PARTNERS HUMAN RESEARCH COMMITTEE
PROTOCOL SUMMARY

Answer all questions accurately and completely in order to provide the PHRC with the relevant information to assess the risk-benefit ratio for the study. Do not leave sections blank.

PRINCIPAL/OVERALL INVESTIGATOR

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PROTOCOL TITLE

The effects of nicotinamide riboside supplementation on brain NAD+/NADH ratio and bioenergetics

FUNDING

Department Funds

VERSION DATE

12/29/2016

SPECIFIC AIMS

Concisely state the objectives of the study and the hypothesis being tested.

The primary aim of this study is to investigate the effects of exogenously administered nicotinamide riboside (NR) on brain NAD+/NADH ratio and bioenergetics functions in healthy individuals using ^{31}P MRS imaging.

The secondary aim is to investigate the effects of NR on brain structure and neurotransmitter functions using other neuroimaging methods.

BACKGROUND AND SIGNIFICANCE

Provide a brief paragraph summarizing prior experience important for understanding the proposed study and procedures.

NAD+ serves broadly two functions: an enzyme cofactor in redox reactions and a co-substrate for different classes of enzymes. As a coenzyme, NAD+ is essential for energy generation by transferring reducing equivalents from glycolysis (from the activity of glyceraldehyde-3-phosphate dehydrogenase) and from the TCA cycle under the form of NADH. When oxygen is limiting, NADH is converted to NAD+ by reduction of pyruvate into lactate. With oxygen, cytoplasmic NADH transfers its reducing equivalent through the malateaspartate shuttle or the glycerol-3-3-phosphate shuttle to the mitochondrial matrix. These reducing equivalents are oxidized by complex I of the electron-transport chain (ETC), thereby coupling glycolysis and the TCA cycle to ATP synthesis via oxidative phosphorylation [metabolizma sensoru, redox, free radical damage vb.]. As a co-substrate, NAD+ is consumed by three classes of enzymes -(i) the sirtuins (SIRTs), (ii) the adenosine diphosphate (ADP) ribose transferases (ARTs) and poly (ADP-ribose) polymerases (PARPs), and (iii) the cyclic ADP ribose (cADPR) synthases (CD38 and CD157)- which are involved in DNA repair, gene expression, regulation of energy metabolism, and calcium mobilization, aging/longevity, carcinogenesis, and immunological functions [15, 19].

Despite the crucial roles of NAD+ and NADH (together termed NAD metabolites) in cellular metabolism and physiology, their noninvasive in vivo detection is extremely challenging. In vivo P MR spectroscopy (MRS) provides a critical window into membrane phospholipid metabolism and high-energy phosphate molecule dynamics. In addition to commonly observed signals such as phosphocreatine (PCr), inorganic phosphate (Pi), and the three adenosine triphosphate (ATP) resonances, NAD signals have been identified in ^{31}P spectra. However, because of low concentration of NAD metabolites (below 1 mM), these have previously been considered below the in vivo MRS detection limit. Recently, a ^{31}P MRS-based NAD quantification method has been developed to directly quantify the intracellular NAD+ and NADH concentrations *in vivo* and we implemented this method at our 4T scanner.

Several pathways that NAD+ and NADH involved are also implicated in the pathophysiology of mood disorders and schizophrenia, including mitochondrial dysfunction 1-3, impaired bioenergetics 4, neuro-inflammation 5-8, and oxidative stress 9-12. We investigated the brain NAD+ and NADH levels in SZ patients with chronic, well-established illness as well as first-episode SZ patients who have not been exposed to the confounding effects of chronic medication treatment and chronic psychosis. We also included a first-episode bipolar disorder (BD) cohort, as a psychiatric control group. Our results showed a significant reduction in the NAD+/NADH ratio (Rx) both in chronically ill SZ and in first-episode SZ patients. These findings provide evidence for redox imbalance in the brain in all phases of SZ, potentially reflecting oxidative stress.

NAD+ is consumed by the enzymes and continuously degraded. To generate NAD+, organisms can use de novo synthesis from tryptophan but mainly depend on salvage pathways that involves synthesis from vitamin B3 derivatives nicotinic acid, nicotinamide, and nicotinamide riboside (NR). NR is the most recently discovered salvageable NAD+ precursor and is found in nutrients such as cow milk and yeast containing food products, however, in low quantities. In yeast, assimilation of endogenous NR has been shown to be essential for calorie restriction-mediated life-span extension [14] and exogenously added NR doubles intracellular NAD+ in a nicotinic acid depleted medium, leading to doubling of replicative longevity [7].
NR is also a potent stimulator of NAD+ production in several cultured mammalian cell types, including mouse and human cells. Increases in NAD+ were in some cases as high as 270% of controls [4], levels unprecedented for nicotinamide or nicotinic acid as NAD+ sources.
Several recent studies, including *in vivo* studies in rodents and in human cell cultures demonstrated robust beneficial effects of exogenously administered NR on mitochondrial functions, peripheral and CNS metabolism, DNA damage and inflammatory activity :

[1] In pre-diabetic mice, NR improved glucose tolerance, reduced weight gain, liver damage and the development of hepatic steatosis while protecting against sensory neuropathy. In type 2 diabetes model mice, NR greatly reduced non-fasting and fasting blood glucose, weight gain and hepatic steatosis while protecting against diabetic neuropathy.

[2] NR increased NAD+ levels in muscle and liver, in mice. Animals challenged with high-fat diet were protected from body weight gain, and had enhanced endurance and improved oxidation of fatty acids as a fuel source. NR also markedly improved insulin sensitivity. NR treatment increased mitochondrial biogenesis as measured by higher cristae content in muscle tissue. Consistent with increases in tissue NAD+ levels, sirtuins SIRT1 and SIRT3 appeared to be upregulated, as measured by FOXO1 and SOD2 acetylation levels. In these studies, NR was also shown to have a greater ability to increase NAD+ level than other NAD+ precursors such as NMN, nicotinamide, and nicotinic acid. Nicotinamide riboside increases NAD+/NADH, which could contribute to its ability to enhance mitochondrial oxidative capacity.

[3] High-fat high-sucrose diet was used to elicit chronic hepatosteatosis in mice resembling human fatty liver. This also lead to lowered hepatic NAD+ levels driving reductions in hepatic mitochondrial content, function, and adenosine triphosphate (ATP) levels. NR added to this diet prevented and reverted non-alcoholic fatty liver disease by inducing a SIRT1- and SIRT3-dependent mitochondrial unfolded protein response, triggering an adaptive mitohormetic pathway to increase hepatic b-oxidation and mitochondrial complex content and activity.

[4] NR supplementation increases total NAD+ in primary cortical astrocytes obtained from non-transgenic and mutant human superoxide dismutase (hSOD1) overexpressing mice. Because total NAD+ increases more than total NADH levels (about 100 and 35% change, respectively), treatments also modify the NAD+/NADH ratio. Mitochondrial NAD+ is also increased, but no significant change was observed in mitochondrial NADH. In the same study, NMN treatment confers astrocyte resistance to oxidative stress as reflected by reduced vulnerability to H2O2 treatment. Likewise, overexpression of NAMPT or mNAMPT also provides protection and reduces mitochondrial ROS production in response to H2O2. Moreover, NMN treatment and overexpression of NAMPT or mNAMPT also appear to decrease mitochondrial ROS levels under basal conditions.

[5] Treatment with NR treatment increased NAD+ concentration in muscle stem cells (MuSCs) and the number of MuSCs in young and old mice. In old mice only, NR treatment reduced nuclear DNA damage, pro-inflammatory proteins, increased TCA cycle genes, oxidative phosphorylation, mitochondrial membrane potential and increased abundance of ATP in MuSCs. In further experiments, NR also increased the number of neural and melanocyte stem cells, and slightly increased mouse lifespan.

[6] NR treatment of cultured fibroblasts increased mitochondrial membrane potential and DNA content, however, did not affect expression levels of nDNA- or mtDNA-encoded respiratory complex subunits.

[7] Increasing cochlear NAD+ levels by NR prevents noise-induced hearing loss and spiral ganglia neurite degeneration. These effects are mediated by the NAD+-dependent mitochondrial sirtuin, SIRT3.

[8] NR given to a mouse model of mitochondrial myopathy increased cytochrome c oxidase activity, amount of oxidative phosphorylation enzymes and the number of mitochondria in skeletal muscle. Similarly, in brown adipose tissue, NR increased mitochondrial number and size, and mtDNA amount.

Despite the consistent evidence indicating that NR increases NAD+/NADH ratio in several tissues with concomitant effects summarized above, no study to date has investigated the *in vivo* effects of NR in human CNS. In this study, we propose to administer NR to healthy human subjects and measure the NAD/NADH ratio and bioenergetic functions in the brain using ^{31}P MRS. We hypothesize that NR treatment will increase the NAD/NADH ratio in the healthy human subjects and improve energy metabolism.

RESEARCH DESIGN AND METHODS

Briefly describe study design and anticipated enrollment, i.e., number of subjects to be enrolled by researchers study-wide and by Partners researchers. Provide a brief summary of the eligibility criteria (for example, age range, gender, medical condition). Include any local site restrictions, for example, "Enrollment at Partners will be limited to adults although the sponsor's protocol is open to both children and adults."

We plan to study the levels of chemicals associated with cellular energy metabolism; kinetics of enzymes involved in cellular energy metabolisms in brains of healthy people before and after use of nicotinamide riboside (NR) for 2 weeks. In addition, we will also collect data on the structure of the gray matter and white matter; resting state functional brain activity; levels of brain chemicals including glutamate and GABA; and white matter integrity. We will collect whole brain data where possible and focus on the medial prefrontal cortex and parietal cortex as two regions of interest where whole-brain data collection is not possible due to technical limitations. Data collection for each of these modalities is identical to our ongoing patient MRI studies which are all IRB approved. We hypothesize that bioenergetics parameters will change after the use of NR. Investigation of these parameters in healthy people will give us a chance to determine the alterations related to drug itself, independent from the pathophysiology of the illness.

Enrolment: The study will enroll up to 60 healthy people with no individual history of psychiatric illness and family history of psychotic and mood disorders. Family history will be obtained via self-report only. Participants will be recruited via online advertisements and flyers as well as approaching healthy individuals who previously participated in our studies. Flyers will be distributed throughout the hospital. All advertisements and flyers will be approved by the PHRC.

Participants will be pre-screened with an IRB-approved telephone interview (attached) prior to coming to the Brain Imaging Center. An investigator associated with the program approaches participants with information about the study. If the participant is interested, he/she will be invited to hospital for study visit. During the study visit the investigator (Dr. Chouinard; Dr. Baker; Dr. Shinn; Dr. Murphy; one of them will be present in each steps of the study according to their availability) will give information about the study again and conduct the consent process.

There are no restrictions on subject recruitment based on sex, ethnic background or health status other than those mentioned below. Individuals who do not speak English will not be allowed to participate in this study.

Inclusion Criteria

1. Age: 18-65 year-old
2. Male or female

3. Without psychiatric diagnosis according to a structured psychiatric interview (SCID; First et al 1994)
4. Without history of a psychotic disorder and/or mood disorder among parents, siblings, or children, as obtained via self-report only.

Exclusion Criteria

1. Significant medical or neurological illness.
2. Diagnosis diabetes mellitus (DM), uncontrolled hypertension (HTN), severe hypotension, coronary artery disease (CAD), metabolic syndrome, glaucoma, liver impairment, decreased renal function, respiratory disorders, uncontrolled peptic ulcer disease.
3. Taking any other medications, including over the counter supplements with the exception of oral contraceptives for women
4. Pregnancy. Females of child-bearing age must be using an effective contraceptive method.
5. History of smoking, substance abuse or dependence.
6. Contraindication to MR scan *(claustrophobia, cardiac pacemakers, metal clips and stents on blood vessels, artificial heart valves, artificial arms, hands, legs, etc., brain stimulator devices, implanted drug pumps, ear implants, eye implants or known metal fragments in eyes, exposure to shrapnel or metal filings, other metallic surgical hardware in vital areas, certain tattoos with metallic ink, certain transdermal patches, metal-containing IUDs*)
7. Medical condition that would prevent blood draws, including current anti-coagulant or anti-aggregant therapy, tendency for abnormal scarring (e.g. keloids).
8. Difficulty in swallowing capsules.

Briefly describe study procedures. Include any local site restrictions, for example, "Subjects enrolled at Partners will not participate in the pharmacokinetic portion of the study." Describe study endpoints.

This study will be comprised of three main parts. All participants will be asked not to use alcohol or any other substances and drugs during study period.

1. <u>The First Part of the Study</u>

The first part of the study will be carried out within 10 days of study entrance and consists of 3 separate visits which will take about 6 hours in total. This part of the study will consist of the following procedures:

A. Consenting procedures (20 minutes)

B. Clinical evaluation (60 minutes)

i. Structured Clinical Interview for DSM-IV (SCID; (First et al., 1997)) to establish absence of Axis I psychiatric diagnoses

ii. Montgomery-Asberg Depression Rating Scale (MADRS)

iii. Young Mania Rating Scale (YMRS)
iv. Beck Anxiety Inventory (BAI)
v. MOCA (Montreal Cognitive Assessments)
vi. Pittsburgh Sleep Quality Index
vii. Sleep Log
viii. Edinburgh Handedness Survey
ix. North American Adult Reading Test (NAART)
x. DIGS Ethnicity Cards (including Parents and Grandparents)
xi. Medical Questionnaire
xii. Baecke Questionnaire of Habitual Physical Activity

C. Urine toxicology screen (5 minutes) and urine pregnancy test (5 minutes) prior to each MRI procedure
D. MRI procedure:
i. Diagnostic MRI scan at 3T (15 minutes)
ii. Common Anatomic Protocol (CAP) at 3T (MR Scan at 3T-1): Structural MRI, resting functional MRI and DTI + long MPRAGE at 3T (28 minutes)
iii. MRS protocol to quantify Glu and GABA at 3T (MR Scan at 3T-2) (30 minutes)
iv. Phosphorous MRS/MT at 4T (MR Scan at 4T- 1) (90 minutes)
v. Diffusion Tensor Spectroscopy (DTS) at 4T (MR Scan at 4T- 2) (90minutes)
E. Blood draw of 30 ml (10 minutes)
i. HCG, CBC, LFT, BUN, U&E, Lipid Panel, HgA1c, Insulin, Prolactin
F. Assessment of vital signs (blood pressure, heart rate, temperature), weight, height, waist and hip circumference (5 minutes)
G. EKG (10 minutes)
H. Filling out a food diary form (All participants will be asked to fill out a food diary form for the last 4 days before starting their medication process)

Participants will go into the medication process after completion of all procedures in this part of the study. Some participants may be excluded based on their psychiatric and medical conditions and laboratory tests at the end of the initial investigations.

2. <u>The Second Part of the Study</u>

The second part of the study in which participants will take daily NR will be carried out within the next 3 weeks. There will be 4 separate visits during this period on the 1st, 2nd, 5th and 9th days.

Participants will take daily 1000 mg NR orally, in the morning, for 16 consecutive days. NIAGEN®, the commercially available form of nicotinamide riboside, will be used as medication. The medication will always begin on

weekdays except Friday. On the 1st and 2nd day of the medication, participants will be asked to take their drugs at McLean Hospital as some side effects may occur more often at the beginning of the medication. Observation of medication intake will only take place on these days. After they take their drugs we will monitor them closely for 1 hour to observe any side effects. The participant will then be able to leave the hospital, in case of absence of any side effects at the end of the monitoring period. If the participants do not have any unacceptable side effects in the first two days, then they will be able to take the remaining doses at home. We will provide them sufficient number of drugs to take at home. In case of unacceptable side effects, participation will be terminated.

Additional visits will be on the 5th and 9th days. These visits will not include observation of drug intake but will include assessment of weight, vital signs (blood pressure, heart rate, temperature), blood glucose and will take about 30 minutes. In addition, a blood draw will be carried out on the 5th day to measure CBC, LFT, BUN, U&E. These follow-up visits will take place in an interview room at the Admissions Building 3rd floor where our research offices are located. During these follow up visits, the participant will be interviewed by a physician (Dr. Chouinard; Dr. Baker; Dr. Shinn; Dr. Murphy; one of them will be present in each steps of the study according to their availability) to assess for any developing adverse effects from the medication. If there is any concern for treatment- emergent adverse effects, drug administration will be discontinued.

Participants will also be asked to fill out a food diary for the last 4 days of their medication period (between 13-16 days).

Taking medication regularly is very important for the reliability of this study. However, we recognize that some participants may skip taking their drugs in some days. We will still complete the study with participants who miss up to 3 days of medication administration except any one of the last 3 days, and qualify those participants as having received the intended course of medication (while recording their degree of noncompliance). Participants who miss more than 3 days of medication or on any of the days within the last 3 days will be discontinued from the study.

3. The Third Part of the Study

As we want to investigate the effects of NR on brain structure all participants will undergo final investigations on the last two days of the medication protocol. These two days will not include observation of drug intake. We will split the MR procedure into two days. Participants will undergo 4T MR scan on one scan day and all other assessment and 3T MR scans will be done on another day. Days will be determined depending on the availability of the scanners. In addition, there will be clinical evaluation, assessment of vital

signs and blood draw on the first day of the third part of the study (15th day of medication intake).

Third part of the study will consist of the following procedures:

a. Clinical evaluation (30 minutes)
   i. Montgomery-Asberg Depression Rating Scale (MADRS)
   ii. Young Mania Rating Scale (YMRS)
   iii. Beck Anxiety Inventory (BAI)
   iv. MOCA (Montreal Cognitive Assessments)
   v. Pittsburgh Sleep Quality Index
   vi. Sleep Log

b. Urine toxicology screen (5 minutes) and urine pregnancy test (5 minutes) prior to each MRI procedure

c. MRI procedure:

i. Common Anatomic Protocol (CAP) at 3T (MR Scan at 3T- 1): Structural MRI, resting functional MRI and DTI + long MPRAGE at 3T (28 minutes)

ii. MRS protocol to quantify Glu and GABA at 3T (MR Scan at 3T-2) (30 minutes)

iii. Phosphorous MRS/MT at 4T (MR Scan at 4T- 1) (90 minutes)

iv. Diffusion Tensor Spectroscopy (DTS) at 4T (MR Scan at 4T- 2) (90minutes)

d. Blood draw of 30 ml to study CBC, LFT, BUN, U&E, Lipid Panel, HgA1c, Insulin, Prolactin (10 minutes)

e. Assessment of vital signs (blood pressure, heart rate, temperature), weight, height, waist and hip circumference (5 minutes)

THE FIRST PART OF THE STUDY		
VISIT 1 (at McLean Hospital)	**1. Consenting procedure** **2. Clinical evaluation** **3. Urine toxicology screen and urine pregnancy test** **4. Blood drawn** *(HCG, CBC, LFT, BUN, U&E, Lipid Panel, HgAlc, Insulin, Prolactin)* **5. Assessment of vital signs** *(blood pressure, heart rate, temperature),* **weight, height, waist, hip circumference** **6. EKG** **7. Diagnostic MR Scan at 3T**	
VISIT 2 (at McLean Hospital)	**1. Urine toxicology screen and urine pregnancy test** **2. MR Scan at 4T- 1** *(Phosphorous MRS/MT)*	

VISIT 3 (at McLean Hospital)	**1. Urine toxicology screen and urine pregnancy test** **2. MR Scan at 3T- 1** *(Common Anatomic Protocol)* **3. MR Scan at 3T- 2** *(MRS protocol to quantify Glu and GABA)* **4. MR Scan at 4T- 2** *(Diffusion Tensor Spectroscopy)*	
Filling out a food diary for 4 days		
THE SECOND PART OF THE STUDY		
<u>DAY 1</u> **VISIT 1 (at McLean Hospital)**	**1. NR 1000 mg/d, PO** **2. Observation for 1 hour** **3. Assessment of vital signs** **4. Blood glucose test**	
<u>DAY 2</u> **VISIT 2 (at McLean Hospital)**	**1. NR 1000 mg/d, PO** **2. Observation for 1 hour** **3. Assessment of vital signs** **4. Blood glucose test**	
DAY 3-4 (at home)	**1. NR 1000 mg/d, PO**	
<u>DAY 5</u> **VISIT 3 (at McLean Hospital)**	**1. NR 1000 mg/d, PO** **2. Assessment of vital signs** **3. Weight measurement** **4. Blood glucose test** **5. Blood drawn** *(CBC, LFT, BUN, U&E)*	
DAY 6-8 (at home)	**1. NR 1000 mg/d, PO**	
<u>DAY 9</u> **VISIT 4 (at McLean Hospital)**	**1. NR 1000 mg/d, PO** **2. Assessment of vital signs** **3. Weight measurement** **4. Blood glucose test**	**Filling out a food diary between 13th - 16^{h} days**
DAY 10-14 (at home)	**1. NR 1000 mg/d, PO**	

THE THIRD PART OF THE STUDY		
DAY 15 **VISIT 1 (at McLean Hospital)**	**1. NR 1000 mg/d, PO** **2. Clinical evaluation** **3. Blood drawn** *(CBC, LFT, BUN, U&E, Lipid Panel, HgAlc, Insulin)* **4. Urine toxicology screen and urine pregnancy test** **5. Assessment of vital signs, weight, waist, hip circumference** **6. MR Scan at 3T- 1** *(Common Anatomic Protocol)* **7. MR Scan at 3T- 2** *(MRS protocol to quantify Glu and GABA)*	
DAY 16 **VISIT 2 (at McLean Hospital)**	**1. Urine toxicology screen and urine pregnancy test** **2. MR Scan at 4T- 1** *(Phosphorous MRS/MT)* **3. MR Scan at 4T- 2** *(Diffusion Tensor Spectroscopy)*	

For studies involving treatment or diagnosis, provide information about standard of care at Partners (e.g., BWH, MGH) and indicate how the study procedures differ from standard care. Provide information on available alternative treatments, procedures, or methods of diagnosis.

N/A

Describe how risks to subjects are minimized, for example, by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk or by using procedures already being performed on the subject for diagnostic or treatment purposes.

Participants will be monitored very closely by research stuff members including a physician (Dr. Chouinard ; Dr. Baker; Dr. Shinn; Dr. Murphy; one of them will be present in each steps of the study according to their availability) throughout the whole study. The presence of a qualified physician in all steps of the study will maintain the level of observation and guard against development of negative sequelae. The physician will be responsible for the safety of the subjects and interfere to prevent or reduce any unacceptable side effects. If the participants experience a poor reaction to any portion of the study, they are invited to take a break, or to withdraw completely. If they insist on continuing, but they are affected adversely, the research staff member will stop the study intervention at their discretion.

Medication: There are no published long-term studies of NR in humans which document its pharmacokinetic properties. However, we decided to give medication for 2 weeks because longer durations decrease compliance and shorter durations may lead to a failure to reach steady state concentrations in the brain and/or brain changes.

We determined the dose as 1000 mg/day. In a recent study in humans, single dose of 1000 mg/day NR did not induce any side effects despite significant increases in NAD+ and related metabolites. Moreover, 14-day use in rats at doses as high as 5g/kg/day only lead to minor weight reduction and no gross pathological changes.

To protect the participants from potential side effects or toxicities, only the participants who do not have a past or current medical condition will be recruited in this study. At the beginning of the study we will learn about participants' medical conditions and possible risk factors, via clinical evaluation, family histories, blood tests (CBC, LFT, BUN, U&E, Lipit Panel, HgA1c, Insulin, prolactin), EKG, assessment of weight, height, waist circumference, blood pressure and BMI. Participants who have any psychiatric or medical conditions will be excluded. Participants who do not have a diagnosis of any metabolic disease before but have increased levels of glucose, lipid, insulin, HgA1C and prolactin in their laboratory tests and alterations in their EKG results will also be excluded at the beginning of the study. When we identify these subjects, we will contact their primary care physician immediately; and in extreme cases, we may refer the patient to the emergency department for immediate evaluation. For subjects without a primary care physician, but not requiring emergency attention, we will refer the patient to a medical clinic.

To assess very closely the potential side effects of NR, we set up 5 separate visits on the 1st, 2nd, 5th, 9th, 14th days of medication period. A physician (Dr. Chouinard ; Dr. Baker; Dr. Shinn; Dr. Murphy; one of them will be present in each steps of the study according to their availability) will be present to monitor all participants' reactions to the medication and stop the study if they are affected adversely.

MRI/MRS Scan: With respect to the magnetic resonance scanner, we have found that most subjects find that remaining in the scanner is somewhat uncomfortable but acceptable. An MRI technologist will be able to communicate with subjects at all times via intercom. Subjects are informed that they are free to stop at any point. Approximately 4% of total studies are terminated early due to discomfort. Padding around the head will protect subjects from minor mechanical injuries and reduce head motion. Earplugs will be used to minimize the scanning noise of MR scanner.
Subjects are thoroughly screened for MR contraindications both by the research assistant and the MR technologists or authorized personnel, who

must ultimately authorize that it is safe for the subject to go into the scanner. A metal-detecting wand is used to identify any metal in or on the patient of which the patient may be unaware. If any subjects have had a surgical implant, they will not be scanned without proper medical documentation. During the MRI or MRS scans and stimulation paradigms participants are free to stop the experiment any time by communicating the personnel and the experiment will be stopped by the personnel if any adversity is observed. A qualified physician (Dr. Chouinard; Dr. Baker; Dr. Shinn; Dr. Murphy; one of them will be present in each steps of the study according to their availability) will be present in all phases of the screening process.

Women of childbearing age: The effects of nicotinamide riboside on the developing fetus and the pregnant women are not known. While there are also no known risks for fetuses, the safety of MRI for pregnant women, women of childbearing potential, and nursing mothers has not been established. Pregnant women and nursing mothers cannot participate to this study. If the participant is a woman of childbearing potential, she must be using a metal-free IUD, oral contraceptive, barrier methods or must be abstinent (for at least 1 month: time to get the most reliable result with pregnancy test) prior to this study. All women of childbearing age must have a negative blood pregnancy test (instead of urine pregnancy test) at the beginning of the study and urine pregnancy tests prior to MRI procedures. They must go on using the same method of contraception or stay abstinent (if she does not use contraception) during the study.

Blood Draw: Subjects will be monitored for 15 minutes following the blood draw to ensure they are doing well. To minimize the possibility of bruising and infection from the blood proper sterile phlebotomy techniques will be used

Psychiatric Evaluation: Some subjects might feel uncomfortable answering questions during the psychiatric evaluation. They will not be forced to answer if they do not wish to answer any particular question. If suicidality is expressed during the psychiatric interview or in response to study instruments, one of the licensed clinicians involved in the study (Dr. Shinn, Dr, Chouinard, Dr. Baker, Dr. Murphy) will immediately evaluate and refer the patient for appropriate care. In extreme cases, patient will be referred to the emergency department for immediate evaluation.

Describe explicitly the methods for ensuring the safety of subjects. Provide objective criteria for removing a subject from the study, for example, objective criteria for worsening disease/lack of improvement and/or unacceptable adverse events. The inclusion of objective drop criteria is especially important in studies designed with placebo control groups.

Participants who have any psychiatric or medical conditions will not be included in this study.

Participants who do not have a diagnosis of any metabolic disease before but have increased levels of glucose, lipid, insulin, HgA1C and prolactin in their laboratory tests and alterations in their EKG results will also be excluded at the beginning of the study.

Women of childbearing age who are not using a contraceptive method or not staying abstinent (if not using contraceptive methods) or have a positive pregnancy test at any point will not be included.

In case of determining any of these conditions in control visits during medication period, NR administration will be stopped immediately and the study will be terminated for that participant:

- Abnormal vital signs: Heart rate > 100/minute or < 60/minute, or systolic blood pressure > 160 mmHg or < 100 mmHg, or body temperature > 37.5 ^{o}C
- CBC, Chem-7 panel, LFT, U&E, BUN values outside of normal range.
- EKG abnormalities

FORESEEABLE RISKS AND DISCOMFORTS

Provide a brief description of any foreseeable risks and discomforts to subjects. Include those related to drugs/devices/procedures being studied and/or administered/performed solely for research purposes. In addition, include psychosocial risks, and risks related to privacy and confidentiality. When applicable, describe risks to a developing fetus or nursing infant.

Medication: There are no known side effects of NR in humans. A recent study investigated the genotoxicity and toxicology of NR to determine the lowest observed adverse effect level (LOAEL)) and the maximum level of intake at which no adverse health effects are observed (the no observed adverse effect level (NOAEL)), and derive the tolerable upper intake level (UL) which is the threshold above which the risk of adverse effects increase. This study found no genotoxicity at any of the doses applied for the in vitro tests or up to 2g/kg in the in vivo experiment in rats. Single dose at 5g/kg lead to no mortalities, clinical signs, or gross pathological lesions. In 14-days toxicology studies, a minimal reduction in mean body weight was observed in male rats at 2500 mg/kg/day (7–8%) and 5000 mg/kg/day (8–9%). A decrease in overall feed consumption was also observed at 5000 mg/kg/day (8%) in male rats only. 90-days toxicology experiments revealed that at 3000 mg/kg/day NR treatment lead to adverse effects in liver, kidneys, testes, epididymides and ovaries. These effects included increases in clinical chemistry parameters related to hepatocyte damage (ALT, ALP, and GGT) and a corresponding increase in liver weight, centrilobular hepatocellular hypertrophy, and single cell necrosis. In addition, thyroid follicular cell hypertrophy and increased kidney weight with exacerbation of chronic progressive nephropathy were

observed. Statistically significant but minor reductions in sodium and chloride were seen and microscopically associated with hypertrophy of zona glomerulosa in adrenals at 3000 mg/kg. NR administration at 1000 mg/kg/day dose level resulted in treatment-related organ weight changes in liver and kidney and increases in neutrophils, ALT and triglycerides, which were statistically significant in female rats only. Although these changes were considered adverse, based on their dose-dependent responsiveness, the increases in ALT and triglycerides occurred only in one gender and were below the twofold increase that is typically used as the cut-off for a biologically significant effect in the absence of histological results. The kidney weight increases at this dose also occurred in the absence of corresponding histopathology. Therefore, the liver and kidney effects at 1000 mg/kg/day were considered to be treatment related, but mild and potentially adaptive in nature due to prolonged exposure to this form of niacin. There were no treatment-related adverse effects noted at 300 mg/kg/day, although there was a slight decrease (8%) in overall body weight, which was considered adaptive. The NOAEL and LOAEL for NR were determined to 300 and 1000 mg/kg body weight/day, respectively. A UL for human exposure to NR is derived by application of a 100-fold safety factor to the NOAEL determined from this 90-day study; the UL for NR is 3 mg/kg/day or 180 mg/day, assuming a body weight of 60 kg.

In the only published human study of NR, 1000 mg/day was administered to a single human subject and increased NAD+ plasma levels up to 2.7 folds. In a subsequent experiment in the same study, 12 healthy adults used NR at 100 mg/day, 300 mg/day and 1000 mg/day single doses with 1 week wash-out periods in between. Over the total of 36 days of observation of study participants, there were no serious adverse events and no events that were dose-dependent. two individuals self-reported flushing at the 300 mg dose but not at the 100 mg or 1,000 mg dose, and two individuals self-reported feeling hot at the 1,000 mg dose but not at lower doses.

MRI/MRS Scans: In terms of the MRI and MRS scans, unlike X-rays or CAT scans, magnetic resonance (MR) technology does not use ionizing radiation. The MR system requires the use of rapidly varying magnetic gradient fields and strong radio frequency fields, which conform to the guidelines established by the US FDA for time varying magnetic fields in MR devices. This study uses a standard clinical MRI scanner (3 Tesla or 3T), as well as a high field (4 Tesla or 4T) MRI scanner. The 4T scanner is not used for routine clinical studies in children or adults, but the FDA has determined (July 14, 2003) that scanners with magnetic field strengths of less than 8 Tesla (double this scanner) or less do not represent a significant risk to adults, children, or infants aged more than 1 month. There could be adverse effects that are delayed or very mild, such that they have not yet been recognized. Most people experience no ill effects from 3T or 4T scans, but some people do report claustrophobia (fear of being in enclosed small spaces), dizziness, mild nausea, headaches, a

metallic taste in their mouth, double vision, or sensation of flashing lights. These symptoms, if present, disappear shortly after leaving the scanner. No serious adverse effects have been reported to date at any site operating at 3T or 4T field strength.

Blood Draw: Blood draw may lead to a small arm bruise and, in rare cases, clot or infection at the site the blood was drawn. Some people become light-headed during or immediately after a blood draw. These are rare occurrences and in our experience vast majority of subjects tolerate blood draws well.

Psychiatric Evaluation: In terms of the psychiatric evaluation, some subjects might feel uncomfortable answering questions, but they will not be forced to answer if they do not wish to do so. The risk of breach of confidentiality is present by extremely slight, given the precautions we take in de-identifying and protecting our data.

EXPECTED BENEFITS

Describe both the expected benefits to individual subjects participating in the research and the importance of the knowledge that may reasonably be expected to result from the study. Provide a brief, realistic summary of potential benefits to subjects, for example, "It is hoped that the treatment will result in a partial reduction in tumor size in at least 25% of the enrolled subjects." Indicate how the results of the study will benefit future patients with the disease/condition being studied and/or society, e.g., through increased knowledge of human physiology or behavior, improved safety, or technological advances.

Participants are informed that there is no direct benefit to them from participating in this study. It is hoped that future patients might benefit if the results of the study help to understand the effect of NR on brain metabolism. One potential benefit is providing new information to subjects regarding their health information. Structural images taken during the 3T research scan will be read by a radiologist. Any follow up suggested by the radiologist and clinical director of the brain imaging center will be provided at no cost to the subject.

EQUITABLE SELECTION OF SUBJECTS

The risks and benefits of the research must be fairly distributed among the populations that stand to benefit from it. No group of persons, for example, men, women, pregnant women, children, and minorities, should be categorically excluded from the research without a good scientific or ethical reason to do so. Please provide the basis for concluding that the study population is representative of the population that stands to potentially benefit from this research.

All men and women who meet the study criteria may be approached about the study. Subjects of all ethnic and socioeconomic backgrounds are included. Therefore, none of the participants are unfairly burdened with risks; nor do any populations stand to benefit to a greater degree than others.

Pregnant and nursing women are excluded from the study as the effects of NR and long- term risks of MR exposure to the fetus are unknown. For the purposes of this study, subjects who are female and menstruating must be sexually inactive or using a contraceptive measure for at least 1 month prior to entering the study. These subjects must also have a negative blood pregnancy test at study entry and negative urine pregnancy tests prior to each MRI/MRS scan.

When people who do not speak English are excluded from participation in the research, provide the scientific rationale for doing so. Individuals who do not speak English should not be denied participation in research simply because it is inconvenient to translate the consent form in different languages and to have an interpreter present.

Individuals who do not speak English will not be allowed to participate in this study.

For guidance, refer to the following Partners policy:
Obtaining and Documenting Informed Consent of Subjects who do not Speak English
https://partnershealthcare-public.sharepoint.com/ClinicalResearch/Non-English_Speaking_Subjects.1.10.pdf

RECRUITMENT PROCEDURES

Explain in detail the specific methodology that will be used to recruit subjects. Specifically address how, when, where and by whom subjects will be identified and approached about participation. Include any specific recruitment methods used to enhance recruitment of women and minorities.

For subject recruitment, the research coordinators/assistants will post online advertisements and flyers around the McLean campus; describing the study and the procedures involved. Participants who call in about the study will go through a phone screening process, during which they will be informed that everything that is said will remain entirely confidential. If the participants screen through, a mutually agreeable interview time will be set up. The participants will come in and go through the consent process and be administered the Informed Consent by a licensed physician investigator. The subject is then provided with a copy of their signed consent form to review and keep.

Provide details of remuneration, when applicable. Even when subjects may derive medical benefit from participation, it is often the case that extra hospital visits, meals at the hospital, parking fees or other inconveniences will result in additional out-of-pocket expenses related to study participation. Investigators may wish to consider providing reimbursement for such expenses when funding is available

Subjects will be compensated by check in 3-4 weeks for the procedures that they complete. The breakdown of compensation is as follows:

First Part of the Study (total: $230)

- Clinical evaluation: $30/visit
- Blood draw of 30 ml: $15/draw
- Diagnostic MR Scan, CAP protocol (MR Scan at 3T- 1): MRS protocol to quantify Glu and GABA at 3T (MR Scan at 3T- 2): $50/scan
- Phosphorous MRS/MT at 4T (MR Scan at 4T- 1): $60/scan
- Diffusion Tensor Spectroscopy (DTS) at 4T (MR Scan at 4T- 2): $60/scan
- Transportation: $5/day of visits that take place (x 3)

Second Part of the Study (total: $120):

- Medication: $5 per day of administration that takes place (x16)
- Transportation: $5/day of visits that take place (x5)
- Blood draw of 30 ml: $15/draw

Third Part of the Study (total: 225$):

- Clinical evaluation: $30/visit
- Blood draw of 30 ml: $15/draw
- CAP protocol (MR Scan at 3T- 1), MRS protocol to quantify Glu and GABA 3T (MR Scan at 3T- 2): $50/scan
- Phosphorous MRS/MT at 4T (MR Scan at 4T- 1): $60/scan
- Diffusion Tensor Spectroscopy (DTS) at 4T (MR Scan at 4T- 2): $60/scan
- Transportation: $5/day of visits that take place (x2)

If the subject does not complete the entire study he/she will be compensated for the procedures listed above that were completed. The subjects will be compensated a total of up to $ 575 for all study procedures. If the subject cannot combine the diagnostic MRI scan with the CAP 3T scan, he/she will be paid $25 for a freestanding diagnostic scan at the 3T scanner.

If the subject travel to McLean Hospital and is somehow unable to complete any part of the study, he/she will be compensated $25 for his/her time and travel expenses incurred during the trip here.

For guidance, refer to the following Partners policies:
Recruitment of Research Subjects
https://partnershealthcare-public.sharepoint.com/ClinicalResearch/Recruitment_Of_Research_Subjects.pdf

Guidelines for Advertisements for Recruiting Subjects
https://partnershealthcare-public.sharepoint.com/ClinicalResearch/Guidelines_For_Advertisements.1.11.pdf

Remuneration for Research Subjects
https://partnershealthcare-public.sharepoint.com/ClinicalResearch/Remuneration_for_Research_Subjects.pdf

CONSENT PROCEDURES

Explain in detail how, when, where, and by whom consent is obtained, and the timing of consent (i.e., how long subjects will be given to consider participation). For most studies involving more than minimal risk and all studies involving investigational drugs/devices, a licensed physician investigator must obtain informed consent. When subjects are to be enrolled from among the investigators' own patients, describe how the potential for coercion will be avoided.

For participants, consent is obtained in a private interview room. The physician (Dr. Chouinard, Dr. Baker, Dr. Shinn, Dr. Murphy; one of them will be present in each steps of the study according to their availability) and a research assistant will thoroughly review the consent form with the participant. Subjects are given as much time as they need to look over the consent before signing it. If subjects ask for time to consider participating in the study, they are given as much time as they need. Typically, the physician and the research assistant will check back the next day and/or give the subject a phone number to call if the subject is interested in participating.

NOTE: When subjects are unable to give consent due to age (minors) or impaired decision-making capacity, complete the forms for Research Involving Children as Subjects of Research and/or Research Involving Individuals with Impaired Decision-making Capacity, available on the New Submissions page on the PHRC website:
https://partnershealthcare.sharepoint.com/sites/phrmApply/aieipa/irb

For guidance, refer to the following Partners policy:
Informed Consent of Research Subjects:
https://partnershealthcare-public.sharepoint.com/ClinicalResearch/Informed_Consent_of_Research_Subjects.pdf

DATA AND SAFETY MONITORING

Describe the plan for monitoring the data to ensure the safety of subjects. The plan should include a brief description of (1) the safety and/or efficacy data that will be reviewed; (2) the planned frequency of review; and (3) who will be responsible for this review and for determining whether the research should be altered or stopped. Include a brief description of any stopping rules for the study, when appropriate. Depending upon the risk, size and complexity of the

study, the investigator, an expert group, an independent Data and Safety Monitoring Board (DSMB) or others might be assigned primary responsibility for this monitoring activity.

NOTE: Regardless of data and safety monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for protecting the rights, safety, and welfare of subjects under his/her care.

Subjects are thoroughly screened by a research assistant and a qualified physician (Dr. Chouinard; Dr. Baker; Dr. Shinn; Dr. Murphy; one of them will be present in each steps of the study according to their availability) prior to their enrollment in the study to ensure subject safety. The physician closely monitors the participants during the medication period. Subjects are carefully assessed before, during, and after the MRI scans by the research assistant and MR technologist or authorized personnel to ensure that they suffer no adverse reactions. The physician will be present in all phases of the screening process. Subjects are carefully assessed before, during, and after the blood draw by the research assistant to ensure that they suffer no adverse reactions. Participants may experience an emotional reaction in response to the clinical evaluation portion of the study; however, this risk is mitigated by the investigators' offering the subjects the possibility to take a break or withdraw from the study. The PI closely oversees all of the study activities and intervenes wherever necessary.

Describe the plan to be followed by the Principal Investigator/study staff for review of adverse events experienced by subjects under his/her care, and when applicable, for review of sponsor safety reports and DSMB reports. Describe the plan for reporting adverse events to the sponsor and the Partners' IRB and, when applicable, for submitting sponsor safety reports and DSMB reports to the Partners' IRBs. When the investigator is also the sponsor of the IND/IDE, include the plan for reporting of adverse events to the FDA and, when applicable, to investigators at other sites.

NOTE: In addition to the adverse event reporting requirements of the sponsor, the principal investigator must follow the Partners Human Research Committee guidelines for Adverse Event Reporting

As soon as a member of research staff, or the PI, learns of an adverse event, he/she will first consider the safety of the participant. If the participant is not safe the member of the research staff/PI will take appropriate steps to stabilize the subject. Members of the research staff will defer to the PI's judgment. If the participant is safe, then the person who learned of the event will call the PI and/or other members of the research staff who had contact with the participant. They will decide what course of action to take with the participant in order to deal with the event. Principal investigator will then submit an adverse event report to the PHRC within 5 working days/7 calendar days of the date that the investigator first becomes aware of the problem.

MONITORING AND QUALITY ASSURANCE

Describe the plan to be followed by the principal investigator/study staff to monitor and assure the validity and integrity of the data and adherence to the IRB-approved protocol. Specify who will be responsible for monitoring, and the planned frequency of monitoring. For example, specify who will review the accuracy and completeness of case report form entries, source documents, and informed consent.

NOTE: Regardless of monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for ensuring that the study is conducted at his/her investigative site in accordance with the IRB-approved protocol, and applicable regulations and requirements of the IRB.

Dost Ongur, the Principal Investigator (PI), will be responsible for monitoring the validity and integrity of the data, as well as adherence to the protocol. The study coordinator and PI will report any deviations from the protocol immediately to the IRB.

For guidance, refer to the following Partners policies:

Data and Safety Monitoring Plans and Quality Assurance
https://partnershealthcare-public.sharepoint.com/ClinicalResearch/DSMP_in_Human_Subjects_Research.pdf

Reporting Unanticipated Problems (including Adverse Events)
https://partnershealthcare-public.sharepoint.com/ClinicalResearch/Reporting_Unanticipated_Problems_including_Adverse_Events.pdf

PRIVACY AND CONFIDENTIALITY

Describe methods used to protect the privacy of subjects and maintain confidentiality of data collected. This typically includes such practices as substituting codes for names and/or medical record numbers; removing face sheets or other identifiers from completed surveys/questionnaires; proper disposal of printed computer data; limited access to study data; use of password-protected computer databases; training for research staff on the importance of confidentiality of data, and storing research records in a secure location.

NOTE: Additional measures, such as obtaining a Certificate of Confidentiality, should be considered and are strongly encouraged when the research involves the collection of sensitive data, such as sexual, criminal or illegal behaviors.

All subject scans and clinical data are coded with research numbers that do not contain any identifying information. The code linking the research number to personal identifying information is stored in a password-protected encrypted computer file on a locked computer in a locked office at McLean. Information about the subjects" diagnoses, medical and other relevant study

information will be maintained in computers accessible only to the investigators and research staff that they designate. The data may be maintained for an indefinite period of time since scientific progress may indicate that new analyses be carried out on previously obtained data. The data will be collected and stored via the secure web-based application REDCap. The application complies with HIPAA regulations and will store the information securely, protected by web authentication, and Secure Sockets Layer (SSL) encryption. Results of the research specific to any subject will not be provided to anyone including the subject, except for in the case where the subject"s structural scan is abnormal. In this case, the PI is required to inform the subject of the abnormal scan and schedule the necessary follow-ups cans at McLean. Blood samples will be encoded and only Dr. Öngür and his research staff will have access to the code key. If third-party uses for blood samples are planned, we will submit a proposal for such uses for IRB review and approval prior to initiating them. The code key linking the sample to personal identifiers will never be provided to such third parties and third-party investigators will have to agree not to attempt to gain access to such identifiers.

SENDING SPECIMENS/DATA TO RESEARCH COLLABORATORS OUTSIDE PARTNERS

Specimens or data collected by Partners investigators will be sent to research collaborators outside Partners, indicate to whom specimens/data will be sent, what information will be sent, and whether the specimens/data will contain identifiers that could be used by the outside collaborators to link the specimens/data to individual subjects.

N/A

Specifically address whether specimens/data will be stored at collaborating sites outside Partners for future use not described in the protocol. Include whether subjects can withdraw their specimens/data, and how they would do so. When appropriate, submit documentation of IRB approval from the recipient institution.

None of the samples will be used for a future use not yet described in the protocol, as of now. But science is always changing and such uses may emerge in the future. If there was a future use, a request would be submitted to the IRB for permission. Subjects can choose to withdraw their sample at any point in time from analysis. If a subject wishes to withdraw their sample, they will be instructed to write and sign a letter to that effect.

RECEIVING SPECIMENS/DATA FROM RESEARCH COLLABORATORS OUTSIDE PARTNERS

When specimens or data collected by research collaborators outside Partners will be sent to Partners investigators, indicate from where the specimens/data will be obtained and whether the specimens/data will contain identifiers that could be used by Partners investigators to link the specimens/data to individual subjects. When appropriate, submit documentation of IRB approval and a copy of the IRB-approved consent form from the institution where the specimens/data were collected.

N/A