Protocol Title: NAPS: **Nia**cin for **P**arkinson's Disease

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1. Objectives

Describe the purpose, specific aims, and hypothesis:
(1) examine the blood, urine and spinal fluid of persons with Parkinson's to look for evidence of inflammation and; (2) whether 18 months of vitamin B3(niacin or niacinamide) supplementation may reduce the inflammation and/or improve symptoms.

Blood samples will be analyzed through Enzyme Linked Immuno Sorbent Assay (ELISA). We will examine GPR109A levels in WBCs, NAD/NADH ratio in RBCs, inflammatory and non-inflammatory cytokines (Serum and CSF levels of following twenty-seven cytokines EGF, FGF2, Eotaxin, TGFa, GCSF, Flt3L, GMCSF, IFNg, IL10, MCP3, IL12P40, IL12P70, IL13, IL15, sCD40L, IL1RA, IL1a, IL1b, IL5, IL6, IL7, IL8, MIP1a, MIP1b, TNFa, TNFb and IP10), and niacin metabolites in plasma and urine. Blood samples will be analyzed through FACs, Seahorse, and protein (total and phosphorylated) analysis to understand biomarkers of PD and effects of niacin in PD.

2. Background

Describe the background and rationale for the study:
Inflammation plays a central role in Parkinson's disease (PD) pathology [1] as evidenced by the presence of microglia in the substantia nigra in post-mortem samples [2] as well as activated microglia and cytokines in clinical and animal studies [3]. The use of non-aspirin non-steroidal anti-inflammatory drugs was found to reduce the risk of PD [4]. We recently identified an anti-inflammatory receptor GPR109A that is upregulated in PD [5]. Niacin has a high affinity for this receptor, suggesting that it (niacin) may play an important role in reducing inflammation in PD. We also found that individuals with PD have a chronic niacin deficiency [5]. Using seed funding from the local PD chapter, we obtained pilot data which suggested that restoring the deficiency via over-the-counter (OTC) supplementation reduced inflammation and decreased the severity of the disease symptoms [6]. In this VA-funded study, we will determine the effect of 18 months OTC niacin or niacinamide supplementation on inflammation (as assessed in the blood and spinal fluid) and severity of the PD symptoms.

References
1. Barnum CJ, Tansey MG (2010) Modeling neuroinflammatory pathogenesis of Parkinson's disease. Prog Brain Res 184: 113–132.
2. Banati RB, Daniel SE, Blunt SB (1998) Glial pathology but absence of apoptotic nigral neurons in long-standing Parkinson's disease. Movement Disorders 13: 221–227.
3. Crotty S, Fitzgerald P, Tuohy E, Harris DM, Fisher A, et al. (2008). Neuroprotective effects of novel phosphatidylglycerol-based phospholipids in the 6-hydroxydopamine model of Parkinson's disease. European Journal of Neuroscience 27: 294–300.
4. Gagne JJ, Powers MC (2010) Anti-inflammatory drugs and risk of Parkinson's disease: A meta-analysis Neurol 74: 995–1002.
5. Wakade, C., Chong, R. K., Thomas, B., Bradley, E., & Morgan, J. (2014). Upregulation of GPR109A in Parkinson's disease. PloS ONE, 9(10), e109818.
6. Wakade, C., Chong, R. K., Bradley, E., & Morgan, J. (2015). Low-dose niacin supplementation modulates GPR109A levels and NAD/NADH ratio and ameliorates PD symptoms. Clinical Case Reports. doi: 10.1002/ccr1003.1232.

3. Inclusion and Exclusion Criteria

List the inclusion/exclusion criteria:

Inclusion criteria

PD subjects will be adult men and women diagnosed with idiopathic mild to moderately severe PD. The majority of PD subjects are expected to be > 60 years old. Disease severity is defined as modified Hoehn & Yahr Stages I-IV (while "On"). PD is defined according to the UK Brain Bank Criteria made at least six months prior to recruitment to the study. PD features include the presence of at least two of the four cardinal clinical manifestations of the disease, which are tremor, rigidity, bradykinesia, and disturbances of posture or gait, without any other known or suspected cause of Parkinsonism. Subjects should be stabilized on PD medication for at least 3 months before enrollment into the study. Subjects' PD drug prescriptions will not be altered nor withheld during the study. The patient will have signed informed consent.

Exclusion criteria

Subjects will be excluded if they present with significant cognitive deficits. A MMSE score of ≤25 is considered substantial global cognitive impairment.

Subjects will be excluded if they had previous brain surgery or other severe neurological problems – intracerebral hemorrhage, traumatic brain injury, central nervous system malignancy, active CNS infection, significant stroke, Alzheimer disease or any type of implanted stimulator including but not limited to Deep Brain Stimulator (DBS) or pacemaker. All subjects must be without evidence of dementia, defined as a score > 24 the Mini-Mental State Examination and able to understand test instructions. Subjects must not have functional blindness (inability to participate in gait and visuomotor assessments) or lower limb amputation higher than the forefoot or any orthopedic problem that precludes performance of physical tests. Subjects must not have known allergy to vitamin B3.

Significant cardiac, pulmonary, hepatic, gastrointestinal, or renal disease (e.g., New York Heart Association Class III or IV congestive heart failure; endocarditis; pulmonary insufficiency symptomatic at rest or with mild physical exertion; acute or chronic hepatitis; renal failure requiring dialysis; second and third degree AV block or sick sinus syndrome), or uncontrolled/advanced diabetes are also exclusionary factors.

Subjects will be excluded if they are taking B3 but will be included if they are taking B complex that has very low dose B3 (25 mg) which has minimal effects on GPR109A (based on our unpublished observation).

Overall, Dr. John Morgan will exercise his clinical judgment to exclude a subject from the study if, in his opinion, he feels that a patient presents with a set of comorbidities which renders them unsuitable for the study.

4. Number of Subjects/Records/Samples Collected

Indicate the total number of subjects to be accrued/records reviewed/samples collected across all sites:

100 No new recruitment.

5. Recruitment Methods

Describe when, where, and how potential subjects will be recruited:

VA clinic (including medical records), word of mouth.

6. Procedures Involved

a. *Describe the procedures involved to include those procedures that are standard evaluation and/or care and those that are solely for research purposes:*

Standard procedures: basic physical examination for strength, speed, coordination, tremor, flexibility, rigidity and reaction time

Research procedures: questionnaire and tests to document health and cognitive status,

Blood (2-3 tsp), urine and spinal fluid samples, over-the-counter (non-prescription) vitamin B3 (Niacin or Niacinamide) supplementation.

Blood Collection

Ten ml blood samples will be collected by venipuncture following standard procedures and processed within 4 hours as described earlier (Wakade et al., 2014 doi.org/10.1371/journal.pone.0109818). Briefly, purple-top ethylenediaminetetraacetic acid (EDTA) tubes will be used to collect the blood sample and immediately kept in ice. Whole blood will be spun and leukocytes (WBCs) will then be collected and placed in fresh tubes, re-suspended in 4 ml of ACK Lysing Buffer (Lonza cat # 10-548E) and be incubated for 10 min at room temperature before being spun again at 300 ×g for 5 min. The process will be repeated one more time. Supernatant will be discarded from the clean WBCs pellet. WBCs pellets will be washed twice with 1 ml of PBS and WBCs pellets will then be stored at −80 °C until further analyses (Wakade et al., 2014).

Analytical flow cytometry

As described previously (Stranahan et al., 2016), whole blood cells will be incubated with antibodies for surface markers including CD11b, CD68, F4/80 (M1), CD206 (M2), and GPR109a. Next, cells will be fixed and permeabilized using fix/perm concentrate (eBioScience) before incubation with antibodies for intracellular staining of IL-10 (functional M2 s). Cells will then be washed and run through a four-color flow cytometer (FACS Calibur). Data will be collected using Cell Quest software. Samples will be double-stained with control IgG (isotypes) and cell markers to assess any spillover signal of fluorochromes. Gating will exclude dead cells and debris using forward and side scatter plots.

Western Blot

WBCs will be separated as described previously (Wakade et. al 2014). WBCs will be subjected to lysis by RIPA buffer with a protease inhibitor cocktail and protein concentration will be measured by Bradford reagent (Bio-Rad). Again the samples will be subjected to western blot using Bio-Rad 4–15% SDS-PAGE then transferred to PVDF membrane followed by incubation with respective antibody overnight and developed with an ECL kit (Giri et al., 2007).

Saehorse

Oxygen consumption rate (OCR) is measured before and after the addition of inhibitors to derive several parameters of mitochondrial respiration. Initially, baseline cellular OCR is measured, from which basal respiration can be derived by subtracting non-mitochondrial respiration. We will study OCR before and after niacin treatment in PD.

b. *Describe and explain the study design:*

Baseline, 6 month, 12 month and 18 months repeated measures placebo-controlled study

Group 1 – Niacin Arm: 30 PD subjects	**Group 2 – Niacinamide Arm**: 30 PD subjects	**Group 3 – Placebo Wait-listed Arm**: 30 PD subjects
Oral 100 mg fixed dose twice daily x 18-months (200 mg total / day) with assessments @ baseline, 6 month, 12 month and 18 months	Oral 100 mg fixed dose twice daily (200 mg total / day) x 18-months with assessments @ baseline, 6 month, 12 month and 18 months	Oral placebo twice daily x 18-months with assessments @ baseline, 6 month, 12 month and 18 months

c. *Describe the procedures performed to lessen the probability or magnitude of risks:*

Use of slow-release supplement to minimize potential skin itching

d. *Describe the duration of an individual subject's participation in the study and the time involved:*

18 months supplement study with a baseline, 6 month, 12 month and 18 months each lasting about 1 to 1.5 hr.

7. Data and Specimen Management

a. *Describe the data analysis plan, including any statistical procedures:* ☐ N/A

We will use the Unified Parkinson's Disease Rating Scale (UPDRS) III (Motor Section) as the primary pre- and post-treatment outcome assessment that is recommended for PD studies. Significant changes in outcome measures will be determined by performing a Mixed model analysis of variance, with Groups as the independent factor and Time as the repeated factor.

b. *When applicable, provide a power analysis:* ☐ N/A

Based on the preliminary data using unpaired simple effects analyses, the minimum number of subjects needed per group to detect a statistical significance @ p = .05, 2-tailed and 80% power of detection are as follows:

Outcome measures	**Effect size (based on Cohen's d)**	**# Subjects/group**
UPDRS motor	1.46	14
Sleep efficiency (pilot data)	0.97	8

The sample sizes are based on a large effect size, i.e., a grossly observable and clinically meaningful treatment effect. We have demonstrated that this is a reasonable expectation based on the actual effect sizes of the human preliminary data that we have shown. We used simple effects analyses as

the basis for the sample size calculations because any sample size calculations based on the full design would require knowledge of the intra-class correlation across the treatments, as well as the carry-over, group, and period effects.

Correlation statistics. Based on the preliminary data's correlation coefficients (r) obtained via simple linear independent correlation tests comparing the variables versus disease severity (UPDRS motor section), the minimum number of subjects needed per group to detect a statistical significance @ p = .05, and 80% are as follows:

Outcome measures	**r**	**# Subjects/group**
GPR109A levels	0.59	20
Total Sleep Time	-0.57	18
Time to Fall Asleep	0.79	8
Sleep Efficiency	-0.73	10

Based on the simple effects and correlation power analyses above, we propose to collect 30 PD subjects per group (total 90) to account for potential subject dropouts, experimental/data corruption and/or an unexpected increase in the variability of the data. We plan to recruit about 100 subjects to take into account potential drop-outs and an unexpected increase in the variability of the data which impacts the estimated statistical power.

c. *Describe how data and specimens will be handled:* ☐ N/A

i. *What information will be included in that data or associated with the specimens?*

Please refer to 6a above. All data will be coded.

ii. *Where and how data and/or specimens will be stored?*

Specimens: Core lab. Data: Secured computer. ICD will be stored in a locked cabinet. NOTE: All data including specimens will be coded. Specimens will be stored at the VA in room 6B-127, and data will be stored in room 6B-128 (PI's Office).

iii. *How long will the data and/or specimens be stored?*

Indefinitely

iv. *Who will have access to the data or specimens?*

Research team

v. *Who is responsible for receipt or transmission of the data and/or specimens?*

Chandramohan Wakade (PI)

vi. *How will data and/or specimens be transported?*

Manually

8. Provisions to Monitor the Data to Ensure the Safety of Subjects

This study involves no more than minimal risk study and this section is not required. ☐ **N/A**

The plan might include establishing a data monitoring committee and a plan for reporting data monitoring committee findings to the IRB and the sponsor.

a. Describe the plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe. Subjects will be contacted the day after their study test participation to make sure that they did not experience unexpected or severe reactions to the vitamin supplement and optional lumbar puncture. A log will be kept to document the contact and relevant information from the subject. Subjects will also be given a 24-hour hotline to report any adverse event that they think is related to the study. The CSR&D DMC will review the study data every six months for adverse event occurrence, safety monitoring, overall performance and data generation. Any recommendations will be submitted to the IRB. Study is completed with recruitment. This is only the analysis of collected samples. No identifiers are used in analysis.
b. Describe what data are reviewed, including safety data, untoward events, and efficacy data. Safety and untoward events related to the vitamin supplement and optional lumbar puncture will be reviewed. NA
c. Describe how the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls with participants). Safety information will be collected by telephone calls or emails with the participants. NA
d. Describe the frequency of data collection, including when safety data collection starts. Data will be collected by the researchers at baseline, 6 month, 12 month and 18 months of the study. Subjects will be instructed to contact the researchers as soon as possible if they experience any adverse events that they think is related to the study. NA
e. Describe who will review the data. Dr Chandramohan Wakade will be the main person to review the data. He will consult with Dr Sharad Purohit if needed. The DSMB will also review the data every 6 months.
f. Describe the frequency or periodicity of review of cumulative data. Cumulative data will be reviewed by the lab every month to ensure adherence to the approved protocol. Every 6 months it will be reviewed by DSMB.

g. *Describe any conditions that trigger an immediate suspension of the research.*

If a subject reports an unexpected adverse event, the study will be suspended. The study will resume if it is determined that the event is unrelated to the study.

9. Withdrawal of Subjects ☐ N/A

a. *If applicable, describe anticipated circumstances under which subjects will be withdrawn from the research without their consent.* Click here to enter text.	☒ N/A
b. *If applicable, describe any procedures for orderly termination.* Click here to enter text.	☒ N/A
c. *If applicable, describe procedures that will be followed when subjects withdraw from the research, including partial withdrawal from procedures with continued data collection.* A subject who withdraws from the study on their own accord will be replaced by another. A subject who partially withdraws from procedures will remain in the study as long as they continue taking the supplement.	☐ N/A

10. Risks to Subjects

a. *List the reasonably foreseeable risks.*
NA

Low-level temporary discomfort or pain during the blood and lumbar draw. Contraindications for the lumbar puncture: Although there are no absolute contraindications to performing the procedure, caution will be used in patients with the following conditions: 1) Possible raised intracranial pressure; 2) Thrombocytopenia or other bleeding diathesis (including ongoing anticoagulant therapy); 3) Suspected spinal epidural abscess. The side effects associated with lumbar puncture may include following: 1) Discomfort or pain during the procedure; 2) Bleeding into the spinal cord, particularly in people who take blood thinners or have a low platelet count (thrombocytopenia); 3) Temporary headache as a result of CSF leakage; 4) Infection; 5) Nerve damage; 6) Cerebral herniation; 7) Temporary back pain.

We will take a conservative approach in deciding which patient to include from the lumbar procedure. Overall, Dr. John Morgan will exercise his clinical judgment to exclude a subject from the procedure if, in his opinion, he feels that the subject presents with a set of conditions which renders them unsuitable for the procedure.

b. *If applicable, describe any costs that subjects may be responsible for because of participation in the research.* Click here to enter text.	☒ N/A

c. *If applicable, describe risks to others who are not subjects.* Click here to enter text.	☒ N/A

11. Potential Benefits to Subjects

Describe the potential benefits that individual subjects may experience from taking part in the research.

NA

Niacin is an over-the-counter supplement. Our preliminary data indicates that PD patients will benefit from the supplementation. The dosage selected is 100 mg twice daily for 18 months. This dosage is low and do not produce any side effects. If at all, the PD patients will be benefitted by its actions.

12. Confidentiality

Describe the procedures for maintenance of confidentiality.

All data will be kept in locked files and information on computer databases including cloud storage will be coded to protect patient identity. The only place where the subject's identity is revealed is the Informed Consent form. Subjects' identity will be coded in all other sources of documentation (hard copies and electronic files).

13. Consent Process

If you are obtaining consent of subjects describe the consenting process.

Subjects who indicate an interest to participate in this study will be tested at the VA clinic. Blood and urine samples will be collected at the VA lab. The nature of the information in the informed consent will be a brief overview of the study, tests involved, benefit and risks as well as the subject's right to terminate the session at any time. The informed consent will also state that each participant agrees to freely and voluntarily participate in the study. The document will also state that any other financial impact is the participant's sole responsibility.

You will receive a $15 VA voucher redeemable at the VA cashier and the payment will be made at the time of each visit. You will receive an additional $20 for 18-month visit (so total of $50). You will receive an additional $50 at each time if you also participate in the spinal fluid sampling (which is initially and at 18-month visit, so total of $100).

14. Compensation for Research-Related Injury ☐

This section is not required when research involves no more than Minimal Risk to subjects.

a. *Describe the available compensation in the event of research related injury.*

The VA will provide any necessary medical treatment should you be injured by participation in this study and you will be treated for the injury within this facility, with limited exceptions, at no cost to you. An exception would be situations where this facility would not be capable of furnishing the care or services the subject requires. This

requirement does not apply to treatment for injuries that result from noncompliance by a research subject with study procedures.

Click here to enter text.

15. Resources Available ☒ N/A

a. Describe the availability of medical or psychological resources that subjects might need as a result of an anticipated consequences of the human research.
Click here to enter text.

b. Describe your process to ensure that all persons assisting with the research are adequately informed about the protocol, the research procedures, and their duties and functions.

Documentation from the VA facility will be provided that research team members have undergone training in the following areas: Good clinical Practices; the ethical principles of human research protection; Privacy; Cybersecurity; and VA Research Data Security and Privacy.