Measurement of Insulin Levels in CSF of Healthy Adults after a Single Intranasal Dose

Short title: INI-CSF

Clinical Study Protocol

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Sponsor

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This study will be conducted in compliance with the protocol, IND regulations and other applicable regulatory requirements.

HealthPartners® Neuroscience Center

PROTOCOL SIGNATURE PAGE

I have read this protocol and agree to adhere to the requirements. I will provide copies of this protocol and pertinent information to the study personnel under my supervision and my local ethics committee/institutional review board (EC/IRB). I will discuss this material with them and ensure they are fully informed regarding the study medication and the conduct of the study according to this protocol, applicable law, applicable regulatory requirements including 21 CFR parts, 50, 54, 56, 312 and 812, general standards of good clinical practice and local EC/IRB requirements.

_____
Principal Investigator

_____
Date

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Definitions

Adverse event (AE)	Any undesirable patient experience that may include but is not limited to an abnormal sign, symptom, illness, abnormal laboratory value, or other medical event.
Cerebrospinal Fluid (CSF)	Clear liquid that flows around the brain and spinal cord.
Columbia-Suicide Severity Rating Scale (C-SSRS)	A scale designed to quantify the severity of suicidal ideation and behavior.
Data Safety Officer (DSO)	An independent individual with relevant experience assigned to review safety data to monitor for incidence of trends that would warrant termination of the trial.
Intranasal (IN)	A method of drug delivery that is particularly applicable to delivering centrally acting medications into the central nervous system.
Intranasal Insulin (INI)	Human insulin that is administered to the nose in effort to directly target delivery to the central nervous system
Lumbar Puncture (LP)	A medical procedure which involves inserting a needle between two vertebrae of the lower back to collect fluid surrounding the spinal cord and brain.
Serious adverse events (SAE)	Any symptom, sign, illness or experience that develops during the study and results in a life-threatening situation, hospitalization, significant disability, and other events determined by the investigator to be significant.

Protocol Summary

PROTOCOL TITLE	Measurement of Insulin Levels in CSF of Healthy Adults after a Single Intranasal Dose
SHORT TITLE	INI-CSF
STUDY PHASE	Phase I
STUDY OBJECTIVES AND PURPOSE	
Primary Objectives: • To measure the concentration of insulin that reaches central nervous system in humans after intranasal administration	
Secondary Objectives: • To describe the effect of single intranasal insulin dose on serum insulin in healthy adults	
STUDY DESIGN	
Study Type	Pharmacokinetics
Control Type	None
Study Indication Type	Pharmacokinetics
Blinding Schema	Unblinded
Study Drug	Regular, Human Insulin [rDNA origin] USP solution for injection 100 units/mL
Device	SipNose
Route	Intranasal
Study Design	Open-label, randomized
Planned Duration of Subject Participation	4-5 weeks (Treatment duration: 1 dose)
PRIMARY ENDPOINTS	
• Cerebrospinal Fluid (CSF) Insulin Levels at pre-defined times (0-40 Min)	
SECONDARY ENDPOINTS	
• Serum Insulin levels at pre-defined times (0-40 Min)	

<table>
<tr><th colspan="2">INVESTIGATIONAL PRODUCTS, DOSE AND MODE OF ADMINISTRATION</th></tr>
<tr><td>Investigational Product</td><td>Regular insulin (Novolin-R) in 2 different doses (80, and 40 IU) (see Table 2)

<table><tr><th>INI Dose</th><th>CSF Collection Times</th></tr><tr><td>40 IU (n=6)
2 x 200 uL</td><td>0, 10, 20, 30, 40 min</td></tr><tr><td>80 IU (n=6)
2 x 200 uL
Wait 2 min
2 x 200 uL</td><td>0, 10, 20, 30, 40 min</td></tr></table></td></tr>
<tr><th colspan="2">SUBJECT SELECTION</th></tr>
<tr><td>Targeted Accrual</td><td>Up to 12 subjects. We estimate a need to consent 25 participants to reach this goal.</td></tr>
<tr><th colspan="2">INCLUSION CRITERIA</th></tr>
<tr><td colspan="2">1. Subject is between ≥18 and ≤ 35 years of age
2. Subject’s body mass index is between >=18.5 and <=24.9, or can safety undergo a lumbar puncture at the discretion of the radiologist
3. Subject must be proficient in speaking English to comply with instructions and measures for the study
4. Subject can provide written informed consent
5. Female subjects must have either: (1) a negative pregnancy test at the screening and treatment visits OR (2) be at least 2 years post-menopausal / surgically sterile.</td></tr>
<tr><th colspan="2">EXCLUSION CRITERIA</th></tr>
<tr><td colspan="2">1. Subject has medical history and/or clinically determined disorders: chronic sinusitis, previous nasal and/or oto-pharyngeal surgery and severe deviated septum and/or other anomalies.
2. Subject has history of any of the following: active and significant central nervous system, psychiatric illness, pulmonary, or cardiovascular disorders or any other clinically relevant abnormality that inclusion would pose a safety risk to the subject as determined by investigator
3. Subject has participated in a clinical trial investigation within 3 months of this study.
4. Subject has an insulin allergy
5. Subject has Insulin-dependent diabetes
6. Subject is pregnant or breast feeding
7. Contraindication to spinal tap or other safety factors that preclude lumbar puncture in the investigator’s opinion</td></tr>
</table>

1 Introduction

1.1 Background and Rationale

1.1.1 Intranasal Insulin

Intranasal (IN) delivery offers a non-invasive route to deliver large molecules such as insulin directly to the brain while minimizing systemic exposure. Peptides, proteins, vaccines, drug treatments and ions of various sizes are able to pass along the olfactory and trigeminal nerves and are deposited directly into the CNS without having to pass through the blood-brain barrier (BBB), which may degrade or limit the amount arriving at the target [1-8]. CSF insulin levels in healthy adults have been detected as early as 10 minutes following IN delivery (Appendix A) while not measurably increasing systemic blood-glucose levels [3,9]. Similar observations of the safety of intranasal insulin (INI) I have been made in memory impaired adults [10,11].

Originally thought to exist solely in the periphery, insulin has since been determined to be instrumental in the overall health and function of the CNS [12]. Central insulin and insulin receptors (IRs) have been established as differing from that of the systemically occurring counter parts that specifically regulate glucose utilization. In rodents, insulin receptors and insulin-sensitive glucose transporters are selectively co-localized in brain areas responsible for memory, thus providing a platform for insulin signaling whereby selective increases in cerebral glucose utilization could modulate memory [13,14]. Consistent with evidence of insulin functioning as a neuromodulator for memory-related function is the high-density of IRs in the hippocampus and cerebral cortex, brain regions integral to the formation, retention and recall of information [12,15]. Systems with impaired insulin signaling pathways have demonstrated inhibition of acetylcholine biosynthesis and subsequently have incurred debilitating effects on neuronal plasticity [16,17]. Increased insulin resistance and glucose intolerance has been observed in a multitude of neurodegenerative processes including Alzheimer's disease [14], Parkinson's disease [18], and Huntington's disease [19].

Clinical studies with INI in the last 20 years have studied it as a therapeutic option for multiple diseases including Alzheimer's dementia (AD), Parkinson's disease, and Post-traumatic stress disorders. In addition, studies in healthy humans and patients have shown effects on metabolism, including reductions in food intake, body weight, improvements of glucose uptake, and possible influence on mood, addiction, and sleep [20]. Recent studies have shown that INI also reduces white matter damage in the brains of patients with Alzheimer's Disease[21]. Appendix A provides an overview of the multiple studies conducted with INI. In Appendix B, Tables 1 (acute INI, 38 studies) and 2 (chronic INI, 18 studies) from the Schmid review provide key effects of INI on functional outcomes such as cognition, or memory.

The delivery of drugs to the CNS remains a development challenge mainly due to the blood-brain barrier limiting access. Commercially available aerosol nasal devices such as the LMA MAD device are not specifically engineered to facilitate nose-brain delivery, and consequently deposit most of the drug within the lower nasal cavity, resulting in suboptimal CNS penetration. Furthermore, there is concern about variable, user-dependent drug delivery due to dependency upon subject positioning and inconsistent volume ejection.

1.1.2 Intranasal Insulin and Devices

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1.1.3 Safety of Intranasal Insulin

Novolin R has been approved for subcutaneous or intravenous use since 1991. The most common side effect noted with these two routes is hypoglycemia (Regular Insulin, Drug Monograph; Appendix D). A recent review of safety of IN human insulin trials revealed no safety concerns, with no serious adverse events or symptomatic hypoglycemia in a total of 1092 individuals studied[28]. In Appendix B, Tables 1 (acute) and 2 (chronic) of this review provide a summary of the human studies with INI. The majority of the studies used regular Insulin (Novolin R or Actrapid, Humulin R, Insuman Rapid, H-insulin 100) with similar excipients and none are believed to be in a range that causes harm [29].

Study of pharmacokinetics of INI in mice showed only 3% of INI entered the circulation and no peripheral metabolic effects were detected up to a day after IN administration[30]. Treatment with 40 IU INI in humans found no significant changes in circulating insulin levels, suggesting that INI does not pass into the circulation [3,9]. A recent study in cardiac surgery patients, intranasal administration of insulin at doses of 40 IU and 80 IU did not cause hypoglycemia intraoperatively[31]. Though some studies with higher doses (160IU) have shown small detectable increase in insulin levels, no significant change in blood glucose levels were noted[32]. Notably, intranasally administered insulin improves memory in healthy adults and Alzheimer's patients without altering blood levels of insulin or glucose [11,33-39].

The most common side effect with INI administration noted is transient local or nasal irritation. In Appendix B, please refer to Table 3 of the Schmid et al., 2018 review for a summary of the local or nasal effects of INI in humans. In 2012, Craft et al reported, that number of adverse events was higher for the 20 IU and 40 IU when compared to the placebo group; however, there were no SAE's and mostly minor AEs such as rhinitis were reported (Table 4, [35]). Similar minor AEs were reported in the 2017 by Craft et al using INI (Supplementary table 2, [39]). Similar findings were reported in recent meta-analyses[40] and the most recent trial on INI[26], which showed no clinically relevant adverse events during the chronic administration of 40 IU of insulin with two different administration devices. Overall, INI at the proposed dose in this protocol, is expected to be safe with limited side effect profile. We do plan to monitor the subjects for hypoglycemia.

2 Summary of Device Description

2.1 Intranasal SipNose Device Overview

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2.1.1 Intranasal SipNose Device

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2.1.1.1 Reliability

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2.1.1.2 Safety

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2.1.1.3 Device-Drug Contact and Safety

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3 Study Objectives

3.1 Primary Objective

- To measure the concentration of insulin that reaches cerebrospinal fluid in humans after intranasal administration

3.2 Secondary Objective

- To describe the effect of single intranasal insulin dose on serum insulin in healthy adults

4 Study Endpoints

4.1 Primary Endpoints

- Cerebrospinal Fluid Insulin Levels at pre-defined times (0-40Min)

4.2 Secondary Endpoints

- Serum Insulin levels at pre-defined times (0-40 Min)

4.3 Safety

- Incidence and severity of serious adverse events (SAEs) and adverse events (AEs)
- Frequency of change in clinically-significant vital signs
- fingerstick glucose level <70mg/dl

5 Study Design

This Phase 1 is a single center, open-label, randomized pharmacokinetic study designed to describe the central nervous system delivery of intranasal insulin to CSF using anIN delivery device.

Table 1: Study Design

INI Insulin Dose	Collection Times
40 IU (n=6) *2 x 200 uL (0.2ml)*	0, 10, 20, 30, 40 min
80 IU (n=6) *2 x 200 uL (0.2ml)* *Wait 2 min* *2 x 200 uL (0.2ml)*	0, 10, 20, 30, 40 min

After written informed consent has been obtained, subjects will be screened to assess study eligibility based on study inclusion/exclusion criteria. Subjects who are eligible at the end of the screening visit will be randomized and assessed for outcome measures, including lumbar puncture and CSF/blood draw following a single dose of INI administered by the trained research staff. Randomization will be stratified by gender to ensure a balanced distribution of male and female participants within each group. A Block randomization scheme will be employed to ensure that the final group sizes are equivalent.

5.1 Justification for Study Design, Dose and Endpoints

5.1.1 Justification for Study Design and Dose

In this study, we will be measuring the concentrations of insulin in the CSF of 12 healthy adults after IN administration of one of two doses of insulin: 40 IU and 80 IU (Table 2). Multiple clinical trials of INI have ranged from 10 IU -160 IU for a variety of disorders, have been found to be safe in healthy adults[45] and are the basis for dose selection in this study. The higher dose (80 IU) may be the maximum feasible dose for

clinical studies, while the lower dose is the most encountered dose in clinical studies (both acute/chronic). The INI doses proposed are both safe, and are known to improve function or outcomes in the respective disorders. In addition, our research group has completed (NCT01436045, NCT02432716, NCT02503501, NCT04028960) or has ongoing (NCT04115384, NCT04251585) INI based clinical trials with similar doses and designs that are summarized in Appendix F.

Figure 7. REDACTED

5.1.2 Justification for Endpoints

The key objective of this study is to provide essential information on the ability of device to target INI to the brain and spinal cord as measured in CSF collected by lumbar puncture, which is the primary endpoint for the study. Born et al (2002)[3] (Appendix E) demonstrated detection of insulin after intranasal administration of 40 IU using a metered dose spray pump. Our study will measure the concentrations of insulin that reach the CSF following administration using an intranasal delivery system. The secondary endpoint will provide additional information regarding the concentration of insulin in serum.

5.2 Study Duration

Study participation will last approximately 4-5 weeks, consisting of a screening/baseline visit, treatment/lumbar puncture visit, a follow-up/final assessment visit.

5.3 Study Drug Administration

5.3.1 Drug Administration with SipNose

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5.3.2 Device Dose Loading and Administration

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6 Study Population

6.1 Eligibility Criteria

6.1.1 Inclusion Criteria

A subject will be included for consideration in this study only if all the following criteria are met:

1. Subject is between ≥18 and ≤ 35 years of age
2. Subject's BMI is between >=18.5 and <=24.9, or can safely undergo a lumbar puncture at the discretion of the radiologist
3. Subject must be proficient in speaking English to comply with instructions and measures for the study
4. Subject can provide written informed consent
5. Female subjects must have either: (1) a negative pregnancy test at the screening visit and treatment visit OR (2) be at least 2 years post-menopausal / surgically sterile.

6.1.2 Exclusion Criteria

A subject will *not* be included for consideration in this study if any of the following criteria are met:

1. Subject has medical history and/or clinically determined disorders: chronic sinusitis, previous nasal and/or oto-pharyngeal surgery and severe deviated septum and/or other anomalies.

2. Subject has history of any of the following: active and significant central nervous system, psychiatric illness, pulmonary, or cardiovascular disorders or any other clinically relevant abnormality that inclusion would pose a safety risk to the subject as determined by investigator
3. Subject has participated in a clinical trial investigation within 3 months of this study.
4. Subject has an insulin allergy
5. Subject has Insulin-dependent diabetes
6. Subject is pregnant or breast feeding
7. Contraindication to spinal tap or other safety factors that preclude lumbar puncture in the investigator's opinion

7 Study Assessments and Procedures

A summary of study events and procedures is outlined in the Study Visit Table (Table 3).

7.1 Demographic and Baseline Assessments

7.1.1 Screening Visit 1

The following procedures will be performed at this visit: This may also be divided into two parts, with virtual and in-person visits. Virtual activities (e.g., consent, review of medical history/medications) can be completed on a separate day(s) from other study procedures, provided that consent occurs within 3 months of the lumbar puncture and all other screening activities are completed within the specified window (21-7 days prior to lumbar puncture visit/Visit 2). All activities will take approximately 2 hours to complete. No study procedures, virtual or otherwise, will be completed until a fully executed consent form has been obtained. Participants will be given the option to have a dose of sterile saline administered by research staff using the device if they want to experience the sensation of the device before the lumbar puncture visit.

- Obtain written informed consent Review Inclusion/Exclusion Criteria
- Review medical history, as it pertains to inclusion/exclusion criteria
- Pregnancy test for women of childbearing potential
- Review guidance on the use of effective birth control from the time of study enrollment until 30 days ***after*** the final visit.
- Obtain subject's demographic information (date of birth, gender, race, and ethnicity).
- Collect vital signs, height and weight
- Obtain details of medications taken over the course of the last 30 days
- Physical/neuro examination including examination of the nares and nasal passages
- Blood draw for laboratory investigations
- C-SSRS
- 12-Lead Standard ECG
- Saline test dose (optional)
- Pre-procedure instructions
- Provide Information sheet provided relating to pre-lumbar puncture hydration
- Visit #2 scheduled after 2 weeks ±7 days

7.1.2 Lumbar Puncture Visit 2: In-Person Visit (2 week ± 7 days after Visit 1)

The subject will be check in at the Regions Hospital outpatient care unit (OPCU). The visit will be at the General Radiology Department (where the lumbar puncture will be performed and study data collected) and will return to the OPCU for post-operative monitoring.

The following procedures will be performed at this visit:

- Review Inclusion/Exclusion Criteria
- Collect concomitant medication information and record AEs/SAEs
- Collect vital signs and weight
- Pregnancy test for women of childbearing potential
- Review guidance on the use of effective birth control until 30 days study ***after*** the final visit.
- Glucose finger stick test (once before study drug administration and twice after)
- Clinician will discuss procedure with the subject
- Lumbar puncture and collection of CSF for insulin
    - CSF samples will be obtained at predefined times (0-40 min). CSF samples will be collected FIRST at all time points.
- Blood draw and collection of serum for insulin

  Serum samples will be obtained at predefined times (0-40 Minutes). CSF Samples will be collected SECOND at all time points.
- Study Drug administration
- Nasal Irritation Scale
- Patient will lay on back for a total of 2 hours post-lumbar puncture
- Visit #3 scheduled within a week ± 5 days

7.1.2.1 Fluoroscopy-Guided Lumbar Puncture Procedure

CSF samples will be collected according to standard operating procedures for fluoroscopy-guided lumbar puncture as observed by the General Radiology department at Regions Hospital. The procedure will be performed by a trained clinician (i.e., an interventional radiologist or an experienced member of their team), who will obtain verbal consent (in addition to the research informed consent obtained/documented at Visit 1). CSF will be collected serially using gravity drainage. Sterile extension tubing connected to the LP needle will dispense CSF into sterile collection tubes for pharmacokinetic analysis. Samples will be collected at 5 times: once prior to the administration of a single dose of intranasal insulin and at once each at 10, 20, 30, and 40 minutes post-drug administration. Approximately 1-2ccs of CSF will be collect at all 5 time points for a total of 10ccs of CSF. The tubing connected to the needle will be flushed out between sample collections to avoid contamination between specimen timepoints. The subject will lie in prone position throughout the procedure with appropriate measures taken to minimize discomfort (e.g., provision of warm blankets, use of positioning sponges, etc.).

Subjects will lie on their back 2 hours after the procedure for standard-of-care monitoring with nursing staff, and periodic collection of vital signs. Participants will receive instructions from the clinical team on techniques for preventing and treating post-LP headache (e.g., drinking water and caffeinated beverages, using over-the-counter pain medications). They will also receive guidance on when to seek additional help for their headache and/or any other emergent symptoms requiring immediate medical attention such as fever, persistent headache, and weakness/numbness of the lower extremities. Subjects will receive a safety assessment phone call from study staff 1 day after the procedure.

7.1.3 Phone Visit 1: (following a day after lumbar puncture)

- Study staff phone call to address AEs/SAEs Day 2 (+ 1 days) after visit 2/LP procedure
- There will be screening questions related to headache, bleeding at the site, and infectious symptoms.

7.1.4 Follow-up Visit 3 (Within a week of Visit 2 ± 5 days).

The following procedures will be performed at this visit: This can be divided into two parts, with virtual and in-person visits

- Collect concomitant medication information and record AEs/SAEs
- Physical/neuro examination including examination of nares and nasal passages
- Collect vital signs, weight
- Pregnancy test for women of childbearing potential
- Review guidance on the use of effective birth control from the time of study enrollment until 30 days study ***after*** the final visit.
- 12-Lead Standard ECG
- C-SSRS
- Blood draw for laboratory investigations

7.1.5 Ad hoc Visit if Symptomatic:

- Collect vital signs, Weight
- Physical Examination
- Study staff to address AEs/SAEs

7.2 Early Withdrawal

If subject withdraws from the study after the screening visit, but before the lumbar puncture visit, no further evaluations are necessary.

7.3 Safety

For all safety assessments described below, any clinically significant change will be recorded as an adverse event (AE) or serious adverse event (SAE).

7.3.1 Physical Examination

Complete physical examination including examination of the nares/nasal passages will be performed at visits 1 and 3, or if the subject withdraws or is withdrawn from the study early. Any abnormalities noted at Visit 1, will be documented as part of the subject's medical history.

7.3.2 Neurological Examination

Neurological examination will be performed at visits 1, 3 or if the subject withdraws early. Any abnormalities noted at Visit 1, will be documented as part of the subject's medical history.

7.3.3 Vital Signs:

Vital signs will be recorded at all three study visits. Diastolic blood pressure will be measured at the disappearance of Korotkoff sounds. Vitals sign will be monitored by clinical staff during screening and lumbar puncture visits of the study.

Serum Glucose will be examined at visit 1 (screening) and visit 3 (safety follow-up). Fingerstick glucose tests will be performed at visit 2 (LP procedure). A blood glucose < 70 mg/dL will be considered clinically significant. The likelihood of peripheral hypoglycemia is low based on numerous clinical trials performed showing no effects of INI on peripheral glucose.

7.3.4 Weight:

Body weight will be measured at all three study visits.

7.3.5 Laboratory Samples:

All the laboratory samples will be drawn independent of fasting or non-fasting status.

When a patient withdraws from the study, samples collected prior to the date of withdrawal may still be analyzed, unless the patient specifically requests that the samples be destroyed or local laws require destruction of the samples. However, if samples have been tested prior to withdrawal, results from those tests will remain as part of the overall research data. Samples will be collected and processed according to the standard procedures. Key planned labs include:

- Basic Metabolic Panel
- Complete Blood Count
- Blood Coagulation Tests

7.3.6 Point-of-Care Glucose Testing:

At Visit 2, the participant will receive a single dose of intranasal insulin administered prior to their lumbar puncture. Point-of-care fingerstick glucose testing will be performed once before the administration of the study drug (baseline glucose reading) and twice after the administration of the drug according to the following schedule:

(1) 15 minutes after study drug dosing (between the 2nd and 3rd CSF/serum samples are collected)
(2) 45 minutes study drug dosing (after the final CSF/serum samples are collected)

7.3.7 Pregnancy Test

All women of childbearing potential will have blood collected to test for pregnancy. This will be performed at the screening visit (Visit 1), treatment visit (Visit 2), and final follow-up visit (Visit 3). A negative pregnancy test (urine) must be confirmed on the day of Visit 2 prior to the administration of the study drug.

7.3.8 Contraceptive Counseling

All study participants (male and female) will be counseled on the use of effective forms of birth during study participation and in the 30 days following the final study visit. If a pregnancy occurs during this time period (in either the subject or the subject's partner), the participant will be asked to inform the study team immediately.

7.3.9 Suicidality: Columbia Suicide Severity Rating Scale (C-SSRS)

The C-SSRS is a suicidal ideation rating scale to evaluate suicidality. It rates an individual's degree of suicidal ideation on a scale, ranging from "wish to be dead" to "active suicidal ideation with specific plan and intent"[47]. The C-SSRS will be administered by trained raters at specified time points, as indicated in Table 3 as well as when clinically indicated. Any subjects demonstrating evidence of suicidality will prompt

immediate consultation with the site's on-call psychiatrist for assistance with decision-making and potential referral to behavioral health services.

7.3.10 Nasal Irritation Scale

Study team will ask subjects if they are experiencing any nasal irritation (schedule see Table 3) on a Likert scale of 0-10, with 0 being no irritation and 10 being severe irritation. The scale will be administered 5 times at the following predetermined intervals: Once immediately before study drug administration, and again at 20 and 40 minutes post-administration. Research staff will also observe whether the subject has experienced any bleeding (Yes/No). If Yes, they will be asked to categorized the bleeding as: a) Bleeding which stops within 1 minute; b) Bleeding, taking 1-5 minutes to stop; c) Bleeding for 4-60 minutes, does not require medical intervention; d) Bleeding which requires medical intervention.

7.3.11 Pharmacokinetic Sampling

The study team will attempt to collect the CSF and serum samples as close in time as possible for each PK time point. Given differences in how serum/CSF are extracted for this study, there may be a slight difference between collection start and stop times. To ensure consistency across participants, the study team will initiate collection of the CSF samples first, followed by the serum samples, at each of the 5 PK time points.

7.3.11.1 CSF Sampling

It is our intention to collect samples at pre-defined times following a single dose administration. CSF will be collected at 0, 10, 20, 30 and 40 minutes for all study participants. CSF samples will be assessed for insulin using a one-step sandwich immunoassay with a limit of detection of 0.1 µIU/ml or 0.7 pmol/L

7.3.11.2 Serum Samples

It is our intention to collect samples at pre-defined times following a single dose administration. Serum will be collected at 0, 10, 20, 30 and 40 minutes for all study participants. Serum samples will be assessed for insulin using the same one-step sandwich immunoassay with a limit of detection of 0.1 µIU/ml or 0.7 pmol/L.

Table 2: Study Visit Schedule

Assessment/ Procedure	**Visit 1: Screen**	**Visit 2: Lumbar Puncture (LP)**	**Phone Visit 1: Safety Check**	**Follow-up Visit 3**	**Ad Hoc Visit if Symptomatic**
Schedule – Week and Windows	-2	1	Following day after LP	Within 1 week of Visit 2	
		± 7 days	+ 1 day	± 5 days	
Informed consent	**X**				
Review of inclusion/exclusion criteria	**X**	**X**			
Medical History, demographics	**X**				
Physical/Neuro exam with examination of nares and nasal passages	**X**			**X**	**X**
Vital Signs (Blood pressure, heart rate, weight)	**X**	**X**		**X**	**X**
Height	**X**				

Basic Metabolic Panel (Na, K, CO2, CL, BUN, Creatinine, Glucose and Ca)	X			X	
Labs INR/PT/PTT	X			X	
Complete Blood Count	X			X	
Pregnancy Test (in females)	X	X		X	
Counseling on effective birth control	X	X		X	
Randomization		X			
Lumbar Puncture		X			
Glucose Finger Stick (0 Min, 15 Min, 45 Min)		X			
Nasal Irritation Scale (0, 20, 40 min)		X			
CSF Samples and Insulin Levels (0-40 Min)		X			
Serum insulin Levels (0-40 Min)		X			
C-SSRS, 12-Lead Standard ECG	X			X	
Pre-Procedure instructions	X				
Study Drug Administration (single dose)		X			
Concomitant Medication	X	X	X	X	X
Adverse Events	X	X	X	X	X

8 Investigational Product(s)

8.1 Description of Investigational Product

The research staff will utilize the following investigational products:

- Intranasal delivery route
- Regular insulin (Novolin-R –U-100; Insulin Full Prescribing Information; Appendix C)
- SipNose Device

8.2 Handling and Storage

The study drug will be kept at study site per label recommendations and institutional Standard Operational Policy, specifically, but not limited to temperature controlled secure area.

To ensure that a stable temperature and/or conditions are maintained, site staff will verify and document temperature at a minimum of three times per week or monitored continuously if automated systems such as TempTrak are used. An electronic log will be securely stored at the study site. Study staff will be responsible for safeguarding and maintaining the master log.

8.3 Packaging and Labeling

Only designated study staff will be responsible for handling all investigational drug and devices; study participants will not be preparing/administering the drug themselves. The study drug will not be individually labeled; the study team may use the same vial of insulin for multiple participants in order to minimize waste.

All study drug will be labeled according to the following specifications:

- Protocol identifier/IRB approval/account/study number

- Quantity statement
- "Caution: New Drug—Limited by Federal (or United States) law to investigational use"

All study devices will be individually packaged and labeled by the manufacturer as follows:

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8.4 Occupational Safety

No known significant safety risks exist to site personnel in direct or indirect contact with the study drug. As the lumbar puncture will employ fluoroscopy, clinical (non-research) staff will be exposed to radiation. The fluoroscopy technicians and radiologist will follow standard clinical protocols for radiation safety (e.g. wearing lead Personal Protective Garments). The amount/duration of radiation exposure for this research LP is not greater than what would be experienced during a routine LP. As fluoroscopy is only needed to assist with needle placement, study staff will not need to be present for this part of the procedure and can step out of the procedure room while the fluoroscopy machine is in use. Research staff can then reinter the room to assist with pre-dose sample collection/fingerstick glucose testing after the machine has been deactivated.

9 Concomitant Medications and Non-Drug Therapies

9.1 Permitted Medications

Any medication not listed in list of Prohibited Medications (Table 4) will be permitted during this study. A record will be kept by site staff detailing doses and indication of any concomitant medications used by subjects.

9.2 Prohibited Medications

Any subject with insulin-dependent diabetes taking insulin will be excluded from the study. Additional medications that are exclusionary and/or should be avoided during the study period are outline below in Table 4.

Table 3: Prohibited Medications

Prohibited Medications
Barbiturates
Benzodiazepines
Insulin
Muscle Relaxants
Opioids
Beta Blockers

10 Subject Completion and Withdrawal

10.1 Subject Completion

Subjects completing all study visits (4) will be considered to have completed study.

10.2 Subject Withdrawal

Subject may withdraw from study at any time for any reason without penalty or be terminated from the study by the clinical investigator (see provisions for termination by study team.) Investigational team will

document the reason(s) for withdrawal. In the event a subject chooses to withdraw from study before Visit 3, the safety procedures described in 7.3 will be performed (ideally within 3 days following subject's decision to withdraw). For all subjects who withdraw, all final safety assessments will be collected as possible regardless of time elapsed since previous visit. In addition to the termination visit, subjects who withdraw early will be contacted within 7 days by study staff via telephone to assess development of new and/or ongoing AEs and concomitant medications. Efforts will be made to recruit subjects to replace any withdrawals before lumbar puncture (or subjects for whom we are unable to get sufficient for analysis) so as to maintain n=6 per group.

Subject's participation may be terminated at the discretion of the investigator. Individuals may be withdrawn for the following reasons:

- Clinically significant adverse events
- Lost to follow-up
- Protocol violations
- Inability to tolerate study medication
- Other

11 Adverse Events (AE) and Serious Adverse Events (SAE)

11.1 Definition of AE

An adverse event is any symptom, sign, illness or experience which develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- Results in study withdrawal
- Is associated with clinical signs or symptoms
- Leads to treatment or to further diagnostic tests
- Is considered by the investigator to be of clinical significance

11.2 Definition of SAE

Adverse events are classified as either serious or non-serious. A serious adverse event is any event that results in:

- Death, Life-threatening situation
- Hospitalization or prolongation of hospitalization
- Disability or incapacitation
- Other events determined by investigator to be medically significant in which subject's well-being is jeopardized (e.g. events that have high likelihood of escalating to the point of meeting criteria outlined above)

11.2.1 Clinical Laboratory Abnormalities & Other Abnormal Assessments as AEs & SAEs

Any new abnormal, vital, examination, or laboratory finding judged clinically significant by the investigator will be documented as an AE or SAE, if meeting the definitions for such. Abnormal lab findings or other abnormal assessments associated with the disease under study will not be considered AEs or SAEs unless more severe than expected, as judged by the investigator.

11.2.2 Time Period and Frequency of Detecting AEs and SAEs

Upon consenting, a subject is considered to be a participant in the study, and until that person either withdraws or completes study, AEs and SAEs will be recorded. The investigational team will promptly report any AE/SAE as required per federal guidelines.

11.2.3 Device Failures and Malfunctions

Device Quality Control. All failures and malfunctions of the device will be documented.

12 Data Analysis and Statistical Considerations

12.1 Statistical Analysis Overview

The population demographics (age, gender, etc.) and clinical values (vital signs, labs etc.) will be reported using the appropriate summary measures (e.g. mean, standard deviation, and proportions).

12.2 Statistical Analysis of Primary and Secondary Endpoints

Primary Endpoints: We will utilize immunoassay to analyze the CSF samples to determine the levels of Insulin concentration at the specified times. We will graph these values across time for each subject using line graphs. We will also graph the sample average Insulin concentration across time, and at different doses with error bars and report concentrations as mean ± SE. Finally, the concentrations will be analyzed using common pharmacokinetic measures including the peak concentration (Cmax), time to peak concentration (Tmax), and area under the curve (AUC). This type of analysis is very similar to that done in Dhuria's 2009 publication in the *Journal of Pharmaceutical Sciences,* on which Leah Hanson and William Frey II were co-authors. Similar analysis will be conducted for secondary endpoints.

AEs and SAEs will be categorized and summarized by study group. We will descriptively note if any group was more likely to have experienced a particular AE/SAE or generally experienced more AEs/SAEs throughout the course of the study.

13 Study Conduct Considerations

13.1 Regulatory and Ethical Considerations, Including the Informed Consent Process

The study will be conducted in accordance with GCP. Subject privacy requirements will also be observed as well as the fundamental concepts of the Declaration of Helsinki (e.g. IRB approval of the study, obtaining informed consent from all subjects, and meeting reporting requirements). The clinical trial will be registered on the clinicaltrials.gov website.

13.2 Data Safety Monitoring Plan

A data safety officer (DSO) will be an independent individual who is not participating in the trial and has no direct affiliation with the research team. The Data safety monitoring plan will be established prior to initiation of the study. The DSO responsibilities include but are not limited to the following:

- Monitoring the study for compliance to the protocol.
- Stopping the study if the rate of SAE's raises safety concerns. The details will be specified in the data safety monitoring plan.

During the course of the trial, the DSO will review accumulating safety data to monitor for incidence of trends that would warrant termination of the trial. The frequency of the DSO meetings, responsibilities, membership, and procedures will be documented in the data safety monitoring plan.

13.2.1 Stopping Criteria

Previous research suggests that the incidence of peripheral hypoglycemia in patients given INI at the dosages examined in this trial (40IU and 80IU) is low. However, the study team will obtain point-of-care blood glucose readings before and after administration of the single dose of intranasal insulin and use this information to determine the safety of continued study participation. Although the study team will document the blood glucose readings <70mg/dL as a safety event, levels this low will not necessitate withdrawal/study stoppage. A reading of <54mg/dL will be considered clinically significant regardless of symptoms.

For individual subjects: Study activities will be stopped for participants with Hypoglycemia readings <54mg/dL as measured by point-of-care fingerstick glucose tests. If this value is reached, hypoglycemia rescue measures will be performed, and no additional CSF or blood samples will be collected. If a participant exhibits the following symptoms of dangerous hypoglycemia in the absence of clinically significant value, their participation will be similarly stopped: blurred vision, confusion, slurred speech, cold clammy skin, etc or that requires hypoglycemia rescue measures.

The study team will follow our institution's standard clinical protocol for the treatment of hypoglycemic events. Measures taken to restore glucose levels to >70mg/dL may include oral treatments (e.g., fruit juice or oral glucose gel) as appropriate. If a patient is unresponsive or otherwise not able to take oral treatments, they may be given IV dextrose or intramuscular glucagon.

Participants with glucose levels >54 but less than <70 will not need to be withdrawn based on these values alone. A reading in this range will trigger an ad-hoc glucose test reading for safety monitoring in participants that are otherwise asymptomatic/mildly symptomatic but not requiring rescue measures.

For dosing cohorts: Participants will be randomized to one of two arms for this study, receiving either a 40IU or 80IU dose of insulin. If two participants in either arm (40IU or 80IU) experience a clinically significant glucose level (<54mg/dL) and/or experience severe/possibly life-threatening symptoms of hypoglycemia as defined in the protocol, the study will be paused and the Data Safety Officer will review data related to these events and recommend a course of action for the study. These options may include, but are not limited to: (1) Adjusting the arm to a lower dose for future enrollees (e.g., reducing the amount from 80IUs to 40IU, or 40IU to 20IUs, etc.), (2) Arm termination, and (3) Study termination.

13.3 Quality Assurance

In the event of a regulatory agency audit or inspection, site will allow the auditor/inspector access to all records documented and facilities utilized in conducting the study. Site will also make accommodations (e.g. time, schedule) to discuss findings, concerns, and questions with auditor/inspector.

13.4 Study Closure

Upon completion of all subject visits, data entry and analysis, investigator will inform local IRB of study closure.

13.5 Records Retention

All site records will be maintained and stored in a safe and secure location for a minimum of 15 years post study completion.

13.6 Provision of Study Results and Information to Investigators

Study results will be made available by the study statistician once analysis is complete.

13.7 Data Management

Data collection/reporting tools will be developed internally (i.e. CRFs and source documents). Data collected and stored electronically will remain confidential and secure (e.g. secured server, encrypted data, password protected file)

13.8 Device and Drug Accountability

A Device Tracking and Drug Log will be maintained at the investigational site. Intranasal devices will be recorded on the log upon delivery to the investigational site and will be stored in a secured area. The Device and Drug Tracking Log will be updated as each device or drug is delivered, dispensed, returned and the reason for the return. Serial numbers, expiration date and model number of devices or batch/lot numbers for drugs delivered to the site will also be recorded.

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Summary of Changes

REDACTED

Appendix A. Intranasal Insulin Studies Table

REDACTED

Appendix B. Schmid et al. 2018, The safety of intranasal human insulin: a review

REDACTED

Appendix C. Novolin R Label Prescribing Information

REDACTED

Appendix D. Regular Insulin, ClinicalKey Drug Monograph

REDACTED

Appendix E. Born et.al 2002 – Sniffing Neuropeptides: A transnasal approach to the human brain

REDACTED

Appendix F. HealthPartners Investigator-Initiated Intranasal Insulin Studies

REDACTED

Appendix G. Insulin Stability Experiment Report & Raw Data

REDACTED

Appendix H. REDACTED