PROTOCOL TITLE: INTRANASAL INSULIN FOR IMPROVING COGNITIVE FUNCTION IN MULTIPLE SCLEROSIS

DATE: November 20, 2020

VERSION: 2.4

SYNOPSIS

This will be a randomized, double-blind, placebo-controlled phase I/II pilot study. Participants will be randomized to intranasal insulin 10 international units (IU) twice a day, 20 IU twice a day, or placebo for 24 weeks. Insulin will be administered intranasally using the novel ViaNase™ controlled particle dispersion system, allowing for direct delivery of the medication to the nasal epithelium, leading to maximal transport to the CNS (Kurve Technology, Lynnwood, WA).[34] Cognitive assessments will occur at baseline and throughout the 24-week trial, as well as for a period of 24 weeks after discontinuation of the intervention, to evaluate the impact of insulin on cognitive performance as well as the longevity of the treatment response. Biomarkers of cellular stress, neuronal injury, oxidative stress, inflammation, and energy metabolism will be assessed at baseline and at subsequent visits. Stored specimens will be acquired for more detailed future mechanistic studies if intranasal insulin does appear to be safe and effective.

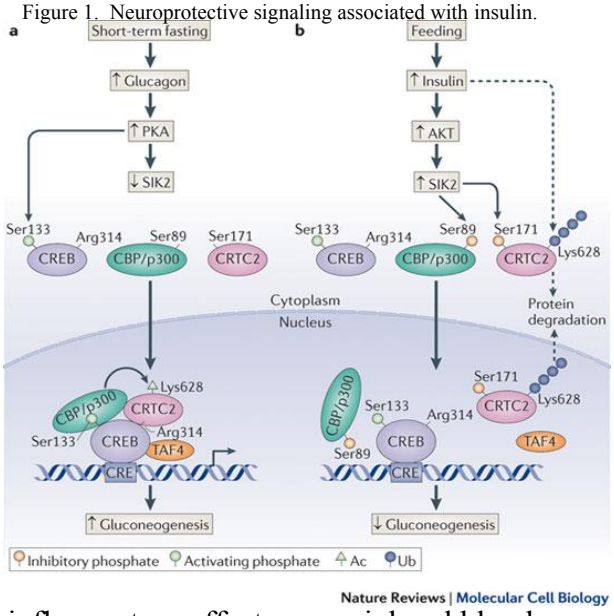
INTRODUCTION

Cognitive impairment is common in and devastating to people with MS. MS is a common, chronic, central nervous system (CNS) disease characterized by inflammation, demyelination, and neurodegeneration. One of the most devastating symptoms of this disease is impaired cognitive function, which is common and present in over 60% of individuals with MS.[1-3] MS-related cognitive impairment is associated with lowered quality of life [4] and reduced functional capacity, including loss of employment,[4-7] impaired social relationships,[8] compromised driving safety,[7] and poor adherence to treatment.[9] Impaired cognitive functioning has been observed early in the disease, sometimes even before diagnosis,[10] and cognitive function has been shown to decline longitudinally, both over the short- and long-term.[11-13] Several cognitive domains are impacted in people with MS, including attention, memory, executive functioning, and especially processing speed.[2, 10, 14-18]

To date, multiple pharmacologic interventions have been assessed with disappointing results. There was no significant difference between treatment and placebo for cognition in randomized control trials of donepezil,[19] aminopyridines,[20] gingko biloba,[21] and memantine.[22] Psychostimulants demonstrated some efficacy, but only in secondary outcome measures.[23] Behavioral interventions show promise but are understudied.[24] Furthermore, cognitive rehabilitation is often time consuming, costly, and not universally available. ***Hence, there is an urgent need to identify or develop novel therapies that can help improve cognitive function in MS.***

Insulin is critical for helping with regulation of multiple CNS functions including brain metabolism, neurite outgrowth, neurotransmitter channel activity, neuronal survival, and learning and memory.[25 ,26] There are insulin receptors throughout the brain, with robust concentrations located in eloquent areas including the olfactory bulb, hippocampus, cerebral cortex, and cerebellum. Insulin is present at high levels in the brain and when these levels are decreased, there may be learning and memory impairments.[25, 27, 28] The cognitive impairment that can ensue in the context of low brain insulin levels may be related to its ability to protect neurons from various insults including oxidative stress, ischemia, and glutamate-related excitotoxicity.[25] Moreover, insulin's anti-inflammatory effects, as detailed below, may also impact brain health via suppressing concentrations of chemokines, cytokines, and other molecules that may provoke ongoing CNS inflammation and damage in disease states.

Figure 1. Neuroprotective signaling associated with insulin.



Insulin has biologically relevant anti-inflammatory and neuroprotective CNS effects. At the cellular level, insulin regulates various neuronal functions including receptor expression, trafficking, and survival pathway activation. Insulin signaling in neurons exerts a neuroprotective effect, especially under oxidative stress, via the phosphoinositide-3 kinase (PI3K/AKT) pathway (Figure 1) which, downstream, induces the cAMP response element binding protein (CREB). CREB regulates the expression of several genes that are crucial for learning and memory, including brain derived neurotrophic factor (BDNF),[29] an important promoter of neuronal survival pathways, resulting in regulating learning and memory. This neuroprotective effect has been seen in models of Alzheimer's, Parkinson's, and Huntington's Diseases.[30] Insulin also exerts anti-inflammatory effects on peripheral blood

mononuclear cells (PBMCs) by altering inflammatory markers (e.g. monocyte chemoattractant protein-1, chemokine receptor-5) that could ultimately help minimize ongoing nervous system injury.[25]

Insulin's ability to regulate energy metabolism could also result in a protective effect on neurons via impacting oxidative stress and excessive energy demands. Insulin appears to directly mediate brain glucose metabolism maximally within the cortex, and with any disturbance in the communication between insulin and neuronal glucose metabolism, there may be impaired adenosine triphosphate (ATP) synthesis followed by neuronal apoptosis.[31, 32] Moreover, the oxidative stress that results from excessive energy demand leads not only to the production of reactive oxygen species (ROS), but also to ceramide production. These signaling pathways are connected and may be altered upstream by insulin's impact on brain glucose metabolism. For example, several apoptosis-inducing treatments both activate the sphingomyelin (SM)-ceramide pathway and generate oxidative stress.[33] Inflammatory cytokines relevant to MS (e.g. tumor necrosis factor [TNF]-α) can also induce ceramide formation and ROS. Chemical scavengers prevent elevations in ROS and also SM-ceramide pathway activation. Finally, the use of pyrrolidine dithiocarbamate or N-acetyl cysteine, both antioxidants, restricts ceramide formation and cell death due to TNF-α in rat astrocytes or human breast carcinoma cells.

The impact of insulin in the nervous system, especially as it relates to cognitive health, deserves more attention, as it may be a treatment for disease that cause CNS dysfunction from several pathologies, including MS.

Insulin administration has been shown to improve memory and learning in healthy people and in those with neurodegenerative diseases (see Table 1). Intranasal insulin has shown to have neuroprotective and restorative effects in several human clinical trials. In a recent randomized, double-blind, placebo-controlled study with over 100 people suffering from amnestic mild cognitive impairment or Alzheimer's Disease, intranasal insulin preserved cognitive function in both selective cognitive domains and more globally compared to placebo.[34] The insulin-treated patients also did not show a decline in measures of brain metabolism, whereas the placebo-treated participants did show decreases in fluorodeoxyglucose ^{18}F uptake in various lobes (parietotemporal, frontal, precuneus, and cuneus). Further, improvements in episodic memory persisted for several weeks after treatment cessation. Overall, these findings suggest that intranasal insulin not only affects cognitive function acutely, but that over time, there may be associated structural changes that lead to a more permanent treatment benefit. Since cognitive dysfunction is very common in MS and can be devastating, a treatment intervention (i.e., intranasal insulin) that can help both acutely and longitudinally is worth pursuing.

Table 1. Summary of past and ongoing human intranasal insulin trials. *Adapted from [34-50]*

REF.	INTRANASAL INSULIN DOSE	SUBJECTS	ASSESSMENT
Benedict 2004	160 IU (long-term)	Healthy	Word list (immediate, delayed recall
Reger 2006	20 or 40 IU (acute)	Probable AD or MCI vs. healthy	Story recall (immediate, delayed recall) Word list (immediate, delayed recall)
Benedict 2007	20 IU Aspart vs. 20 IU Regular (long term)	Healthy men	Word list (immediate recall) Word list (delayed recall)
Benedict 2008	160 IU (acute)	Healthy, normal weight, no meds	Digit span, object location (immediate recall) Mirror tracing (immediate recall)
Hallschmid 2008	160 IU (long-term)	Obese men	Word list (delayed recall) Word list (immediate recall)
Reger 2008	20 IU (long term)	AD or MCI	Memory score (immediate/ delayed recall ratio) Voice onset time (immediate/delayed recall ratio)
Reger 2008	10, 20, 40, 60 IU (acute)	AD or MCI vs. healthy	Story recall (immediate recall or delayed recall) Word list learning (immediate & delayed recall)

Krug 2010	160 IU (acute)	Healthy women	Digit span (immediate recall) Object location(immediate recall)
Fan 2012	I40 IU (acute)	Schizophrenic	Hopkins Verbal Learning Test
Craft 2012	10 or 20 IU BID	AD or MCI	Verbal Memory Composite
Craft 2012	20 or 40 IU	AD or MCI	Story recall (delayed recall)
McIntyre 2012	40 IU (long term)	Euthymic with bipolar disorder	California Verbal Learning Test, second edition Process Dissociation Task
Burns 2012	40 IU (acute)	Early AD	Functional MRI activation; cognitive battery
Novak 2013	40 IU (long term)	Diabetic	Brief Visuo-spatial Memory Test-Revised Verbal fluency measures
Fan 2013	40 IU (long term)	Schizophrenic	Cognitive battery
Craft 2013	20 IU BID	AD or MCI	Cognitive battery
Haley 2013	20 IU	AD	Cerebral glutamate levels; cognitive battery

AD= Alzheimer's Disease; MCI=mild cognitive impairment; IU=international units; BID=twice daily

Intranasal insulin is extremely safe and tolerable in other populations, allowing for concentrated delivery to the nervous system. Intranasal insulin has a long-standing safety track record that spans more than a decade. *No significant adverse events were reported in the largest intranasal insulin study to date or in any other study that has used intranasal insulin* (see Table 1). An intranasal delivery system provides a non-invasive way to bypass the blood-brain barrier and allow rapid delivery of a medication to the CNS via the olfactory and trigeminal perivascular channels.[34] The main advantage of the delivery system is reducing systemic side effects via limiting a medication's exposure to peripheral organs and tissues. In addition, first-pass metabolism is avoided. Intranasal insulin, as seen in previous human studies, does not adversely affect systemic (blood) glucose or insulin, and it increases CSF insulin to biologically protective concentrations within 30-40 minutes.[51]

Intranasal Insulin for Cognition in MS is an Innovative Treatment Approach-Summary

Insulin appears to activate biologically relevant pathways within the nervous system for regulating and improving learning and memory. **The direct and indirect effects of insulin on energy metabolism, oxidative stress, and modulation of inflammatory and neuronal pathways, along with the data from clinical trials in people with other neurodegenerative disorders, prove the rationale for investigating it in people with MS who suffer with cognitive impairment.** As noted above, reduced levels of insulin and/or insulin activity may contribute to a number of pathological processes that may ultimately lead to long-term disability in neurodegenerative diseases. Restoring brain insulin to normal levels and increasing insulin sensitivity in the brain may provide therapeutic benefit to people with MS. Herein, we thus propose the first clinical trial in MS to determine the safety and tolerability as well as the effectiveness of intranasal insulin as a therapeutic intervention for cognitive dysfunction. Our study will investigate associated changes in putative biomarkers (see Preliminary Data below) that may corroborate clinical outcomes and provide data regarding the mechanisms by which insulin acts. These measures, along with neuropsychological testing, will be incorporated with safety data in order to accurately assess the potential utility of intranasal insulin for MS cognitive impairment.

OBJECTIVES/AIMS

Primary Objective: To evaluate the safety and tolerability of intranasal insulin in people with MS

Secondary Objectives: The main secondary objective is to evaluate if intranasal insulin improves cognition in people with MS, as assessed by the SDMT. Additional secondary objectives are to determine if intranasal insulin is associated with changes in additional components of the Minimal Assessment of Cognitive Function in MS (MACFIMS), the Multiple Sclerosis Functional Composite (MSFC) score, health-related quality of life

(Functional Assessment of Multiple Sclerosis [FAMS]), the Expanded Disability Status Scale (EDSS) score, and changes in biomarkers of oxidative stress, neuronal injury, cellular stress, and metabolism.

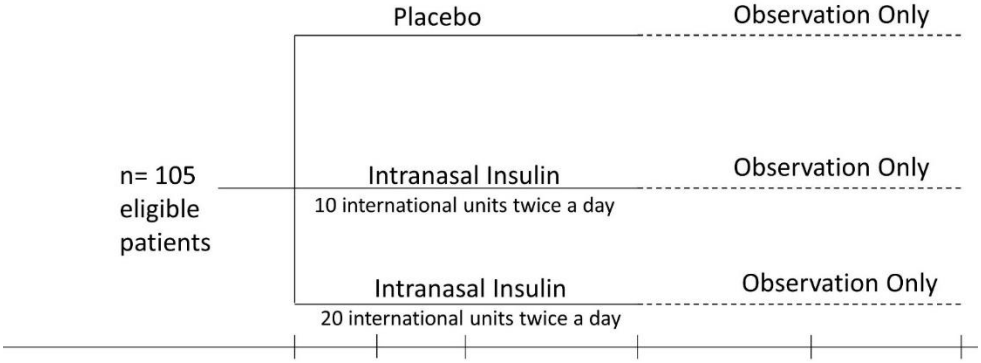
Aim 1. To evaluate the safety and tolerability of intranasal insulin in people with MS.
Hypothesis: Intranasal insulin has been shown to be safe in a pilot clinical trial of patients with mild cognitive impairment and Alzheimer's Disease, but its safety and tolerability in MS patients are unknown. *We hypothesize that intranasal insulin will be safe and tolerable for people with MS.*

Aim 2. To evaluate if intranasal insulin improves cognition in people with MS.
Hypothesis: Intranasal insulin has been shown to improve multiple aspects of cognition in several randomized, blinded clinical trials of healthy people and in those with mild cognitive impairment and Alzheimer's Disease. *We hypothesize that intranasal insulin will improve cognition in MS patients who are cognitively impaired.*

Aim 3. To evaluate the impact of intranasal insulin on measures of oxidative stress, axonal injury, cellular stress, and energy metabolism in MS.
Hypothesis: Although there is not a single biomarker for MS, there are some surrogate measures of interest that may well be impacted by treatment with insulin. We will perform an exploratory analysis to evaluate the effect that intranasal insulin has on these measures, with the hypothesis that *intranasal insulin will improve markers of oxidative and cellular stress, neuronal injury, metabolism, and inflammation in people with MS.*

STUDY DESIGN

Design: The design of this phase II, randomized, double-blind, placebo-controlled trial is as follows:



Week	0	6	12	24	36	48
Study visit	*	*	*	*	*	*
MACFIMS	*		*	*	*	*
Biomarkers	*	*	*	*	*	*

Participants: The eligibility criteria for the trial are provided in the table below:

Eligibility Criteria	Rationale
Meets 2010 criteria for multiple sclerosis	Study is focused on individuals with MS
No relapse in past 3 months	To avoid relapse-induced cognition confounding
Age 18-70 years	To ensure results generalizable
At least mild cognitive impairment (1.0 standard deviation or greater below the published mean SDMT z-score, or a score of <34 on the processing speed test [PST]	To ensure that we are able to detect improvements in cognition if insulin does in fact have this effect

Capacity to learn and self-administer intranasal insulin, or presence of a caregiver with such capacity who is willing to do it for the duration of the trial	To ensure that subjects are able to take the medication as prescribed
Untreated/on the same MS therapy for at least 2 months, with no anticipated change in the next year	To avoid change in measures due to change in medication
No current, active major depression	May impact results of cognitive testing
If on tricyclic antidepressant or anticonvulsant, on stable dose for 6 weeks or more; if on oxybutynin or tolterodine, on stable dose for > 6 months without plans for changing dose in next year	May impact results of cognitive testing
If taking selective serotonin (± norepinephrine) reuptake inhibitors, pregabalin, gabapentin, sympathomimetic, monoamine oxidase inhibitor, antipsychotic, amantadine, cholinesterase inhibitor, memantine, modafanil, armodafinil, or evening short-acting benzodiazepines, on stable dose for 6 weeks or greater	To minimize impact on cognitive testing without impairing recruitment/limiting generalizability (MS patients use these therapies commonly)
Not pregnant or nursing, and willing to prevent pregnancy during study if of childbearing potential	Risks of intervention to developing fetus or breastfeeding infant unknown
No or stable THC use in past 6 weeks; no other illicit drug or alcohol abuse in past 3 months*	To ensure participants can adhere to treatment/ avoid impact of substances on cognitive testing
No known history of diabetes mellitus or insulin resistance	To avoid confounding due to global glucose dysregulation or use of systemic insulin
No active liver disease, stage IV/V kidney disease or severe metabolic derangements	To prevent differential metabolism of insulin
No CNS disorder other than MS or headache	To avoid confounding due to insulin effect on other disorder
No issue making participation not in best interest of patient	To prevent any undue harm to patient
During COVID-19 pandemic and related periods of slowdown or shutdown, willing to comply with state/local recommended social distancing and other suggested COVID-19 related safety measures. Additionally, if female of childbearing potential, to prevent pregnancy	To ensure minimization of risks to participants

Recruitment: Eligible subjects with MS will be recruited from the Johns Hopkins MS Center, at which over 3,000 MS patients, who come from diverse racial/ethnic and socioeconomic backgrounds and from a wide catchment area, are seen annually. We will also screen potentially eligible subjects using an IRB-approved Telephone Screening Script. This will allow us to screen patients who may or may not meet the study's eligibility criteria. If a patient meets the eligibility criteria via telephone screening, we will schedule the patient to come in for a baseline visit. The study coordinator will call only patients of physicians on the study team. Patients interested in participating will be emailed or mailed the written consent form to review in advance.

Since we began routinely administering the iPAD-based correlate of the SDMT, the Processing Speed Test (PST) for all MS Center patients since early 2017 and impaired cognition based on the SDMT is one of the main eligibility criteria, we will quickly be able to focus recruitment efforts on those who have evidence of mild cognitive impairment, eliminating the need for an extra screening visit. More than 80 people with MS who are currently enrolled in various studies in the center already have SDMT data and thus will also be easily screened

for eligibility, even if they haven't had a recent clinic visit. For MS Center patients that have not taken an SDMT or PST test, we will use an oral consent process to administer the SDMT test and determine eligibility. SDMT or PST test results obtained within 3 months of the baseline visit will be considered as proof of eligibility for the study and may be screened in person or by phone. The study coordinator will only approach MS Center patients of physicians on the study team or those referred by their treating physician. In addition to the above recruitment strategies, we will obtain home-based PST screening test for our MS patients that have iPADs (Air, Air 2, and iPAD 2017), as the PST app is now available. This will allow for remotely screening potential subjects that have transportation and/or mobility limitations. If at any time point, enrollment is less than 85% of the target, we will interview subjects who have declined to participate to determine the deterrents to enrollment; we will then implement alterations in recruitment needed to enhance enrollment.

Study Treatment: Intranasal insulin (Novolin R, Novo Nordisk, Princeton, NJ) will be evaluated in two doses (10 IU twice a day, 20 IU twice a day) and compared to the sterile diluent (Eli Lilly and Company – NDC:00002-0800) (1:1:1 randomization, stratified by relapsing versus progressive MS). The 20 IU dose will consist of insulin. The 10 IU dose will be a diluted mixture comprised of normal saline and insulin. The study drug, insulin, has a distinct smell. In order to preserve study blinding, we will use the Lilly sterile diluent, which has a similar odor as the insulin, to preserve blinding. The study drug and placebo (Lilly diluent) will be prepared by the Johns Hopkins Investigational Drug Service and will appear identical to maintain blinding. A nasal drug delivery device (Kurve Technology, Lynnwood, WA) will be utilized to ensure optimal exposure of the olfactory epithelium. The treatment regimen was chosen to match that of a recent successful pilot study in Alzheimer's Disease.[34] Evaluating two doses will allow for the possibility that any benefit of insulin may be non-linear.

Treatment Preparation: The study pharmacist will transfer either insulin; sterile diluent (placebo); or normal saline into 10cc empty syringes to preserve study blinding. The syringes will be labeled syringe A or syringe B, respectively. At the baseline visit, subjects and/or caregivers will be instructed on how to fill the cartridges by adding one A and one B syringe to the cartridge; gently mix the contents and be shown how to administer the doses using the ViaNase device. Due to the 28-day shelf life of the insulin/diluent, the subjects need to know how to refill their own study products prior to their next study visit.

Methods to Maximize Adherence: Given data from prior studies, it is anticipated that adverse events will not reduce adherence. The study coordinator will help each participant set reminder alarms on watches or phones; those who do not have access to such devices will be given a medication alarm watch for use during the study. Study staff will also identify a patient advocate and to educate that person to help the study participant maintain adherence. To maximize visit adherence, patients will receive a reminder in the week prior to the appointment.

Accountability of Medication: A sufficient amount of medication will be supplied at the baselineand week 12 visits to ensure the supply will last until the next study visit, otherwise, refills will be mailed to the participants. Participants will be asked to return unused medication at each study visit for adherence assessment. The Johns Hopkins Investigational Drug Unit will maintain adequate records of the disposition of the study drug and accounts of any destroyed study drug.

Study Personnel: All personnel (except the statistician) will be blinded to treatment assignment. The treating physician will assess eligibility criteria, obtain informed consent, obtain and maintain the medical history and medication record, monitor patients' safety, conduct the physical examination, and evaluate and report adverse events. The examining physician will perform the blinded EDSS. The blinded study neuropsychologist will administer the MACFIMS to participants. The blinded study coordinator will be responsible for the study's administrative duties, obtaining vital signs, conducting the MSFC and administering the depression, suicidality, and health-related quality of life measures, and drawing and processing blood samples as well as urine

pregnancy testing, when indicated. The laboratory technician will conduct the biomarker analyses for Aim 3. Other personnel include Drs. Haughey and Calabresi, who will interpret biomarker data. A statistician from the Johns Hopkins School of Public Health will create the randomization schedule and perform final data analyses.

Study Visits: The visit schedule is detailed below. Visits will take place within ± 5 days of the target date.

	Week 0	Week 6	Week 12	Week 24	Week 36	Week 48
Written consent	X					
Verify eligibility	X					
Teaching and First dose administration and observation	X					
Medical history, relapse assessment	X	X	X	X	X	X
Medication review	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X
Physical, neurologic (EDSS) exams	X		X*	X*		X*
MS Functional Composite	X	X	X	X	X	X
Adverse event assessment		X	X	X	X	X
Biomarker evaluation	X	X	X	X	X	X
Stored blood for future studies	X	X	X	X	X	X
DXA scan	X					
A pregnancy test (if woman of childbearing potential)	X	X	X	X		
MACFIMS battery	X		X	X	X	X
Sleep questionnaires	X		X	X		X
Depression and quality of life	X	X	X	X	X	X
Suicidality evaluation	X	X	X	X	X	X

*EDSS only

Schedule Modification during COVID-19 (will be in effect unless JHU operations have returned to normal, a COVID-19 vaccine has been developed, or evidence of substantial likelihood of herd immunity from COVID-19 due to a high proportion of previously-infected individuals). Additionally, due to COVID-19 and staffing shortages during the holiday season, we will allow study visits within ± 10 days of the target date if needed to ensure the safety of the participants.

	Week 0	Week 6: Virtual	Week 12**	Week 24	Week 36: Virtual	Week 48: Virtual[%]
Written consent[&]	X					
Verify eligibility	X					
Teaching and First dose administration and observation	X					
Medical history, relapse assessment	X	X	X	X	X	X
Medication review	X	X	X	X	X	X
Vital signs	X		X	X		
Neurologic (EDSS) exam	X					
MS Functional Composite	X		X***	X***		

Adverse event assessment		X	X	X	X	X
Biomarker evaluation	X		X	X		
Stored blood for future studies	X[$]		X[$]	X[$]		
DXA scan	X					
Pregnancy assessment*	X*	X	X	X		
MACFIMS battery	X		X	X		X
Sleep questionnaires	X		X	X		X
Depression and quality of life	X	X	X	X	X	X
Suicidality evaluation	X	X	X	X	X	X

*Pregnancy test at baseline; document last menstrual period/adequate pregnancy prevention plan at virtual follow-ups (for women of childbearing potential)
**will convert to virtual follow-up with activities equal to week 6 if stay-at-home orders recur
Items that are in **bold** will be done either virtually or in-person until operations are fully normalized with respect to COVID-19 pandemic; in addition, some aspects of the MACFIMS neuropsychological battery can (and will) be done virtually as well
***timed 25-foot walk only
[&]Consent will be reviewed at length virtually within the week prior to the day of the visit, with all questions asked at that time. At the baseline visit, patients will be given the opportunity to ask any remaining questions and can sign during the teleconsent, if capable, or will sign at the time of the baseline visit.
% if COVID-19 pandemic is lifted, we will still complete this visit in-person; we will accept a study window that extends 3 months beyond week 48 if there is indication at that time, even if the pandemic is still affecting safety, that it will soon be safe again (e.g. vaccine developed or clear evidence of critical percentage of population has already been infected, fully operational at JHU)

If a severe COVID-19 outbreak occurs again such as occurred in the spring of 2020, we will conduct all follow-up assessments by video or phone until such time as it is safe to pursue the modified schedule, as above, or resume the originally-planned schedule.

All study visits will be scheduled for the morning to reduce variability in cognitive function related to the time of day. At baseline, fasting laboratory testing will be performed, including a hemoglobin A1c test and insulin level. All subjects will then be given breakfast prior to completing cognitive testing. For follow-up visits, all subjects will complete the MACFIMS battery after eating a usual meal (if the participant came without having eaten, breakfast will be provided to them) and prior to taking the morning dose of study treatment. Unscheduled visits will occur if any safety issue arises.

In order to ensure subjects (or their responsible caregivers) are able to use the medication as instructed, all subjects and caregivers (when relevant) will be instructed on the use of the inhaler and will be observed by a health care provider when performing their first dose administration at the time of the first visit.

Risks

Intranasal insulin

Intranasal insulin has been shown to be safe and tolerable in other populations and patient groups, but it has never been studied in people with MS. The most common side effects related to irritation of the nasal passages. Giving insulin through the nose did not result in lower circulating glucose levels in other studies, but the first 15

people in this study will be monitored for this potential risk by testing a fingerstick blood glucose level periodically for 90 minutes after the first dose of intranasal insulin (see **Safety** section under **Outcomes** on page 10).

Insulin, given by the more traditional subcutaneous route, has the following potential risks:

Hypoglycemia: Common symptoms of hypoglycemia include sweating, dizziness or lightheadedness, shakiness, hunger, a fast heartbeat, tingling of the hands, feet, lips or tongue, trouble concentrating, confusion, blurry vision, slurred speech, anxiety, irritability, or mood changes, or headache. Very low blood sugar can cause loss of consciousness, seizures, or temporary or permanent brain problems or death. Mild hypoglycemia can be treated by drinking or eating something sugary right away (fruit juice, sugar candies, or glucose tablets).

Hypokalemia: Hypokalemia can lead to breathing problems, low heartbeat, or death.

Allergic reaction: Common symptoms of an allergic reaction include a rash all over the body, trouble breathing, a fast heartbeat, sweating, and feeling faint.

Weight gain or swelling of the arms and legs.

While these are all risks potentially associated with subcutaneous administration of insulin, none of these risks has been demonstrated to be the case in the published trials of intranasal insulin. Thus, by avoiding the traditional route of administration, it is anticipated that these risks will be minimized.

Blood Draw
Taking blood may cause discomfort, bleeding or bruising where the needle enters the body. In rare cases, it may result in fainting. There is a small risk of infection. To minimize the risks, blood will be drawn by a trained study team member/phlebotomist.

Confidentiality
Despite the best efforts of the research team, there may still be a risk if information about participants were to become known to people outside of this study.

Questionnaires
Participants may get tired or bored when we are asking questions or they are completing questionnaires. They do not have to answer any question they do not want to answer.

DXA scan
DXA testing is painless and involves exposure to radiation. This research study includes exposure to radiation from x-rays or gamma rays. This radiation exposure is for research purposes only and is not part of the participant's medical care. X-rays and gamma rays can damage cells, but at low doses, the body is usually able to repair these cells.

The radiation exposure that participants will get in this research study is 0.001 rem. This is less than the 0.3 rem that the average person in the United States gets each year from natural sources like the sun, outer space, air, food, and soil. The risk from the radiation exposure in this research study is very small. The radiation exposure described here is what participants will get from this research study only. It does not include any exposure

participants may have received or will receive from other medical tests outside of this study that are a part of their medical care.

Benefits
There is no known benefit of intranasal insulin, and thus there is no known risk of being in the placebo group. All patients who are already on a medication for MS will be allowed to continue that medication during the study.

Payment and Remuneration
Participants will receive a parking coupon at each visit.

Costs
There are no costs to participants for taking part in this study.

Outcomes:

Safety Since this medication was extremely well-tolerated in several published studies,[34-50] we anticipate the same will be true for people with MS. In prior studies, there was no effect of intranasal insulin on serum glucose levels. However, we will monitor the first 15 participants for a period of 90 minutes after their first dose of the medication to evaluate serial finger stick blood glucose levels. If there is a clinically significant reduction in these levels, we will convene a Data Safety Monitoring Board (DSMB) meeting to adjust the protocol. We will also record and, when appropriate, report all patient-reported adverse events, as described below. Subjects and their caregivers will be required to keep an electronic diary of adverse events, which will be reviewed at each study visit. As mandated by the FDA, the Columbia Suicide Severity Rating Scale will be administered at each study visit.

Cognitive Assessments The MACFIMS battery includes the following: The Symbol Digit Modalities Test (SDMT**)** is commonly used in MS to assess processing speed.[1, 52-54] The oral version of the test is recommended for MS since the written version may be confounded by upper extremity weakness.[53, 55, 56] There are three alternate versions of this test with strong psychometric properties.[37] A 3.5-4 point raw score difference on the SDMT has been identified as clinically meaningful.[6, 57] The Controlled Oral Word Association Test (COWAT) measures phonemic fluency.[58] The California Verbal Learning Test, Second Edition (CVLT-II**)** is a verbal learning and memory test.[55, 59] The Brief Visuospatial Memory Test – Revised (BVMT-R**)** is a visual, nonverbal test of learning and memory.[60] The Rao-version of the Paced Auditory Serial Addition Test (PASAT) evaluates processing speed, working memory, and basic addition skills.[58] Visual-spatial abilities are assessed with the Judgement of Line Orientation Test (JLO).[58, 61] The Delis-Kaplan Executive Function System (DKEFS) tests executive functioning, concept formation, and cognitive flexibility.

Exploratory Biomarkers Plasma and/or PBMCs will be collected at baseline, week 12, and week 24 visits (except if COVID-19 related shutdown does not permit follow-up blood collection or lab-based processing) and will be isolated and cryopreserved for batch analyses in the labs of Dr. Haughey or Calabresi. Inflammatory (cytokines, lymphocyte subsets), metabolic (Kreb's cycle substrate, fatty acids), cellular stress (ceramides, sphingomyelin) and neurotrophic (BDNF) measures, as well as markers of oxidative stress (protein carbonyls, 8-isoprostane, nitrotyrosine, and 4-hydroxynonenal adducts [lysine and histidine]), will be quantified. Since our data show oxidative stress biomarkers and elevated ceramides in plasma of MS patients and recent work shows

the brain has lymphatics, explaining how brain metabolites return to the periphery, blood biomarkers are a relevant and convenient way to monitor CNS metabolism.[62]

Additional Outcomes and Assessment of Covariates In order to identify and, if needed, adjust for comorbid depression, the Beck Depression Inventory will be administered at each study visit. Health-related quality of life will also be assessed utilizing the FAMS. We will also evaluate how overall sleep quality in people with MS impacts health-related quality of life. We will also evaluate for occult insulin resistance or differences in metabolism in several ways. We will evaluate dual-energy x-ray absorptiometry (DXA) at baseline, which will help determine body composition, in particular bone density. We will also calculate an insulin resistance index, the homeostatic model assessment (HOMA-IR),[63] using baseline fasting glucose and insulin. Statistical models exploring these body composition and insulin resistance parameters as potential confounders or mediators will be evaluated. Finally, the MACFIMS, safety, depression, and health-related quality of life outcomes will be monitored after discontinuation of the insulin (or placebo) to evaluate for any decline in cognitive function associated with medication discontinuation.

Safety/Adverse Event Monitoring An adverse event (AE) is any occurrence or worsening of an undesirable or unintended sign, symptom (or abnormal laboratory test), or disease temporally associated with the use of a medicinal product or intervention, whether or not it is considered related to the product/intervention. We will use the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0 to report and grade all adverse events, whether or not they are related to disease progression or treatment. The relationship between an AE and the intervention will be determined by the blinded study physician and recorded on the appropriate form. The investigator will record all study adverse events in the chart and will treat participants with AEs appropriately, observing them until they resolve or stabilize. AEs will be collected from the start of the study until a participant terminates from the study; those that are unresolved at the time of termination will be followed until they resolve or up to 30 days. An adverse event is considered unexpected when its nature or severity is not consistent with the product information (e.g. protocol or the informed consent form). Serious AEs will be collected from informed consent signing until 30 days after study completion or until 30 days after a participant withdraws from the study. The following process for reporting a serious AE will ensure compliance with the International Conference on Harmonisation guidelines: the Institutional Review Board (IRB) and DSMB will be notified in two business days of a serious AE that is medication-related and unexpected, and standard reporting (15 calendar days) will occur if the event is serious, expected and medication-related, serious, expected and not medication-related, or serious, unexpected and not medication-related. Life-threatening events will be reported within 7 calendar days. Any pregnancy will be reported to the IRB and DSMB, and pregnancies will be followed to their conclusion. Female participants who become pregnant will stop the study intervention. The investigator will report pregnancies to the IRB within two business days. The study medication will be discontinued if an AE grade 3 or higher occurs and is at least possibly related to the medication or if the subject cannot tolerate the medication/ wants to discontinue it. Any death that is at least possibly related to the study will put the study on hold until the DSMB evaluates it.

DSMB: The DSMB consists of an independent neurologist, an endocrinologist, and a statistician. It will convene any time an adverse event ≥ grade 3 occurs and routinely at 25%, 50%, 75%, and 100% enrollment. All serious adverse events will be promptly and simultaneously reported to the IRB, the DSMB and the Independent Research Monitor. Only serious adverse events (SAEs) that would also meet the definition of an Unanticipated Problem Involving Risks to Subjects or Others (UPIRTSOs) that are related to the protocol will be promptly reported to the United States Army Medical Research and Materiel Command Office of Research Protections Human Research Protection Office HRPO (USAMRC ORP HRPO).

Independent Research Monitor: The PI, Dr. Mowry, has appointed Dr. Anne Damian, a trained neurologist, as the Independent Research Monitor. Dr. Damian will provide independent safety review and will have the

authority to stop the research study; remove an individual subject from the research study; and will take any steps to protect the safety and well-being of the subjects until the IRB can assess the problem or event. The independent research monitor must review all unanticipated problems involving risks to subjects or others (UPIRTSO), but not all SAEs. The monitor will review the SAEs if they also meet the definition of a UPIRTSO and will provide an unbiased written report of the event. The Research Monitor will comment on the outcomes of the event or problem and the relationship to participation in the study. Additionally, the research monitor will indicate whether she concurs with the details of the report provided by the principal investigator.

Analytic Plan

Aim 1. Safety data will be analyzed as they are captured in real time. Assessments will focus on AEs (including study treatment tolerability assessments, laboratory evaluations, vital signs, and physical examination). The incidence rate of AEs will be recorded by system organ class, severity, and by relationship to the study treatment. Tolerability analysis will be based on the number (%) of subjects who failed to complete the study due to adverse events. Lab values for each parameter will be summarized by shift tables. For quantitative parameters, summary statistics for actual values and change from baseline will be presented. Changes in the first 24 weeks will be compared between groups using mixed effects regression analyses to account for the longitudinal nature of the data using random subject-specific intercepts and slopes.

Aim 2. Summary statistics (mean ± standard deviation, median and interquartile range, or number and percentages) will be used to characterize the outcomes. For each cognitive outcome, to evaluate variability in the estimated means across groups, assuming that the within-group means are normally distributed, a one-way ANOVA will be conducted at the follow-up visits during the first 24 weeks. If the within-group data are strongly non-Gaussian, a Kruskal-Wallis test will be used. A mixed effects longitudinal model will also be used to test whether any differences across groups exist at the follow-up visits within the first 24 weeks. Further, models accounting for covariates of interest, including the HOMA-IR, body composition, body mass index, age, sex and depression, will be evaluated. For within-person changes in measures at follow-up compared to baseline, paired t-tests will be used. We will also evaluate the SDMT at each study visit and other MACFIMS outcomes at week48 to establish the longevity of any therapeutic response to insulin.

Aim 3. For the biomarker outcomes, to evaluate variability in the estimated means across groups, assuming that the within-group means are normally distributed, a one-way ANOVA will be conducted at the follow-up visits during the active treatment phase. If the within-group data are strongly non-Gaussian, a Kruskal-Wallis test will be used. A mixed effects longitudinal model will also be used to test if differences across groups exist at follow-up visits. For within-person changes in measures at follow-up compared to baseline or before versus after calorie restriction days, paired t-tests will be used. Correlations between the biomarkers will be assessed. A bootstrap of subjects will be used to obtain 95% equal confidence intervals for these measures. We will also evaluate the biomarker changes after study therapy ends.

Sample Size and Power: Sample size estimates are difficult given the novelty of the project but are based on the goal of detecting a benefit of insulin on cognition. In a pivotal study of two doses of intranasal insulin versus placebo in participants with mild cognitive impairment or mild to moderate Alzheimer's Disease, the Dementia Severity Rating Scale score improved over the four-month treatment course in those receiving intranasal insulin.[34] Using the scores in the 10 IU twice a day group, given a two-sided alpha of 0.05 and beta 0.20 (80% power), assuming 3 follow-up cognitive tests and a correlation between baseline and follow-up results of 0.5, 29 subjects per group are required. To account for drop-outs and for the three-arm design, a total of 35 subjects per group is planned. With SDMT as the outcome, for a two-arm design, this sample size confers 80% power (assuming SDMT standard deviation of 9 points and two-sided alpha of 0.05) to detect a 6-point improvement in SDMT which seems reasonable considering that transcranial stimulation with cognitive training

improved SDMT by an average of more than 8 points compared to cognitive training alone in a pilot study of 20 subjects.[64] We do not expect that this study will provide conclusive evidence of efficacy for cognition, but it will provide estimates of the mean and standard deviation of the distribution in change in cognitive tests that will inform the design of definitive, phase III trials. Aim 3 is exploratory, but given the relative abundance differences in MS versus controls in our preliminary data in C22:1, and assuming a similar absolute C22:1 concentration as in women aged 55-64 years,[65] with 35 subjects in a two-arm study of 35 subjects/arm and a 2-sided alpha of 0.05, we will have 80% power to detect a 10 ng/mL difference in ceramide levels.

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