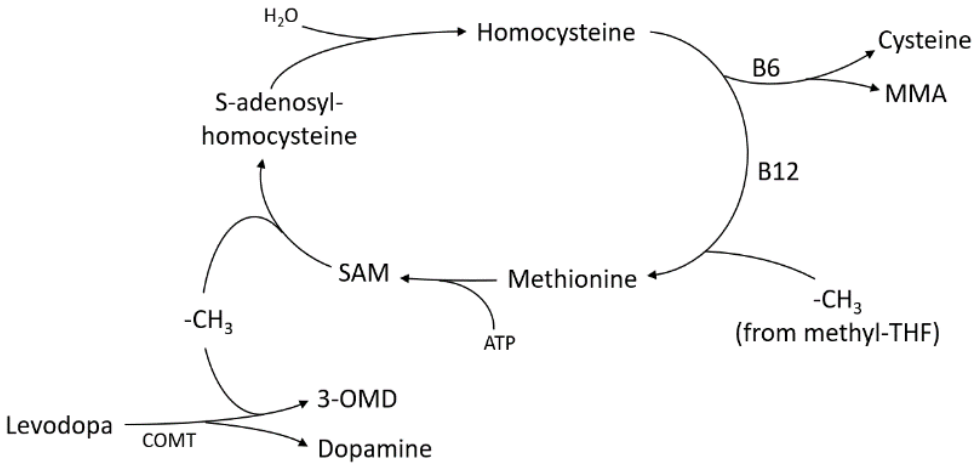

Figure 1. Biochemical relationships between vitamin B6, vitamin B12, homocysteine, methylmalonic acid (MMA) and levodopa [Canissario *et al*. 2021].

We will compare B6 levels from these 144 PwPD with B6 levels from historical controls from the latest publicly available National Health and Nutrition Examination Survey (NHANES), matched for age, sex, and 30-day consumption of: protein (yes/no), food rich in B6 (yes/no), B6 supplements (yes/no), and vitamin supplements (yes/no) [Morris 2008]. Depending on the availability of matches, we will give priority to age and sex for matching criteria with statistical adjustment for other factors. We will exclude people expected to have abnormal B6 levels due to underlying conditions or drug interactions (other than levodopa). We will use a logistic regression model that includes group (PwPD, control) and relevant covariates to estimate the adjusted odds ratio per group (to compare the groups with respect to prevalence of abnormal serum level of vitamin B6). Similarly, we will use a multiple regression model to compare the groups with respect to adjusted mean value of B6 levels (log-transformed). ***Upon completion of Aim 1, we will have estimated the prevalence of abnormal serum levels of vitamin B6 in PwPD.*** A potential problem will be maintaining the accrual of at least 3 PwPD per week. To minimize this problem, we will engage with our clinical and laboratory teams daily, and we will notify all PwPD treated at our Movement Disorders Clinic about this study ahead of time (we serve a total of >4000 PwPD). We will also discuss this study during monthly meetings of the movement disorders division in order for all providers to remain aware and discuss with potential subjects.

Aim 2. Examine the associations between vitamin B6 levels and relevant demographic variables in PwPD.

We will use multiple regression modeling to examine associations between B6 levels (nmol/L, log-transformed) and relevant demographic variables as defined by the NHANES [Morris 2008]:

1) age (years), 2) sex (male/female), 3) race and 4) ethnicity (defined by the U.S. Office of Management and Budget), 5) body mass index (kg/m^2), 6) diabetes (yes/no), 7) smoking (yes/no), 8) alcohol use (yes/no), 9) daily consumption of protein in the last 30 days (yes/no), 10) daily consumption of food rich in B6 in the last 30 days (poultry, fish, greens, tropical fruits) (yes/no), 11) food insecurity (yes/no), 12) B6 supplement use in the last 30 days (yes/no), 13) vitamin supplement use in the last 30 days (yes/no) and 14) history of oral contraceptive pill use (yes/no). We will obtain this information using structured interviews based on standardized questions from the NHANES. By examining the interaction between group (PwPD, control) and each covariate, we will compare associations to those made in historical controls from the latest publicly available NHANES data. Our ***hypothesis*** is that, compared to controls, B6 levels in PwPD will be similarly associated with age, sex, race, ethnicity, body mass index, diabetes, smoking, alcohol use, daily consumption of protein or food rich in B6 in the last 30 days, food insecurity, B6 or vitamin supplement use in the last 30 days, and oral contraceptive pill use. ***Upon completion of Aim 2, we will have examined associations between serum vitamin B6 levels and relevant demographic variables in PwPD.*** To increase the likelihood of obtaining accurate information during the structured interviews, we will send the questionnaire to enrolled subjects ahead of time for preparation.

Aim 3. Examine the associations between vitamin B6 levels and relevant clinical variables in PwPD.

We will use multiple regression modeling to examine the associations between serum B6 levels (nmol/L, log-transformed) and relevant clinical variables in PwPD: 1) age at onset of Parkinson's disease (years), 2) disease duration (years), 3) disease stage (modified Hoehn & Yahr scale), 4) disease severity (Movement Disorders Society sponsored revision of the Unified Parkinson Disease Rating Scale, MDS-UPDRS), 5) levodopa dosage (mg/day), 6) DDCI (carbidopa, benzeraside) dosage (mg/day), 7) levodopa/carbidopa ratio, 8) cathecol-o-methyl-transferase (COMT) inhibitor (entacapone, opicapone) use (yes/no), 9) COMT inhibitor dosage (mg/day), 10) levodopa equivalent daily dosage (mg/day), 11) symptoms or signs consistent with polyneuropathy (yes/no, yes if ≥6 points in the modified Toronto Clinical Neuropathy Score), and 12) symptoms or signs consistent with epilepsy (yes/no). Our ***hypothesis*** is that B6 levels in PwPD will be associated with disease duration, stage, severity, levodopa dosage, carbidopa dosage, symptoms or signs consistent with neuropathy or epilepsy. ***Upon completion of Aim 3, we will have examined associations between serum vitamin B6 levels and relevant clinical variables in PwPD.*** For all aims, we will pay careful attention to evaluation of the assumptions of the regression models (normality, homogeneity of variance, linearity) and identification of outliers, influential observations, and multi-collinearity. We will especially scrutinize the functional form of the covariates since some of the associations might be nonlinear (for example, between B6 level and neuropathy).

Sub study Aim. Explore the clinical use of vitamin supplementation in subjects found to have low serum levels of vitamin B6.

After study completion, we will contact subjects found to have low serum levels of vitamin B6 to 1) ask whether they were started on vitamin supplementation or not, 2) ask what dosage and for how long they were prescribed vitamin supplementation (if applicable), 3) ask whether they per-

ceived any benefits or side effects from vitamin supplementation (if applicable), 4) ask whether any follow-up serum level of vitamin B6 was obtained by their healthcare provider, 5) ask how long after they started taking vitamin supplementation were those levels checked (if applicable), 6) ask if their levels of vitamin B6 increased, decreased or stayed the same (if applicable), and 7) ask the 6 questions of Part IV of the MDS-UPDRS.

4.1 SUBJECT POPULATION

a) Number of Subjects: 144 people living with Parkinson's Disease
b) Gender and age of subjects: Every effort will be made to achieve a balanced inclusion of female and male subjects to reflect the distribution of Parkinson's disease in the general population.
c) Racial and ethnic origin: There are no enrollment restrictions based on racial and/ or ethnic origin. The randomized recruitment process will reflect the naturally occurring proportions in the Rochester community.
d) Vulnerable subjects: Only subjects who are considered capable of providing informed consent will be included in this study. This will be determined using a determination of capacity to consent form. All subjects must sign an informed consent form prior to the conduct of any study-related procedures. Before subjects can provide informed consent, a study team member must determine whether the subject has the capacity to provide consent for participation. A capacity to consent form will be utilized to deem capacity to consent. If the subject is deemed to lack capacity to consent, the subject will not be enrolled.

4.2 STUDY INTERVENTIONS

This study will not test an intervention.

5. INCLUSION AND EXCLUSION CRITERIA

a) Inclusion Criteria:
    a. People with a clinical diagnosis of Parkinson's disease according to the diagnostic criteria of the Movement Disorders Society [Postuma 2015].
    b. Subjects are ≥ 18 years old.
    c. As determined by the study investigators, subjects are able to communicate at a level sufficient to undergo study interviews.
    d. As determined by the study investigators, subjects have the capacity to provide written informed consent and the ability to comply with all study procedures.
b) Exclusion Criteria:
    a. People expected to have abnormal vitamin B6 levels due to underlying conditions (renal disease, bowel disease, rheumatoid arthritis).
    b. People taking medications (other than levodopa) expected to interfere with B6 levels (steroids, cycloserine, hydralazine, isoniazid, theophylline, penicillamine).

6. RECRUITMENT METHODS

Study subjects will be recruited in a random fashion at the Movement Disorders Clinic at the University of Rochester Medical Center in Rochester, NY. Random subject selection will occur 1-2 weeks ahead of scheduled clinic visits as follows:

- We will use Microsoft Excel to create a list of chronological time slots assigned to patient visits to our clinic (including Monday through Friday).
- We will use the "RAND" function of Microsoft Excel to assign a random numeric value to each time slot.
- We will use the "Sort" function of Microsoft Excel to organize the scheduled visits from the highest to the lower numeric value (previously assigned randomly).
- We will contact potential study subjects via phone starting at the highest numeric value (See phone call script), following with the second highest numeric value, and so on until we have enrolled 3-4 PwPD per week.

Upon contacting potential study subjects via phone, the study coordinator and/or study PI will review details of the study and answer any questions (See phone call script). If the potential study subject is interested in participating, we will send him or her the informed consent form via E-mail. Then we will proceed to schedule the participant for a study visit. We will make every effort for these visits to be scheduled on the same day of their scheduled clinic visit.

7. CONSENT PROCESS

The informed consent process will be conducted in a culturally appropriate manner by the principal investigator, research coordinator or an appropriate member of the study team as listed on the protocol. Capacity to consent will be assessed through a capacity to consent form prior to signing the Informed Consent Form (ICF).

The study coordinator and/or study PI will initially contact potential study subjects 1-2 weeks ahead of scheduled clinic visits. During this initial contact, the study coordinator and/or study PI will review details of the study and answer questions.

After sharing a basic overview of the study with potential subjects, the written informed consent document will be shared with those who express interest in participating in this study. This document will describe the purpose of the study, procedures to be followed, risks and benefits of participation. Alternatives to enrollment and the right to withdraw participation at any time will be discussed during the consent process and will be explicitly included within the consent form. The study team will be available over the phone and in-person for any questions that may arise from initial review of the consent form.

Witnessed signature of the informed consent document by a study team member will be required prior to enrollment of any subject. A copy of the signed informed consent document form will be given to each subject and the original copy will be filed in the study binder. The study binder will be stored in a locked cabinet at the University of Rochester Movement Disorder clinic. Only the study coordinator will have access to the locked cabinet. Consent forms will be kept for 6

years after study completion, as required, then destroyed.

The study ICF will ask subjects to indicate whether or not they agree to communicate with study team members through email and, if yes, their email address is to be provided. The ICF also asks subjects to indicate whether or not they wish to be contacted in the future about future research studies or using their de-identified information for future studies. The subjects will also need to indicate whether or not they consent to be contacted about any clinically significant results and if they consent to a member of the study team to contact their primary care provider (PCP) or primary neurologist with any clinically significant results for further evaluation and/or work-up. Subjects will be asked to provide the name of their PCP or primary neurologist whom any clinically significant results should be sent.

Study participation is expected to last one study visit. Therefore, subjects will not be formally re-consented during the study unless there is a change in study protocol.

7.1. Consent Process for Spanish Speaking Subjects

The University of Rochester Movement Disorder's Clinic is home to the UR Medicine Spanish Language Neurology Clinic. The Spanish Language Clinic provides specialty neurologic care to Rochester's Spanish speaking population. Individuals scheduled for clinic visits as part of the Spanish Language Clinic will be included in the randomization process detailed above. Potential study subjects who are seen as part of the Spanish Language Clinic will be provided oral and written study information in Spanish by a certified provider in Spanish who is also a member of the study team. For these potential subjects, these certified provider will determine the potential subject's capacity to consent, and if eligible, obtain consent in Spanish using the Spanish language version of the ICF. A copy of the subject's signed Spanish ICF will be sent home with them. The original Spanish ICF will be placed in the study binder as described in section 7, "Consent Process" of this protocol. The study questionnaire will also be administered in Spanish for the subjects described above.

7.2. Consent Process for Sub Study

After data collection for the main study is completed, the principal investigator will call subjects who were found to have low levels of vitamin B6 and who had consented to be contacted in the future regarding their study results (Please see Sub Study Phone Call Script). During the phone call, the principal investigator will discuss the purpose of the sub study, the potential risks and benefits of participating in the sub study. The principal investigator will then obtain verbal consent before asking the questions corresponding to the sub study.

8. STUDY PROCEDURES

8.1. Enrollment

A randomly selected sample of 144 PwPD will be recruited between July 2024 and June 2025 from the Movement Disorders Clinic at the University of Rochester. To enroll 144 PwPD in one year, we will need to enroll at least 3-4 PwPD per week. As detailed above, we

will use a computer program to randomly select 3-4 of the ~75 PwPD scheduled for an appointment at our clinic every week. In order to facilitate study procedures, we will make every effort to maintain a consistent accrual rate of ~1 PwPD per day.

Once participants are enrolled, we will use REDCap to generate IDs in order to de-identify all data collected. We will maintain a separate file linking the REDCap generated IDs with identifiable information (name, E-mail and phone number).

8.2. Blood Samples

After overnight fasting, blood samples will be obtained from study subjects to measure serum levels of:

- Vitamin B6 (nmol/L)
- Vitamin B12 (pg/mL)
- Homocysteine (µmol/L)
- Methylmalonic acid (µmol/L)
- Folic acid/Folate (ng/mL).

Blood samples will be collected and processed at the University of Rochester, Neurology, Movement Disorders Clinic by an appropriately trained member of the study team. Participants will be asked to provide 3.2mL of blood via standard venous blood draw into 3 SST tubes (yellow top). Processed samples will be sent immediately to URMC Laboratories for measurement of folic acid, vitamin B12 and homocysteine levels (FOL, VB12, HOMCY), as well as to Associated Regional and University Pathologists (ARUP) Laboratories for measurement of vitamin B6 and methylmalonic acid levels (VIT B6, MMAS).

De-identified results will be reported to the study coordinator via URMC email address and entered into REDCap as per standard procedures for research studies.

Table: Blood Sample Normal Values

Test Name	Test Number	Normal Values
Vitamin B6^	VIT B6	20-125 nmol/L
Vitamin B12*	VB12	232-1245 pg/mL
Homocysteine*	HOMCY	≤15 µmol/L
Methylmalonic Acid^	MMAS	≤0.4 µmol/L
Folic Acid/Folate*	FOL	≥4.6 ng/mL

*Samples being processed at URMC laboratories
^ Samples being processed at ARUP laboratories

8.3. Structured Interview

8.3.1. Non-Clinical Structured Interview

The non-clinical part of the structured interview will be conducted by the study coordina-

tor (or a certified provider in Spanish language for subjects who speak Spanish). Data will be collected directly into REDCap. The interview will not be recorded. Demographic and dietary information to be collected includes:

- Age (years)
- Sex (male/female)
- Race (as defined by the U.S. Office of Management and Budget): (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White)
- Ethnicity (as defined by the U.S. Office of Management and Budget) (Hispanic or Latino, non-Hispanic or Latino)
- Body mass index (kg/m^2)
- Diabetes (yes/no)
- Smoking use history (yes/no)
- Alcohol use history (yes/no)
- Daily consumption of protein in the last 30 days (yes/no)
- Daily consumption of food rich in vitamin B6 in the last 30 days (yes/no)
- Food insecurity (yes/no)
- Vitamin B6 supplement use in the last 30 days (yes/no)
- Vitamin supplement use in the last 30 days (yes/no)
- History of oral contraceptive pill use (yes/no).

Dietary aspects will be collected as recommended by standardized questions from the National Health and Nutrition Examination Survey (NHANES) [Morris 2008].

8.3.2. Clinical Structured Interview

The clinical part of the structured interview will be conducted by the study PI or study sub-investigators. Data will be collected directly into REDCap. The interview will not be recorded. This part includes a pre-defined questionnaire that will collect:

- Age at onset of Parkinson's disease (years)
- Disease duration (years)
- Movement Disorders Society sponsored version of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Part I (non-motor symptoms)
- MDS-UPDRS Part II (motor)
- MDS-UPDRS Part IV (motor complications)
- Dosage of levodopa (mg/day)
- Dosage of DDCI (carbidopa, benzeraside) (mg/day)
- Levodopa/carbidopa dosage ratio
- Cathecol-o-methyl-transferase (COMT) inhibitor (entacapone, opicapone) use (yes/no)
- COMT inhibitor dosage (mg/day)
- Levodopa equivalent daily dosage (mg/day)
- modified Toronto Clinical Neuropathy Score
- Symptoms or signs consistent with epilepsy (yes/no)

8.4. Focused Neurological Examination

8.4.1. MDS-Unified Parkinson Disease Rating Scale

In addition to Parts I, II and IV of the MDS-UPDRS, which will be obtained as part of the structured interview mentioned above, a focused neurological examination will be performed by a certified rater of the MDS-UPDRS in order to obtain Part III of the MDS-UPDRS at the Movement Disorders Clinic at the University of Rochester. Data will be collected directly into REDCap.

8.4.2. Toronto Clinical Neuropathy Score

The primary measure of neuropathy will be the modified Toronto Clinical Neuropathy Score (TCNS). This focused examination will be performed by a trained rater at the Movement Disorders Clinic at the University of Rochester. Data will be collected directly into REDCap.

8.5. No data obtained as part of this study will be included in the subject's medical record. If a clinically significant result is evidenced (e.g., lab test result, previously unknown evidence of neuropathy or epilepsy), this will be communicated directly to the study subject and, if previously consented, to the subject's PCP or primary neurologist.

Table 1: Schedule of Assessments. All study activities should be completed on the same day.

	Study Visit (URMC's Movement Disorders Clinic)
1. Informed consent	X
2. Enrollment	X
3. Obtain blood samples (after overnight fasting)	X
4. Conduct Structured Interviews	X
5. MDS-UPDRS	X
6. TCNS	X

URMC = University of Rochester Medical Center, MDS-UPDRS = Movement Disorders Society-sponsored revision of the Unified Parkinson's Disease Rating Scale, TCNS = Toronto Clinical Neuropathy Score

Return of Results:

If any previously unknown abnormalities in the blood work or previously unknown neuropathy are detected, the study subject will be notified immediately and, if permission is given, this information will be shared with the relevant healthcare providers for treatment and/or further workup. Consent for the return of individual clinically relevant results to the subject and/or their

primary care provider will be discussed during the consent process and a written indication will be required.

8.6. Sub study data collection:

After completion of the study, we will contact subjects found to have low levels of vitamin B6 who had consented to be contacted in the future to ask:

8.6.1. Whether they were started on vitamin supplementation or not.
8.6.1.1. If yes: What dosage and for how long have they been taking it for.
8.6.1.2. If yes: Whether they perceived any benefits or side effects from vitamin supplementation.
8.6.1.3. If yes: What were the benefits or side effects they perceived.

8.6.2. Whether any follow-up serum level of vitamin B6 was obtained by their healthcare provider.

8.6.2.1. If yes: How long after they started taking vitamin supplementation
8.6.2.2. If yes: If those follow-up levels increased, decreased, or stayed the same. What was the value of the follow-up levels.

8.6.3. Repeat the 6 questions of the MDS-UPDRS Part IV (motor complications) regarding time spent with dyskinesias, functional impact of dyskinesias, time spent in the OFF state, functional impact of fluctuations, complexity of motor fluctuations, and painful OFF-state dystonia.

9. RISKS TO SUBJECTS

Obtaining the blood sample, usual risks of sustaining a local injury on the site used to obtain the blood sample. The possible side effects from blood drawing include faintness, inflammation of the vein, pain, bruising, or bleeding at the site of puncture. There is also a slight possibility of infection. In order to minimize risk, only appropriately trained members of the study team will obtain blood samples. Fasting prior to the blood draw could increase the risk of subjects feeling uncomfortable symptoms, including dizziness or light-headedness, weakness, hunger, nervousness or restlessness. In order to minimize the duration of discomfort, we will make snacks and drinks available to subjects immediately upon completion of the blood draw.

If research data is shared with unauthorized users, you may be at risk of loss of the privacy of your health data. This risk is intended to be minimized by protections put in place by the Principal Investigator. All laboratory, demographic and clinical information will be collected in a de-identified manner using an alphanumeric study ID to maintain subject privacy and confidentiality. All sharing of de-identified laboratory, demographic and clinical information, for the purposes of presentation, abstract, manuscript or grant preparation, will be done using a Box folder maintained and secured by the University of Rochester IT team. Any presentation, abstract, or manuscript will be made available to the sponsor and RSRB as part of routine annual reports.

Risk of excessive burden and stress posed to study subjects by the blood draws, the structured interview, and the focused examination as part of this study. To minimize burden, all study activities will occur at the Movement Disorders Clinic and be conducted only my appropriately trained members of the study team. Participants will be able to take breaks and skip any questions that are uncomfortable for them.

Alternative to Participation:

The alternative would be not to participate. Subjects may request their Primary Care Provider order the labs specified in this study.

10. POTENTIAL BENEFITS TO SUBJECTS

Detection of previously unknown abnormalities in levels of vitamin B6, vitamin B12, homocysteine or methylmalonic acid. Detection of previously unknown neuropathy or epilepsy. If any of these are detected, the study subject will be notified immediately and, if permission is given, this information will be shared with the relevant healthcare providers for treatment and/or further workup.

11. COSTS FOR PARTICIPATION

There will be no cost to participate in this study.

12. PAYMENT FOR PARTICIPATION

Subjects will not be paid for their participation.

13. SUBJECT WITHDRAWALS

Subjects can discontinue participation in the study at any time. If a subject chooses to self-withdraw from the research study for any reason, they will forego all study-related procedures, including blood samples, they will continue to receive usual routine clinical care and we will analyze any data collected prior to withdrawal. This is outlined in the consent form.

As subjects are not being randomized into groups nor is there a cap on recruitment, subjects who withdraw for any reason will not need to be replaced.

14. PRIVACY AND CONFIDENTIALITY OF SUBJECTS AND RESEARCH DATA

To minimize a breach in subject privacy, all data obtained from the subjects enrolled will be kept confidential. All subjects will be given a de-identified study number generated by REDCap after they consent to study procedures. These REDCap study numbers will be linked to personal information (name, E-mail and phone number) on a separate file that will be password-protected and located in UR Box. Only the principal investigator and research coordinator will have access to this file in UR Box. Screening data will be deleted if subjects do not meet inclusion criteria.

Study data will be collected using a REDCap database specifically designed for this study. Laboratory, demographic, and clinical information will be maintained in REDCap until the study is completed. Only individuals on the study team who are listed on the protocol will have access to the data in REDCap. Once the study is completed, de-identified data will be exported from REDCap to UR Box for analysis.

Data will be stored for 6 years after the completion of the study.

All sharing of de-identified laboratory, demographic, and clinical information for the purposes of presentation, abstract, manuscript, or grant preparation, will be done using a BOX folder maintained and secured by the University of Rochester IT team per University IT Policies. Permissions to the BOX folder will be limited to GCP certified research personal mentioned in this protocol. All presentation, abstract, or manuscript will be made available to the sponsor and RSRB as part of routine annual reports.

Subject laboratory, demographic, or clinical information will not be released without written permission of the subject, except as necessary for monitoring by the RSRB, NIMH, OHRP, or the sponsor's designee.

If any previously unknown abnormalities in the blood work or previously unknown neuropathy are detected, the study subject will be notified immediately and, if permission is given, this information will be shared with the relevant healthcare providers for treatment and/or further workup.

15. DATA / SAMPLE STORAGE FOR FUTURE USE

Study data and samples will not be used in future research without additional informed consent.

16. DATA AND SAFETY MONITORING PLAN

All protocol deviations will be listed in a protocol deviation log within the study binder. All protocol deviations that involve a safety concern will promptly be reported to the IRB by a member of the study team according to the *Guideline for Reporting Research Events.*

Study related adverse events (AE) and serious adverse events (SAE) are not expected. If the study team is notified by a subject that an AE or SAE did occur, the PI is to be notified immediately upon notification of AE/SAE. Any SAE that could be related to the study will be reported to the IRB within 24 hours of notification.

Study team members will meet on a monthly basis for information/data review and discussion of any safety concerns. During this meeting, a de-identified list of subjects who completed the study following the previous month's meeting will be discussed, confirming all subjects who indicated they wanted their individual clinically significant test results returned and/or sent to their PCP were sent the results.

17. DATA ANALYSIS PLAN

- *Sample size determination:* Study subjects will be 144 adult people living with Parkinson's disease (PwPD). We recently completed a systematic review of the literature and we estimated that the frequency of abnormal vitamin B6 levels in PwPD is ~41% [Modica 2023]. Thus, the sample size of 144 PwPD is necessary to estimate the prevalence of abnormal vitamin B6 levels with 95% confidence and 8% precision (1/5 of the estimated prevalence, based on feasibility).

- *Vitamin B6 levels*: Serum vitamin B6 levels from the 144 PwPD will be compared to the vitamin B6 levels of historical controls from the latest publicly available NHANES, matched for age, sex, and 30-day consumption of: protein (yes/no), food rich in vitamin B6 (yes/no), vitamin B6 supplements (yes/no), and vitamin supplements (yes/no) [Morris 2008]. Depending on the availability of matches, we will give priority to age and sex for matching criteria with statistical adjustment for other factors. We will exclude people expected to have abnormal vitamin B6 levels due to underlying conditions or drug interactions (other than levodopa). We will use a logistic regression model that includes group (PwPD, control) and relevant covariates to estimate the adjusted odds ratio per group in order to compare the groups with respect to prevalence of abnormal serum level of vitamin B6. Similarly, we will use a multiple regression model to compare the groups with respect to adjusted mean value of vitamin B6 levels (log-transformed). Upon completion, we will have estimated the prevalence of abnormal serum levels of vitamin B6 in PwPD.

- *Associations with demographic variables:* We will use multiple regression modeling to examine associations between vitamin B6 levels (nmol/L, log-transformed) and relevant demographic variables as defined by the NHANES: 1) age (years), 2) sex (male/female), 3) race and 4) ethnicity (defined by the U.S. Office of Management and Budget), 5) body mass index (kg/m^2), 6) diabetes (yes/no), 7) smoking (yes/no), 8) alcohol use (yes/no), 9) daily consumption of protein in the last 30 days (yes/no), 10) daily consumption of food rich in vitamin B6 in the last 30 days (poultry, fish, greens, tropical fruits) (yes/no), 11) food insecurity (yes/no), 12) vitamin B6 supplement use in the last 30 days (yes/no), 13) vitamin supplement use in the last 30 days (yes/no) and 14) history of oral contraceptive pill use (yes/no). We will obtain this information using structured interviews based on standardized questions from the NHANES. By examining the interaction between group (PwPD, control) and each covariate, we will compare associations to those made in historical controls from the latest publicly available NHANES data. Our hypothesis is that, compared to controls, vitamin B6 levels in PwPD will be similarly associated with age, sex, race, ethnicity, body mass index, diabetes, smoking, alcohol use, daily consumption of protein or food rich in vitamin B6 in the last 30 days, food insecurity, vitamin B6 or vitamin supplement use in the last 30 days, and oral contraceptive pill use. Upon completion, we will have examined associations between serum vitamin B6 levels and relevant demographic variables in PwPD.

- *Associations with clinical variables:* We will use multiple regression modeling to examine the associations between serum vitamin B6 levels (nmol/L, log-transformed) and relevant clinical variables in PwPD: 1) age at onset of Parkinson's disease (years), 2) disease duration (years), 3) disease stage (modified Hoehn & Yahr scale), 4) disease severity (Move-

ment Disorders Society sponsored revision of the Unified Parkinson Disease Rating Scale, MDS-UPDRS), 5) levodopa dosage (mg/day), 6) DDCI (carbidopa, benzeraside) dosage (mg/day), 7) levodopa/carbidopa ratio, 8) cathecol-o-methyl-transferase (COMT) inhibitor (entacapone, opicapone) use (yes/no), 9) COMT inhibitor dosage (mg/day), 10) levodopa equivalent daily dosage (mg/day), 11) symptoms or signs consistent with polyneuropathy (yes/no, yes if ≥6 points in the modified Toronto Clinical Neuropathy Score), and 12) symptoms or signs consistent with epilepsy (yes/no). Our hypothesis is that vitamin B6 levels in PwPD will be associated with disease duration, stage, severity, levodopa dosage, carbidopa dosage, symptoms or signs consistent with neuropathy or epilepsy. Upon completion, we will have examined associations between serum vitamin B6 levels and relevant clinical variables in PwPD.

We will pay careful attention to evaluation of the assumptions of the regression models (normality, homogeneity of variance, linearity) and identification of outliers, influential observations, and multi-collinearity. We will especially scrutinize the functional form of the covariates since some of the associations might be nonlinear (for example, between vitamin B6 level and neuropathy).

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