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# Intranasal Insulin for Treatment of Diabetic Polyneuropathy

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# Intranasal Insulin for Treatment of Diabetic Polyneuropathy

by

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A THESIS

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## Abstract

Intranasal insulin administration is a novel approach to slow the progression of diabetic polyneuropathy (DPN). We performed a pilot randomized controlled trial of intranasal insulin in 12 type 1 diabetes mellitus patients with DPN to assess safety. We administered intranasal insulin for 6 weeks using biweekly dose-escalation up to 160 IU/d or intranasal saline. The primary outcome measure was frequency of hypoglycaemia. Frequency of mild (mHG) and serious hypoglycaemic (sHG) events was recorded. Secondary outcomes included clinical (Utah Early Neuropathy Score (UENS)) and laboratory (corneal confocal microscopy and electrophysiology) measures. There were no differences in glycemia between groups after supervised initial administration. The 40 IU/d and 80 IU/d doses were safe and well tolerated with comparable mHG events between groups. One intranasal insulin subject suffered a sHG at home while receiving 160 IU/d. Intranasal insulin was safe and well tolerated at 40 and 80 IU/d.

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2. Dedication

I dedicate this thesis to my wife, Monika, who is my inspiration without whose hard work and dedication to our family none of this work could have been possible. I also dedicate this thesis to my daughters, Mercedes and Anastasia, for whom I wish the blessing of an impassioned career as exciting and rewarding as the one I have.

I also dedicate this thesis to my mother, Elzbieta Nowicka-Korngut, whose passion for psychology and its rich history of early contributions by neurologists led me to choosing a career in neurology, and my father, Leszek Korngut, whose diligence and kindness shaped who I am today.

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## List of Abbreviations

| | |
|---|---|
| ALS | Amyotrophic lateral sclerosis |
| AGE | Advanced glycation endproduct |
| ALT | Alanine aminotransferase |
| ALA | α-lipoic acid |
| ALADIN | Alpha-Lipoic Acid in Diabetic Neuropathy |
| ARI | Aldose reductase inhibitor |
| AST | Aspartate aminotransferase |
| AUC | Area under curve |
| B12 | Vitamin B12; cyanocobalamin |
| β-HCG | Beta-human chorionic gonadotropin hormone |
| BMI | Body mass index |
| BUN | Blood urea nitrogen |
| CBC | Complete blood count |
| CCM | Corneal confocal microscopy |
| CGRP | Calcitonin gene related peptide |
| CNFBD | Corneal nerve fiber branch density |
| CNFBL | Corneal nerve fiber branch length |
| CSF | Cerebrospinal Fluid |
| DM1 | Type 1 diabetes mellitus |
| DCCT | Diabetes Control and Complications Trial |
| DMD | Duchenne muscular dystrophy |
| DPN | Diabetic Polyneuropathy |
| EDIC | Epidemiology of Diabetes Intervention and Complications |
| EFNS | European Federation of Neurological Societies |
| EMG | Electromyography |
| HgbA1C | Glycated hemoglobin |
| HSP27 | Heat shock protein 27 |
| HMRC | Heritage Medical Research Clinic |
| IDDM | Insulin dependent diabetes mellitus |
| IENF | Intraepidermal nerve fiber |

| | |
|---|---|
| IENFD | Intraepidermal nerve fiber density |
| IGF-1 | Insulin-like growth factor 1 |
| IU | International units |
| IU/d | International units per day |
| MDNS | Michigan diabetic neuropathy score |
| mHG | Mild hypoglycemic event |
| MedRA© | Medical Dictionary for Regulatory Activities |
| mRNA | Messenger riboxynucleic acid |
| NADP(H) | Nicotinamide adenine dinucleotide phosphate |
| NATHAN II | Neurological Assessment of Thioctic Acid in Neuropathy |
| NBD | Nerve branch density |
| NFD | Nerve fiber density |
| NFL | Nerve fiber length |
| NIS(LL) | Neuropathy impairment score (lower limbs) |
| NMDs | Neuromuscular diseases |
| NSC | Neuropathy symptoms and change score |
| RCT | Randomized controlled trial |
| siRNA | Short interference riboxynucleic acid |
| SEM | Standard error of the mean |
| sHG | Severe hypoglycemic event |
| SNCV | Sural nerve conduction velocity |
| TSH | Thyroid stimulating hormone |
| TSQM | Treatment satisfaction questionnaire for medication |
| UENS | Utah early neuropathy scale |
| VAS | Visual assessment scale |
| WA | Washington state, United States of America |

# Chapter 1: Diabetic Polyneuropathy – The Problem

## 1.1 Background

Diabetes mellitus (DM) is the most common disorder of glucose metabolism with wide ranging systemic complications, often accompanied by significant disability. It is characterized by hyperglycemia due to abnormal insulin secretion, action or both (1). There are two predominant types of DM. Type 1 (DM1) results from pancreatic β-destruction usually leading to absolute insulin deficiency (2). Type 2 DM (DM2) is associated with progressive reduction in insulin secretion and action (insulin resistance) (3).

The prevalence of diagnosed DM2 in the United States between 2003 and 2006 was 7.8% (4). Canadian data from 2008-09 demonstrates a slightly lower prevalence of combined DM1 and DM2 of 6.8% (5). Reliable data about the prevalence of type 1 DM are not available but its incidence ranges from 10 to 20 per 100,000 person-years in most European regions (6, 7).

Chronic complications of DM include retinopathy, nephropathy and neuropathy. Although predisposing and protective factors have not been completely elucidated it is clear that DM is the major cause of these complications and degree of glycemic control correlates with risk of complications (8).

The diabetic neuropathies are one of the most common manifestations of peripheral nervous system dysfunction and often result in significant neurological deficit, severe pain, and major disability. Broadly, the diabetic neuropathies include acute cranial neuropathies, autonomic neuropathies, acute asymmetric lumbar plexopathies and radiculopathies but the more gradual-onset distal symmetric diabetic polyneuropathy (DPN) is the most common. Despite the

relatively high prevalence and impact on society there is no effective treatment to prevent progression of DPN.

## 1.2 Epidemiology and burden of diabetic polyneuropathy

The global burden of DPN is growing with the increasing prevalence of DM2. The World Health Organization predicts that the prevalence of DM will double between 2000 and 2025 (9). Increased severity of DPN is associated with augmented disability and amplified cost to health care systems (10, 11). Various studies utilizing different clinical and electrophysiologic inclusion criteria for polyneuropathy estimate the prevalence of DPN to be present in 20% to 54% of patients with DM1 and DM2 (12, 13). Inconsistent subject selection and criteria for diagnosis of DPN make more precise prevalence estimates difficult.

The natural history of DPN following its diagnosis is unclear, as no definitive long term data is available. Long term follow up studies exist but the inclusion of other neuropathies is a prominent confounding variable (14). The Rochester Diabetic Neuropathy Study followed 183 patients for two years and found that the Neuropathy Impairment Score did not change in 81%, deteriorated in 10% and improved in 9%. Importantly, the Neuropathy Symptom Score did not change in 92% but improved in half of the remaining subjects (15). Large multi-centre clinical trials can also provide useful natural history data as they employ comprehensive outcome measures to a relatively homogenous subject population exposed to placebo. In the recombinant human nerve growth factor study, 515 subjects were randomized to placebo and were followed for one year utilizing the Neuropathy Impairment Score (Lower Limbs) (NIS(LL)) and the Neuropathy Symptoms and Change (NSC) score. After one year, the NIS(LL) was improved in 34%, 41% were unchanged and 25% were worse. There were no differences detected in the NSC

(16). These studies illustrate the limited data on the natural history of DPN. In particular, the short duration of subject follow up prevents these studies from elucidating the long term natural history of DPN.

### 1.3 Diagnosis

DPN is a chronic progressive length-dependent polyneuropathy that typically begins with positive sensory symptoms in the toes and gradually progresses proximally in a stocking-glove distribution. Painful paresthesias are commonly associated with the sensory symptoms. A careful history elicits the typical symptoms and time course and screens for symptoms that may suggest an alternative etiology for the patient`s complaints. The differential diagnosis for stocking glove distribution sensory complaints includes other causes of length-dependent polyneuropathy, plexopathy, polyradiculopathy, cervical myelopathy, as well as bihemispheric or brainstem lesions. Superimposed chronic inflammatory polyradiculoneuropathy should be considered in patients presenting with an acute-on-chronic progression. Symptoms of prominent neck pain, early weakness, rapid progression or bowel and bladder dysfunction should raise concern for an alternative diagnosis.

Neurologic examination should demonstrate a stocking-glove distribution of sensory loss (loss of light touch, vibration, proprioceptive, pinprick and thermal sensation) to varying degrees related to the severity of the DPN. Careful attention should be given to identifying superimposed entrapment or compressive neuropathies (i.e. carpal tunnel syndrome, ulnar neuropathy at the elbow) as patients with DPN are at increased risk. Motor weakness is typically a late manifestation after years of chronic sensory progression.

Once the working diagnosis of DPN is made, the patient should be screened for other contributing causes of small fiber or axonal polyneuropathy (See Table 1.1). Despite the low yield of the screening investigations, identifying a second contributing cause (i.e. hypothyroidism) allows for prompt treatment to minimize further progression.

**Table 1.1** Screening investigations for other contributing causes of polyneuropathy

| |
|---|
| Complete blood count with differential |
| Electrolyte panel, urea, creatinine |
| Fasting glucose, glucose tolerance test |
| Thyroid stimulating hormone |
| Vitamin B12, fasting methylmalonic acid, fasting homocysteine |
| Serum and 24 hour urine protein electrophoresis with immunofixation |
| Creatine kinase |
| VDRL |
| Rheumatoid factor, Complement C3 and C4 |
| C-reactive protein |

Clinical electrophysiology is essential in confirming the diagnosis of an axonal DPN. Small fiber DPN on the other hand will demonstrate normal nerve conduction studies as these fibers are not interrogated on routine electrophysiology. Typical nerve conduction studies in mild to moderate DPN demonstrate length dependent reduction of amplitudes with only mild slowing of conduction velocities and prolongation of distal latencies consistent with a pattern of axonal loss. Sensory potentials are affected early with motor potentials affected in the more severe stages. Subtle findings of secondary demyelination may be noted as the neuropathy progresses. Overt electrophysiologic demyelination implies an alternative diagnosis in mild to moderate DPN. Electrophysiology can also identify patients with other causes for upper and lower extremity symptoms (i.e. concomitant carpal tunnel syndrome and lumbar spinal stenosis).

More recently, surrogate markers have been validated that can demonstrate anatomical changes prior to detection with electrophysiology. Corneal confocal microscopy (CCM) allows non-invasive quantification of nerve fiber length and density of small fibers of the corneal sub-basal epithelial nerve plexus. Intraepidermal nerve fiber (IENF) assessment allows for quantification of IENF density, branch density, and branch length through a skin punch biopsy. Both CCM and IENF analysis demonstrate progressive reduction of these measures with DPN. The major benefit of CCM is that it is performed in less than 10 minutes and is non-invasive.

## 1.4 Biomarkers

Biomarkers for the diagnosis of DPN are under investigation. Recently, elevated serum heat shock protein 27 (HSP27) levels were associated with a twofold increase in the odds ratio of developing DPN in a case-control study of subjects with DM1 (17). The author has reported that transgenic overexpression of human HSP27 in a mouse model of DM1 was protective against sensory nerve deterioration (18). Reduced levels of neuron-specific enolase mRNA have been associated with polyneuropathy in persons with DM1 and DM2 (19). Both of these potential biomarkers require validation before clinical use is recommended.

### *1.4.1 Epidermal Skin Biopsy*

Epidermal skin biopsy is the best biomarker of DPN and is now used as a diagnostic tool in the clinical setting. Skin punch biopsies are typically obtained 10 cm proximal to the lateral malleolus along the lateral aspect of the lower leg using a 3 or 6 mm disposable circular punch instrument. The tissue is cut into 50-µm-thick sections and stained with the pan-axonal marker

PGP 9.5 allowing quantification of intraepidermal nerve fiber density (IENFD) (20). IENFD measurements are highly reproducible with between different observers and laboratories with low inter- and intra-observer variability (21). Strong correlation of reduced intraepidermal nerve fiber density in the presence of clinically identified peripheral neuropathy has been demonstrated (22).

In subjects with mild DPN prior to development of nerve conduction abnormalities, symptomatic subject IENFD measurements were significantly lower than those in asymptomatic subjects (23). IENFD measurements also correlate with clinical severity of DPN from mild to moderate to severe (24). Correlation with sural sensory nerve action potential amplitude has been confirmed in subjects demonstrating large fiber sensory involvement (25).

Recently, several societies have published guidelines regarding the use of skin biopsy for the diagnosis of polyneuropathy. The European Federation of Neurological Societies (EFNS) guideline provided technical recommendations for performing and interpreting skin biopsies and quantification of IENFD. The EFNS guideline also concluded that skin biopsy is a safe, validated, and reliable technique (level A recommendation) for the determination of IENFD and diagnosis of small fiber neuropathy (26). The American Academy of Neurology guidelines suggested the use of skin biopsy (IENFD) for the diagnosis of distal symmetrical polyneuropathy and small fiber sensory neuropathy in particular (Level C evidence) (27).

Skin biopsy is a minimally invasive and highly sensitive tool for the diagnosis and staging of DPN. Clinical applications include confirmation of clinical diagnosis, particularly in mild cases with normal electrophysiology as well as staging of the severity of the

polyneuropathy. Research applications include confirmation of diagnosis, staging as well as a useful and sensitive outcome measure.

### *1.4.2 Corneal Confocal Microscopy*

Corneal confocal microscopy (CCM) is a novel approach to assessing in vivo peripheral nervous system integrity and is the best validated non-invasive biomarker of DPN (28). Changes in corneal Aδ and unmyelinated C fibers correlate to those seen in the more distal nerve fibers seen in length-dependent DPN. Scanning of Bowman's layer (which contains a prominent nerve plexus, the most densely innervated part of the human body) allows for measurements of nerve fiber density (NFD), nerve fiber length (NFL), and nerve branch density (NBD).

Rosenberg et al identified a correlation between the reduction in number of corneal nerve fiber bundles (NFB) and presence of DPN. Further, significant differences in reduction of nerve fiber bundle numbers correlated with severity of neuropathy (mild to moderate versus severe). No differences were seen between the control group without diabetes and those with diabetes but without neuropathy (29).

Malik et al used CCM to compare 18 subjects with DM1 and DM2 and 18 age-matched controls without diabetes. They identified significant differences between the control and DPN groups. Significant differences were identified between control and moderate and severe diabetes groups on comparison of NFD and NFL. NFL was significantly lower in the severe neuropathy group in comparison to the mild neuropathy group. NBD analysis demonstrated differences between the control and all respective DPN groups (mild, moderate and severe) (30).

Six months following simultaneous pancreas and kidney transplantation several CCM indices (NFD, NFL and nerve fiber tortuosity) were significantly increased suggesting high sensitivity to mild improvements in small fiber integrity (31).

CCM is a validated surrogate marker for DPN that correlates with presence and severity of neuropathy in subjects with diabetes.

## 1.5 Pathogenesis

Traditional views of the pathogenesis of DPN include excessive flux of polyols through the aldose reductase pathway with resulting depletions in nerve and Schwann cell myo-inositol and elevation of protein kinase C expression (32). Microangiopathy and hypoxia of the peripheral nerve trunks, ganglia and spinal cord have also been implicated. Deficiency of growth hormones, nitrative stress from free radicals and abnormal glycosylation of structural neuronal proteins have also been well described (32).

Over the past decade new factors that influence nerve regeneration have been identified and their role in DPN is being elucidated.

### *1.5.1 Receptor for advanced glycation end-products (RAGE)*

The spectrum of advanced glycation endproducts (AGEs) include numerous molecules that are the product of several different chemical reactions. Classically, non-enzymatic glycation produces AGEs (33). AGEs have been associated with the chronic complications of DM including DPN (34, 35). Though the exact mechanisms are unclear an animal model of diabetes in mice lacking RAGE demonstrated attenuated neuropathy (35).

### *1.5.2 Insulin and DPN*

Previous work has demonstrated not only an absolute reduction in plasma insulin levels in persons with DM1 but also in those with DM2 (36). In the Diabetes Complications and Control Trials (DCCT) and subsequent studies, both with patients having DM1 or DM2, the prevalence and severity of DPN was directly related to the intensity of euglycemia-targeted insulin therapy (37, 38). Such evidence suggests that tight control of hyperglycemia is critical in preventing the development of DPN or retarding its progression, but it may also suggest that insulin itself is important in the treatment of DPN.

Insulin receptors have been identified throughout the central and peripheral nervous systems, located in the brain, spinal cord, dorsal root ganglia and upon peripheral nerves (39). The density of insulin receptors and their machinery are modulated during diabetes with demonstrated reduction of insulin receptor protein during diabetes (40, 41).

Animal studies comparing nerve regeneration following crush injury between equally hyperglycemic hypoinsulinemic DM1 and hyperinsulinemic DM2 diabetic rats have demonstrated reduced regenerative capacity in hypoinsulinemic animals associated with reduced Insulin-like growth factor 1 (IGF-1), IGF-1 receptor, TrkA, βII- and βIII-tubulin, and low and medium molecular neurofilament expression (42). This may suggest that impaired insulin signalling in DM1 nerve could have more prominent effects than hyperglycemia on dysregulation of neurotrophic and cytoskeletal protein synthesis.

Attempts to enhance nerve regeneration in experimental animal models of DPN have been successful. Low intermittent doses of insulin administered near diabetic nerve unilaterally correct electrophysiological abnormalities in experimental diabetes and promote regeneration

(43). Systemic insulin also promotes nerve regeneration, and intrathecal delivery of insulin, without systemic glycemic impact, accesses and decorates (when immunolabelled) sensory and motor neurons while reversing electrophysiological and structural features of experimental DPN and promoting reinnervation of epidermal skin fibers (44, 45). As well, intrathecal insulin promotes distal axon regeneration in non-diabetic rodents, demonstrating its potent trophic effect (46). More recent data will be discussed in the treatment of DPN section.

### *1.5.3 Calcitonin gene related peptide (CGRP)*

CGRP is present within the dorsal root ganglia in rats of various backgrounds. Exposure to diabetes reduces CGRP expression in the DRG (47). Following focal sural axonal nerve injury CGRP is expressed within the nerve trunk with findings suggestive of intra-axonal peptide synthesis accompanied by similar expression within adjacent Schwann cells. Severe disruption of new axon outgrowth occurs with administration of siRNA targeting αCGRP (48). CGRP may play a supportive role in axonal regeneration, and deficiency may result in enhanced axonal degeneration in the setting of DM.

## 1.6 Prognosis

Few studies are available that describe the natural history of DPN over a prolonged period of time. One study followed 36 patients for a mean duration of 4.7 years using electrophysiology and a 10 cm graphic rating scale analogous to the Visual Analog Scale (VAS) to assess the severity of symptoms. Some improvement in pain was noted in a minority of the subjects (n=11) with no change noted in the remainder. No patients demonstrated resolution of symptoms. Repeat nerve conduction studies demonstrated mild slowing of the median nerve conduction velocity but no change in peroneal nerve motor conduction velocity. Although the possibility of

vascular insufficiency was ruled out, other possible contributing causes of neuropathy were not. Sensory nerve conductions were not included in the study. Over a four year period, the pain associated with DPN as tested with the graphic rating scale did not worsen (49).

The DCCT, discussed in the Treatment section below, demonstrated slower progression with intensive glycemic control (50).

A subsequent retrospective review of 300 patients with diabetes, 100 with polyneuropathy, who underwent annual assessments revealed that vibration perception threshold increased over the eight year period in those with polyneuropathy despite reasonable glycemic control (51).

Beyond these limited natural history studies, prognosis data is also available from subjects in placebo arms of therapeutic clinical trials. These subjects are well studied with multiple outcome measures but shorter durations, typically 12 months. Progression of polyneuropathy in 472 persons with DM1 and DM2 was demonstrated using several outcome measures in the control group of the Zenarestat multicenter clinical trial. The Michigan Neuropathy Screening Instrument, electrophysiologic markers (sural sensory velocity, median sensory amplitude, and medial distal motor latency) and cool thermal quantitative sensory testing all declined significantly over the 12 month period (52). In the recombinant human growth factor trial, 515 subjects with DM1 and DM2 with DPN in the placebo arm were assessed at baseline and at 48 weeks with the Neuropathy Impairment Score for the Lower Limbs (NIS-LL), CASE IV quantitative sensory testing, and electrophysiology among other outcome measures. 34.0% of subjects in the placebo arm improved, 24.8% worsened and 41.2% did not change at 48 weeks on the NIS-LL. Detailed nerve conduction studies were unchanged (16).

Overall, only limited data are available about the prognosis of DPN. Most patients appear to exhibit slow progression in the setting of appropriate glycemic control. Some patients may stabilize and the available data suggests that some patients may improve over time.

## 1.7 Treatment

Here I focus on the progress in research dedicated to slowing the progression of DPN rather than symptomatic management such as neuropathic pain agents.

### *1.7.1 Glycemic control*

The DCCT randomized 1,441 volunteers ages 13 to 39 with 1 to 15 years of DM1 to standard glycemic control (one to two insulin injections per day) or intensive glycemic control with insulin (at least three insulin injections per day, frequent blood glucose monitoring, and a glycated hemoglobin target of 6% or less). The DCCT followed the original cohort for a mean of 6.5 years while 238 of the patients were followed for a total of nine years (50). The study demonstrated convincingly that intensive glycemic control reduces the risk of developing polyneuropathy as a complication of DM1 (53). The intensive glycemic control group demonstrated early improvement in electrophysiologic indices with subsequent gradual decline that was significantly slower than the conventional glycemic control group. Despite intensive glycemic control, 6.9% of subjects developed new evidence of DPN on history and physical examination suggesting that intensive glycemic control on its own is insufficient to prevent development of polyneuropathy (50). Despite both the intensive and conventional glycemic control groups being encouraged to aim for intensive control after the completion of the trial, the benefits on polyneuropathy remained on follow-up using the Michigan Neuropathy Screening Instrument as part of screening of the subsequent Epidemiology of Diabetes Intervention and

Complications (EDIC) study eight years after the completion of the DCCT study (54). Intensive glycemic control slows the progression of DPN with long-lasting beneficial effect.

### *1.7.2 Aldose reductase inhibitors*

Since the initial description of markedly elevated glucose, sorbitol, and fructose in peripheral nerves and spinal cords of diabetic rats in 1966, the polyol pathway has been implicated in the pathogenesis of diabetic complications and DPN (55). In the two-step polyol pathway, glucose is first converted to sorbitol and nicotinamide adenine dinucleotide phosphate is oxidized (NADPH→ NADP). This first step is catalyzed by aldose reductase. The second step converts sorbitol to fructose and NAD is reduced (NAD→NADH). The second step is catalyzed by sorbitol dehydrogenase. Persistent elevations in serum glucose result in increased flux through the polyol pathway resulting in increased fructose levels and AGE formation, increased cellular sorbitol levels, impaired Na-K ATPase function, and reduced free radical scavengers (i.e. glutathione).

Extensive investigations into the polyol pathway identified several aldose reductase inhibitors (ARIs) that inhibit the polyol pathway (56). These ARIs have extensive human clinical trials. A recent Cochrane review identified 32 randomized controlled trials meeting inclusion criteria for meta-analysis (57). Thirteen studies were included in the final meta-analysis due to insufficient data from the remaining studies. No significant benefit was identified beyond one subgroup analysis of four trials of tolrestat that favored treatment. Beneficial effects on neuropathic symptoms were identified in some trials but this was contradicted in other trials. There was no benefit on electrophysiology or foot ulceration. Three ARIs were withdrawn from human use due to concerning adverse effects (sorbinil, zenarestat, and tolrestat) (58).

Since the publication of the Cochrane review in 2007, several clinical trials have been completed. One of these, a multicenter, randomized, placebo-controlled double-blinded study of ranirestat involving 549 subjects, demonstrated no benefit on clinical, quantitative sensory and sensory electrophysiological outcome measures (59).

Overall, there is no clear evidence that ARIs slow the progression of DPN at this time but further research may demonstrate benefit in a specific subset of patients with DPN.

### *1.7.3 α-lipoic acid*

Increased oxidative stress is implicated in the pathogenesis of DPN. α-lipoic acid (ALA) is a potent antioxidant. In the rat streptozocin model of DM1 and DPN lipoic acid, delivered through intra-peritoneal injection, improved nerve blood flow, electrophysiology of peripheral nerves and reduced peripheral nerve glutathione indicating reduced oxidative stress (60).

Seven randomized clinical trials investigating the efficacy of ALA in alleviating the symptoms and sensory deficits in patients with DPN have been completed. Six of these have been published (NATHAN II unpublished). Four out of five trials demonstrated significant short-term benefit in amelioration of DPN symptoms (61-64). None of the six clinical trials demonstrated clinically significant stabilization of sensory deficits utilizing clinical scales such as the Neurologic Impairment Scale. Two studies demonstrated slowing of deterioration on several electrophysiological parameters (63, 65). A meta-analysis published in 2004 of the clinical trials suggested that daily administrations of 600 mg intravenous ALA was beneficial for reducing positive neuropathic symptoms and sensory deficits over a period of three weeks (66). Only two studies followed patients for six months or more, both beginning with a short-term daily intravenous ALA infusion followed by oral ALA. Both of these studies demonstrated no

benefit in clinically relevant outcome measures (neurologic disability scale (ALADIN II) (65), neuropathy impairment scale, and total symptom score (ALADIN III) (67) at two years and six months respectively.

Intravenous ALA appears to have limited benefit in short-term (< three weeks) symptomatic control of positive neuropathic symptoms associated with DPN but chronic administration of oral ALA has no demonstrated benefit for positive neuropathic symptoms or sensorimotor deficits.

### *1.7.4 C-peptide*

Recent basic laboratory investigations have demonstrated that C-peptide may exert biological effects when administered to patients with DM1 and that this effect may be secondary to stimulation of endothelial nitric oxide synthase (68, 69). One randomized, placebo-controlled, double blinded study of 46 subjects with IDDM and no symptoms of DPN demonstrated improvement in sural sensory nerve conduction velocity and peroneal motor nerve conduction velocity after three months of treatment with daily subcutaneous C-peptide administration 600 nmol/24hr via qid dosing) compared to the placebo group. A small improvement in vibration perception threshold was also identified on quantitative sensory testing without any change in thermal perception thresholds (70). A second randomized, placebo-controlled, double-blinded study of 139 IDDM subjects with symptomatic DPN demonstrated subtle improvement in sural SNCV, neurologic examination and vibration perception threshold improvement for the subjects in the c-peptide groups versus placebo after 6 months of follow up (71). Both studies possessed relatively small sample sizes and did not utilize recognized clinical neuropathy outcome scales. These pilot studies suggest possible benefit that requires further investigation with a larger study

population, longer duration and clinically relevant outcome measures including clinical neuropathy scales, and surrogate markers such as skin biopsy or corneal confocal microscopy.

# Chapter Two: Clinical Development of Novel Therapeutic Compounds

## 2.1. Translational research

The concept of translational research encompasses the spectrum of research across traditional compartments of knowledge development. From a biomedical perspective, translational research can be defined as "the process of applying discoveries generated during research in the laboratory, and in preclinical studies, to the development of trials and studies in humans. The second area of translational research is aimed at enhancing the adoption of best practices in the community" (72).

Translation of research findings from the basic laboratory into clinical studies is a crucial step in development of new therapeutics, diagnostics and technologies. Targeted, efficient and effective translation requires collaboration between basic scientists and clinician investigators, clinical research capacity, validated outcome measures, trial designs, and statistical analysis plans.

The ultimate goal of translational research is the study of compounds, diagnostics or technologies in human studies. The identification of a promising therapeutic lead compound in the basic laboratory is the beginning of the process. Translating new compounds into clinical studies requires adherence to research ethic boards and Health Canada policies and regulations.

The process of translating basic laboratory findings into a therapeutic compound employs various study designs that demonstrate safety and efficacy in cumulative form. Initial Phase I studies typically include pharmacokinetic and clinical safety dose-finding designs in healthy normal subjects. These are generally followed by Phase II studies studying the safety of the compound in the target patient population with small sample sizes that justify progression to

larger studies by demonstrating preliminary safety. Clinical trials compare the compound to a comparator (usually placebo) to further demonstrate safety and efficacy. Clinical trials may occur during Phase II and are usually mandatory during Phase III.

### 2.1.1 *Translational research for new therapies for neuromuscular diseases* (73)

Translational research in NMDs has flourished in recent years due to the emergence of a clearer understanding of disease mechanisms for several NMDs. Major advances in Duchenne (DMD), myotonic, facioscapulohumeral muscular dystrophies and amyotrophic lateral sclerosis have accelerated therapy development of novel molecular targets (74),(75),(76),(77). Specific causative genetic mutations have been targeted in Duchenne muscular dystrophy (DMD) with restoration of dystrophin expression. Both nonsense mutation read-through agents (eg. Ataluren, clinicaltrials.gov NCT01009294) and antisense oligonucleotide-mediated exon skipping have promising DMD human trials underway (78),(79). Translation of laboratory findings into clinical therapeutic applications has recently resulted in a new therapy for Pompe disease (80).

Peripheral nerves and muscles are a particularly physically accessible part of the nervous system. The combination of available tissue samples, improved diagnostic accuracy, genetic breakthroughs, and severity of illness have accelerated research into pathophysiology resulting in numerous molecular targets for therapy development as listed above.

## 2.2. Clinical Trials

A clinical trial can be defined as *"an experiment testing a medical treatment on human subjects"* (81). This simple definition purposefully excludes additional design characteristics (i.e.

randomization, blinding, etc.) thus allowing the definition to apply to clinical trials with varying objectives and designs. The design of the trial should reflect the objective of the trial, the treatment to be studied and the patient population involved. Piantadosi further suggests that the best terminology to distinguish clinical trials from other studies is by their experimental design (versus non-experimental) (81).

The design of a clinical trial can be clearly defined by the various methodological elements of which the study protocol is comprised. The goals of the design of a specific trial are to control random error (the possibility of an erroneous result by pure chance) and bias (the possibility of an erroneous result through a systematic error). A list of the common methodological characteristics of clinical trials are listed and defined in Table 2.1.

**Table 2.1** Important elements of clinical trials to test interventions

| Element | Definition |
|---|---|
| Basic study design | The method used to assess the effect of an intervention versus it's comparator (i.e. placebo). Basic study designs include parallel, cross over, withdrawal, factorial, hybrid, and adaptive (82). |
| Randomization | Each subject is randomly assigned to an intervention group or non-intervention (i.e. placebo or sham) group. Randomization methods include simple, block, stratified and adaptive randomization (82). |
| Blinding | The deliberate masking of whether the subject is receiving the intervention. Blinding can be of the subject, the study co-ordinator/physician or both. Blinding can also include the assessment, classification and evaluation of outcome measures (82). |
| Placebo control | Subjects in the non-intervention group typically receive a placebo designed not to have any significant effect on the trial outcome measures. Generally, the placebo has the same shape, taste, appearance and packaging as the intervention. |
| Allocation concealment | The study team and subject being randomized are shielded from knowing upcoming group assignments (83). |
| Safety monitoring | Sufficient assessment, analysis, and reporting of adverse events to allow interpretation of the risk of a given intervention (82). |

## 2.3 Adverse event monitoring/reporting

Varying definitions of adverse effects exist. The U.S. National Institutes of Health define *adverse reactions* as: "Unfavorable changes in health, including abnormal laboratory findings, that occur in trial participants during the clinical trial or within a specified period following the trial" (84).

Adverse event monitoring can be defined as a systematic process of identifying and organizing adverse events to allow a safety assessment of a specific compound or device (85).

Adverse event reporting can be managed by university research ethics boards, pharmaceutical companies, or governmental agencies (i.e. Health Canada). Adverse event monitoring can be passive where clinicians report serious or unexpected adverse events to the appropriate body. Clinical trials employ active adverse event monitoring where subjects are directly asked about adverse events in general and often about specific adverse events that are of high risk in a given study. Passive adverse event monitoring (aka. reporting) relies on clinician awareness of the program and that they will spend the time to complete forms, etc. and is of much lower yield. It is less resource-intensive and more applicable to approval of a new drug where no specific serious adverse events are expected but reporting is nonetheless indicated perhaps due to limited adverse event data prior to regulatory approval. Active adverse event monitoring where subjects are contacted or where reporting is mandatory is much less commonly employed and much more resource intensive. Patient, disease, and device registries are useful for the active collection of intervention (i.e. compound or device) and subsequent patient outcomes. Otherwise active adverse event monitoring is more suitable for the experimental environment where data is collected on a specific group of patients for a specific time interval such as in a clinical trial.

Certain standard features of clinical trials are also implemented to ensure appropriate safety and adverse event reporting. These include frequent study visits for sufficient opportunity for review and standardized coding of adverse events. Several coding systems for adverse events in clinical trials exist. The Medical Dictionary for Regulatory Activities (MedDRA) (86) is the most commonly used at this time. MedDRA provides a clear coding system for classification of adverse events and their attribution to study drug or placebo. Serious and unexpected AE's where the AE is neither fatal nor life-threatening, must be reported to Health Canada within 15

days after becoming aware of the information, where it is fatal or life-threatening, immediately where possible and, in any event, within 7 days after becoming aware of the information, and within 8 days after having informed Health Canada of the AE, submit as complete as possible, a report which includes an assessment of the importance and implication of any findings.

Attribution is a clinical determination, generally by the study principal investigator, as to the likelihood that an adverse event (AE) is related to a medical treatment or procedure as summarized in Table 2.2.

**Table 2.2** Adverse event attribution categories

| Attribution Categories | Definition |
|---|---|
| Definite | The AE is *clearly related* to the treatment |
| Probable | The AE is *likely related* to the treatment or procedure |
| Possible | The AE *may be related* to the treatment or procedure |
| Unlikely | The AE is *doubtfully related* to the treatment or procedure |
| Unrelated | The AE is *clearly NOT related* to the treatment or procedure |

Following the completion of study visits there is generally a final study visit at least several weeks after the last administration of the intervention (and non-intervention) to capture any late adverse events after completion of all study procedures.

### 2.4 Dose-finding safety studies

Safety, in the context of clinical trials, can be defined as the measurement of adverse effects and wellbeing of study participants. Beyond adverse effects, safety considers the overall impact of the treatment being studied on the well being of the person. Definition of a symptom

attributed to the study treatment or placebo as adverse or not is essential in the course of a study to ensure safety.

Studies examining the safety of new therapeutic agents are essential throughout the clinical development process and for eventual regulatory approval. Preliminary studies often focus on safety and are performed in limited numbers of healthy volunteers (Phase I) or those with the target disease (Phase II). Beyond assessment of pharmacokinetics safety studies capture adverse events and allow determination of whether they are within acceptable limits. Safety studies are often conducted in conjunction with a dose-finding design. Dose-finding safety studies focus on pharmacokinetic properties and dose selection as well as clinical matters (i.e. safety) rather than power, type I error rate and sample size (81). It is not uncommon for these studies to not be powered by sample size calculations. Multiple doses are usually used. If an expected dose is available it can be "bracketed" by larger and smaller doses (81). Research ethics considerations imply that early safety studies use lower doses prior to higher doses and that investigator limit subject exposure to ineffective doses (too low) and potentially toxic doses (too high) and it is important to establish "definitions of dose limiting toxicities" and "decision rules" for doses increases or decreases prior to beginning the study (81). The starting dose or expected optimal dose can be determined from animal studies or previous experience (or pre-existing regulatory approvals for other indications) in the case of re-purposed compounds.

Piantadosi summarizes the history and evolution of dose-finding safety study methodology (81). Dixon and Mood (87) described the classic design of fitting the cumulative normal dose-response model one subject at a time. Quantal (non-continuous) doses are given and moved up

with “success” and down with “failure”. This method was originally designed for explosive sensitivity experiments and is particularly applicable to compounds with a clear and acute dose-response characteristic such as a biochemical marker (i.e. acute blood glucose) or physiological response (i.e. blood pressure) but would not be reasonable to assess for a clinical response that is expected to occur over time (i.e. slowing progression of DPN). Further, where variability in dose-response may be present and a mean group response is required this approach is more resource intensive. Importantly, where dose-response is required over a prolonged time interval (i.e. blood glucose lowering by chronic insulin administration) such quantal (i.e. one subject at a time) data capture is not feasible. Wetherill and Levitt (88) adapted the Dixon and Mood method by increasing the quantal size to 3 subjects. This modification incorporates inter-subject variability in dose-response albeit to a limited extent but has the same inherent limitations as the Dixon and Mood method. In the “modified Fibonacci” method (81), the dose is increased according to an algorithm approximating the Fibonacci sequence (1, 1, 2, 3, 5, 8, 13, 21…) where each successive term is the sum of the previous two and the ratio of successive terms approaches 0.61803. This method is particularly useful to the study of novel compounds with no previous safety data where a very low dose that is not expected to have a biological response is administered initially and then increased as per the sequence. This method is particularly suitable for Phase I studies in normal healthy subjects of compounds that have a very large therapeutic window. Dose-finding in the setting of a compound with more chronic effects generally requires prolonged exposure to the compound at each dose, collection of biochemical and clinical (i.e. adverse effect) data over time, and a comparator group (i.e. placebo) can be useful to interpret adverse effects particularly when variable clinical response to a given dose is expected.

Ultimately, the selected method must be appropriate to the compound selected, its expected therapeutic window from human cell culture and/or animal experiments.

**2.5 Feasibility studies**

While a pilot study can be defined as a "small study for helping to design a further confirmatory study" it does not necessarily include objectives and outcome measures pertaining to feasibility of a future confirmatory study (89). While meeting the criteria for a "pilot trial" this study will also assess preliminary feasibility for a future confirmatory study by examining the 'willingness to participate' of eligible patients at one centre (90). Feasibility studies examine aspects of study design and recruitment to assess the feasibility of a future confirmatory study. A broad array of factors can be examined that related to the target population, geography, expected and unexpected barriers to subject recruitment and completion of study visits as per the study protocol. In general, it is helpful to examine factors that may impede subjects being recruited into, and progressing through the study in question. An assessment of outcome measure variability may also be included. Factors include subject recruitment rate and profile, screen pass rate, effectiveness of randomization and blinding methods used, adherence to the schedule of study visits and procedures, number and type of protocol violations, barriers to appropriate completion of outcome measures, data management, and statistical analysis plan. Other factors such as outcome measure inter-rater reliability between the actual involved raters can be assessed. There are no available guidelines for feasibility assessment of future clinical studies but the above factors should be considered.

## 2.6 Pilot studies

Moore et al., defined pilot studies as "preparatory studies designed to test the performance characteristics and capabilities of study designs, measures, procedures, recruitment criteria, and operational strategies that are under consideration for sure in a subsequent, often larger, study" (91). The authors observed that the "pilot" aspect of a pilot study is often vaguely described and should not be used as a justification for a small sample size, lack of funding and an inadequate study design or analysis plan. The authors recommended that pilot studies: 1. keep the next study in mind; 2. that the pilot study be carefully designed; and 3. that the sample size be justified (91). The authors also summarized issues particularly suited to assessment using a pilot study: "future study conceptualization, study design, sample size, sample selection, data collection, data management and data analysis" (91).

## 2.7 Regulatory Aspects

Health Canada reviews all clinical trial protocols to ensure the protection and safety of participants, assesses the quality of drugs, ensures REB review and approval, reviews adverse drug reactions and verifies the qualifications of Principal Investigators (92). *"The Food and Drugs Act and Regulations provide authority to Health Canada to regulate the sale of drugs for the purposes of use in human clinical trials. Part C, Division 5 of the Regulations defines specific Clinical Trial Application (CTA) and Clinical Trial Application Amendment (CTA-A) requirements for the sale and importation of drugs for use in human clinical trials in Canada."*(92)

Health Canada provides a framework for the progression of a new therapeutic compound through the process of clinical development are summarized in Table 2.3.

**Table 2.3** Health Canada Classification of Clinical Trials (92)

| Phase | Definition |
|---|---|
| Phase I | Initial safety studies on a new drug, including first administration of the drugs into humans, usually conducted in healthy volunteers. |
| Phase II | Clinical trials to evaluate the efficacy of the drug in patients with medical conditions to be treated, diagnosed or prevented and to determine the side effects and risks associated with the drug. |
| Phase III | Controlled or uncontrolled trials conducted after preliminary evidence suggesting efficacy of the drug has been demonstrated. |
| Phase IV | All studies performed after the drug has been authorized by the regulator for the market, and related to the authorized indication. |

New lead compounds emerging from the basic science laboratory generally fit into one of two groups. Novel compounds previously untested in human subjects require Phase I investigations to ensure safety in health subjects and to collect pharmacokinetic, pharmacodynamic and bioavailability data to help confirm the dose range that can be considered for subsequent investigations. The second group consists of "re-purposed" compounds with existing approvals for other indications. As an example, lithium has a long standing approval for use as a mood stabilizer in patients with bipolar disorder. In 2008, a group of Italian researchers reported a powerful effect of lithium on slowing the progression of amyotrophic lateral sclerosis (ALS) in an animal model and a subsequently performed Phase II clinical trial (93). Given the previous approval and availability of existing safety and pharmacological data for lithium, as a member of the Canadian ALS Research Network we participated in a North American Phase III

multi-centred, double-blinded, randomized controlled trial of lithium versus placebo in 250 subjects with ALS. Unfortunately futility criteria were met following a planned interim analysis with 84 subjects randomized (94). Thus while novel compounds require progress through the Phase I and II clinical trial categories, "repurposed" compounds with available Phase I and II data may proceed directly to Phase III. Such requests are reviewed by Health Canada (and the US Food and Drug Administration) on a case-by-case basis through the process of the clinical trial application process. Clinical trials reviewed by Health Canada with no identified concerns are provided a Letter of No Objection which is then provided to the relevant institutional REBs.

Intranasal insulin for the treatment of DPN is a Phase II pilot RCT. This study was designed primarily as a study of safety and tolerability of intranasal insulin in the target population of persons with DM1 and DPN.

**2.8 Institutional Review and Ethics Boards**

The majority of REBs in Canada provide review of both scientific content and adherence to research ethics for submitted studies. All research involving human subjects (except quality improvement studies) are subject to REB review and approval prior to commencement. Scientific review, in general, includes ensuring the following the appropriate and clearly defined: rationale, design, subjects, intervention, outcomes, and statistical analysis plan. Ethical review applies the principles of ethics to the study including issues surrounding privacy and confidentiality (i.e. process of subject identification and recruitment) and equity. The process of REB application and review reduces the possibility of coercion and harm to the community.

Both a Health Canada Letter of No Objection and REB approval are required prior to commencement of a study.

## Chapter 3: Intranasal Insulin – The Intervention

### 3.1 A Brief History of the Discovery of Insulin

Insulin was first purified in 1922 at the University of Toronto by Banting, Macleod, Collip and Best (95). Shortly thereafter insulin became the standard treatment for DM1 and spread quickly around the world. While Banting and MacLeod shared the 1923 Nobel Prize in Medicine and Physiology, they quickly shared their prize money with Collip and Best (95). While Banting and Best received the most international recognition for the discovery, with plenty of debate by medical historians, all four are now recognized as equal contributors (95).

### 3.2 Pharmacology of Insulin

*Extracellular Delivery.* Intranasal delivery of various compounds bypasses the blood-brain barrier resulting in rapid (within minutes) absorption into the central and peripheral nervous system, as well as cerebrospinal fluid. Extracellular delivery, rather than axonal transport, is strongly suspected due to the short (≤10 minutes) penetration time from the nasal mucosa to the brain. Possible mechanisms may involve diffusion through perineuronal channels, perivascular spaces, bulk flow, or lymphatic channels directly connected to brain tissue or cerebrospinal fluid (96).

*Specific Delivery Routes.* Recent evidence demonstrates that intranasally administered therapeutics penetrate the CNS via the olfactory and trigeminal neural pathways (97, 98).

*Systemic Bioavailability of Intranasal Insulin.* Systemic bioavailability of intranasally administered insulin in the absence of absorption enhancers is negligible (99). Negligible

systemic bioavailability of intranasal insulin administered without absorption enhancers is an essential point as CSF penetration of intranasal insulin administered without absorption is well demonstrated. Intranasal insulin administration thus allows effective CSF penetration without significant systemic bioavailability.

Previous studies of intranasal insulin with absorption enhancers have clarified the pharmacokinetics of regular insulin delivered intranasally. Absolute systemic bioavailability of intranasal insulin ranges from 3.4 to 67.5% with various absorption enhancers (99). These studies often describe hypoglycemia as a complication due to higher systemic bioavailability. It is important to note that intranasal insulin administration has not been found to cause hypoglycemia in studies without adjuvant absorption enhancers.

Relative bioavailability comparisons between intranasal (dissolved in a sodium glycholate buffer) and intravenous insulin administration have demonstrated a nasal to intravenous route potency ratio of about 1:8. Time to serum blood glucose level ranged from 5 to 20 minutes and the effect persisted for 30-75 minutes (100).

Relative bioavailability comparisons between intranasal (with di-decanoyl-[alpha]-phosphatidylcholine as an absorption enhancer) and subcutaneous insulin administration demonstrated relative intranasal administration bioavailability of 14.8% and 9.9% at two difference doses of absorption enhancer (101).

*Time to Peak Plasma Insulin Level.* Intranasal insulin administration of up to 150 IU in normal subjects (with di-decanoyl-[alpha]-phosphatidylcholine as an absorption enhancer) demonstrated peak plasma insulin levels at 27.5±5.8 (SD) minutes (101).

*Minimal Effective Insulin Dose Administered Intranasally for Cerebrospinal Fluid (CSF) Penetration.* Intranasal administration of regular insulin without absorption-enhancing adjuvants in normal subjects results in rapid (less than 10 minutes) penetration into the cerebrospinal fluid. The minimal effective dose of regular insulin delivered intranasally is 40 IU (102).

*Peak CSF Insulin Concentration.* Intranasal administration of regular insulin (40 IU) without absorption-enhancing adjuvants in normal subjects resulted in peak CSF insulin concentrations within 30 minutes following intranasal administration (102).

In one Phase I study of 36 normal subjects CSF insulin expressed as area under curve (AUC) with 80 minutes of intranasal insulin administration (40 IU) was significantly elevated (1091.1 pmol/L;±219.8 SEM) in comparison to sterile water placebo intranasal administration (603.2 pmol/L;±34.6 SEM; $p<.05$) (102).

*Duration of Increased CSF Insulin Post-Intranasal Administration.* Intranasal administration of regular insulin (40 IU) without absorption-enhancing adjuvants in normal subjects results in increased cerebrospinal fluid insulin concentrations peaking by approximately 30 minutes and then diminishing to near normal levels at 80 minutes. There is no Phase I CSF insulin concentration data beyond 80 minutes from time of administration available (102).

*Distribution.* Distribution studies of radio-labelled insulin delivered intranasally in mice demonstrated elevated concentrations in cervical spinal cord, dorsal root ganglia, and spinal dura in comparison to mice receiving subcutaneous insulin. Increased concentrations were also demonstrated in olfactory epithelium but not in other tissues such as lung, liver and kidney (103).

*Catabolism.* Insulin is inactivated by enzymatic biotransformations: hydrolyzed by metalloproteinases and reduction, i.e. cleavage of disulfide bonds. Renal insulin elimination is low because, after filtration, it is reabsorbed by the tubule and enzymatic catabolism within the kidney does occur.

## 3.3 Literature review of intranasal insulin administration

### *3.3.1 Safety and tolerability of intranasal insulin in normal and cognitively impaired subjects*

Intranasal administration of insulin has been demonstrated to be a safe and effective technique resulting in increased CSF insulin levels without affecting serum insulin levels in human subjects (102). Circulating glucose and insulin levels also are not affected during prolonged (eight weeks) of intranasal treatment with insulin (160 IU/day) in human subjects (103, 104). Some early concerns arose from animal experiments that suggested that elevated CSF insulin can result in acute sympathoexcitation through its effect on central nervous autonomous centers and result in elevated blood pressure (105). Subsequent human studies have demonstrated an immediate mild rise in blood pressure with high dose (240 IU/day) but no change in blood pressure with daily lower dose administration (160 IU/day) over a period of eight weeks (106). A recent study demonstrated the efficacy of intranasal insulin (40 IU/day) in improving three cognitive outcome measures after 21 days of administration in patients with early Alzheimer's disease without any adverse effects or lowering of blood glucose (107).

### *3.3.2 Nasopharyngeal adverse effects of intranasal insulin*

A randomized, placebo-controlled, double-blinded, cross-over study examined intranasal administration of regular insulin (60 IU once daily) without absorption-enhancing adjuvants in

20 normal subjects described 11 subjects reporting adverse symptoms equally in the treatment and placebo portions of the study (108). Two subjects complained of an unpleasant odour and/or taste after the use of the spray. Nine subjects also felt modest nasal irritation that persisted for a few seconds to a few minutes. One subject reduced the dose frequency due to nasal irritation that lasted 10-20 minutes after each dose. One subject developed spontaneous nasal bleeding once during insulin therapy that resolved quickly. Two subjects reported that they had once had blood in mucus when blowing their nose, one during insulin therapy and the other during the placebo period.

Otorhinolaryngological assessment following completion of the study treatment phase did not identify any irritation of the anterior nasal mucosa on rhinoscopy. Saccharin particle testing for sweet taste recognition, mucociliary clearance time and nasal airway patency measurements utilizing anterior rhinomanometry were unchanged pre- and post-insulin administration (108).

Another randomized-controlled, double-blinded study included 266 children with diabetes receiving intranasal administration of regular insulin (1 IU/Kg or placebo once daily) without absorption-enhancing adjuvants. An otorhinolaryngologist monitored 62 of the children and 2/3 complained of various symptoms of nasal irritation but no objective change in dimensions of nasal passageways were observed by acoustic rhinometry (109).

### *3.3.3 Transient mild hypertension*

Mild elevation of blood pressure has been identified in one Phase I study of intranasal insulin in normal subjects. 95 to 120 minutes following insulin administration mean arterial

pressure and diastolic blood pressure increased by ~12% and ~11% respectively compared to baseline measurements and statistical significance in comparison to the placebo group was observed ($p < .05$ and $p < .04$ respectively). Subsequent blood pressure analyses in the chronic intranasal administration portion of the same study demonstrated no increase in blood pressure between baseline and treatment periods in comparison to the placebo group. The dose of insulin administered intranasally was 240 IU over a two-hour period in the *immediate effect* portion of the study and 40 IU four times daily in the *sub-chronic* portion of the study that lasted eight weeks. Further, the hypothalamic-pituitary-adrenal axis was not stimulated by intranasal administration as indicated by unchanged adrenocorticotropic hormone levels in the subjects (110).

### *3.3.4 Hypoglycemia in normal subjects*

Intranasal administration of regular insulin without absorption-enhancing adjuvants in 20 IU doses every 10 minutes to a total dose of 240 IU over two hours in eight male normal subjects resulted in no hypoglycemic symptoms in comparison to the same eight subjects receiving intranasal sterile water placebo (110).

A second randomized, placebo-controlled, double-blinded, cross-over study examined intranasal administration of regular insulin (60 IU once daily) without absorption-enhancing adjuvants in 20 normal subjects. Two subjects developed one low blood glucose measurement each (< 3.0 mmol/L) but were asymptomatic while on insulin treatment. However, three subjects developed one low blood glucose measurement each while on placebo (108).

Another randomized-controlled, double-blinded study included 266 children without diabetes receiving intranasal administration of regular insulin (1 IU/Kg or placebo once daily)

without absorption-enhancing adjuvants. Throughout a mean treatment duration of 1.7 years there were no hypoglycemic events (109).

### *3.3.5 Hypoglycemia in subjects with diabetes*

Prior investigations of intranasal insulin administration in subjects with diabetes were designed to elucidate whether intranasal delivery could result in adequate systemic glycemic control to obviate the daily subcutaneous insulin requirements. These studies examined systemic delivery through the intranasal portal and due to the low bioavailability of insulin delivered intranasally several absorption-enhancing adjuvants were developed and invariably utilized throughout these studies. Several studies of bile salts, surfactants, polysaccharides, or glycocholate and methylcellulose as absorption-enhancing adjuvants resulted in increased systemic bioavailability of insulin but the amount of insulin remained insufficient for appropriate systemic glycemic control, or nasal irritation was significantly exacerbated and with some, due to the increased bioavailability, hypoglycemic events were more frequent (111).

## 3.4 Rationale for choosing intranasal insulin for DPN

Over the past decade, the Zochodne and Toth laboratories have demonstrated within several animal models of diabetes that low doses of insulin influence the behaviour of motor and sensory axons and neurons without modulating glycemia (43, 112). Over the last two decades, insulin receptors have been identified throughout the central and peripheral nervous systems, located in the brain, spinal cord, dorsal root ganglia and upon peripheral nerves (39). The density of insulin receptors and their machinery are modulated during diabetes with demonstrated reduction of insulin receptor protein during diabetes (40, 41).

Low intermittent doses of insulin administered near diabetic nerve unilaterally corrects

electrophysiological abnormalities in experimental diabetes and promotes regeneration (43). Systemic insulin also promotes nerve regeneration, and intrathecal delivery of insulin, without glycemic impact, accesses and decorates (when immunolabelled) sensory and motor neurons while reversing electrophysiological and structural features of experimental DPN and promoting reinnervation of epidermal skin fibers (112, 113). As well, intrathecal insulin promotes distal axon regeneration in non-diabetic rodents, demonstrating its potent trophic effect (114). Insulin is also capable of reversing mitochondrial abnormalities in sensory neurons exposed to diabetes (115). More recent work has demonstrated that intranasal insulin accesses the brain, cerebrospinal fluid, spinal cord, and dorsal root ganglia (103). This method of delivery is much more practical than intrathecal injection or intrathecal port delivery systems. Intranasal insulin, as demonstrated with intrathecal administration, reversed features of experimental DPN (103). Overall these findings indicate potent actions of insulin itself upon the regeneration of peripheral neurons and in the reversal of both electrophysiological and behavioural changes of DPN.

### 3.5 Rationale for dosage selection

The study drug doses of 20, 40, and 80 IU of insulin delivered intranasally twice daily were chosen based on Phase I normal subject safety data demonstrating no effect on systemic blood glucose levels and significant CSF penetration of insulin.

Phase I safety data also demonstrated that 40 IU four times daily in the for 8 weeks was well tolerated without significant hypoglycemia in normal subjects (110).

### 3.6 Summary

The global burden of DPN is growing with the increasing prevalence of DM. While persons with DM1 only constitute a fraction of the overall DM population their early age of onset often results in accumulation of complications including DPN. Beyond optimal glycemic control there is no treatment to prevent or slow the progression of DPN. Basic laboratory investigations reveal that insulin has a role as a sensory nerve growth factor and that intranasal administration of insulin results in improved penetration to the cerebrospinal fluid and the sensory neuron cell bodies (aka. dorsal root ganglia). Intranasal insulin resulted in slowed progression of DPN in animals receiving intranasal insulin compared to subcutaneous insulin. Intranasal insulin without absorption enhancers has been found to be safe and tolerable in healthy subjects and those with cognitive impairment but has not been studied in subjects with DM1.

## Chapter Four: Study Methodology

### 4.1 Objectives and hypotheses

4.1.1 *Primary objective:* Examine the safety of intranasal insulin in subjects with DM1 and DPN

4.1.1.1 *Primary hypothesis: Intranasal insulin is safe in subjects with* subjects with DM1 and DPN

4.1.2 *Secondary objective:* Examine the tolerability of intranasal insulin in subjects with DM1 and DPN

4.1.2.1 *Secondary hypothesis*: Intranasal insulin is well tolerated in subjects with DM1 and DPN

4.1.3 *Additional secondary objective:* Examine for preliminary evidence of a treatment effect of intranasal insulin in subjects with DM1 on their DPN

4.1.3.1 *Additional secondary hypothesis:* Intranasal insulin is an efficacious treatment for DPN

### 4.1 Feasibility Objectives

*4.1.2 Objective 1:* Examine the willingness of participants to participate.

4.1.2.1 Hypothesis 1: It will take 6 months to recruit 12 subjects into the study at an average recruitment rate of 2 subjects/month

*4.1.3 Objective 2:* Examine the probability of consented participants to successfully pass screening into the study.

4.1.3.1 Hypothesis 2: We estimate that 40% of screened subjects will be eligible to continue in the study to the baseline visit.

*4.1.4 Objective 3:* Intranasal insulin administration compliance.

4.1.4.1 Hypothesis 3: Subjects will be compliant with self-administering the intervention

*4.1.5 Objective 4:* Study procedure compliance will be examined qualitatively to determine whether future study design adjustments should be considered.

4.1.5.1 Hypothesis 4: Subjects will be compliant with study procedures

## 4.2 Study design and plan

This study is a randomized, placebo-controlled, double-blinded, dose escalation Phase II pilot trial of intranasal insulin in the treatment of DPN. The primary objective of our study was to determine the safety and tolerability of intranasal insulin delivery in subjects with DPN. The secondary study objective is to determine whether intranasal insulin is efficacious in slowing the progression of DPN based upon the preliminary data collected.

This study employed dose-finding safety methodology based upon doses previously observed to be safe and well-tolerated in healthy subjects (up to 80 IU twice daily) (116). The range of doses was determined to be 20 IU (the minimum demonstrated effective dose for CSF penetration) twice daily through 80 IU twice daily. Due to cost only three doses could be used and an intermediate dose of 40 IU twice daily was selected rather than 60 IU twice daily primarily for safety considerations being that the increase from the first dose to the second dose tested would be smaller.

### 4.3 Study centre

The Heritage Medical Research Clinic at the University of Calgary (address below) was used for this study. Subject visits took place in the facility and the clinical trial nurse coordinator booked and facilitated each visit. Location: Heritage Medical Research Clinic, Teaching Research Wellness Building, 5th Floor, 3280 Hospital Drive NW, Calgary, Alberta, T2N 4Z6, T: 403 220-3659, F: 403 283-8731.

### 4.4 Equipment and devices

All equipment required is locally available to the investigators:

1. Confocal microscope and technician, Alberta Children's Hospital

2. Clinical electrophysiology (Cadwell); Foothills Medical Centre Clinical Neurophysiology Laboratory; Dr. Douglas Zochodne, Director

3. ViaNase Atomizer Device (Bothell, WA) for intranasal administration of active treatment compound (Regular Insulin) and placebo (saline)

4. Blood glucose monitoring equipment (glucometer (Abbott Diabetes Care, Mississauga, ON, Canada) and strips (Abbott Diabetes Care)

### 4.5 Study duration

The study duration for each subject is 11 weeks with 6 weeks of active study drug or placebo exposure.

## 4.6 Selection of the study population

### *4.7.1 Recruitment*

Subjects were recruited from the University of Calgary Neuromuscular Clinic, Clinical Neurophysiology laboratory and the Diabetes/Metabolism and Endocrinology clinics. Interested subjects were invited to a screening visit and were provided with a letter of information and consent. Subjects were recruited by the study physicians, treating physicians and the study research nurse (S. Mawani).

### *4.7.2 Target population*

The target population is adults (ages 18-70years) with DM1 and DPN.

### *4.7.3 Inclusion criteria*

1. Patients classified as having DM1 according to the Canadian Diabetes Association Criteria (117).
2. Patients clinically defined as having DPN:
    a. Meeting at least two of the following conditions: (1) clinical signs of polyneuropathy; (2) Symptoms of nerve dysfunction; (3) Nerve conduction deficits in at least 2 nerves.
3. Aged 18 through 70 years (inclusive).
4. Body Mass Index (BMI) <30 $kg/m^2$.
5. Reduced but detectable sural nerve potentials (Onset to peak amplitude < 6.0 uV).

### *4.7.4 Exclusion criteria*

1. Any other possible etiology contributing to the neuropathy:
    a. History of prolonged untreated hypothyroidism.
    b. Presence of untreated $B_{12}$ deficiency.
    c. Presence of a paraproteinemia, detected using serum protein electrophoresis with a minimal threshold detection of 2 g/L.
    d. Use of a neurotoxic medication with a clear association with peripheral neuropathy within the past one year based upon clinical impression of association.
    e. Previous exposure chemotherapeutic agents with a clear association with peripheral neuropathy at any time.
2. History of two or more severe hypoglycemic episodes within the previous six months.
3. History of clustering of hypoglycemia episodes within the previous 12 months.
4. History of active or recent (<five years) malignancy.
5. History of systemic or local nasal disease that would complicate the use of intranasal insulin.
6. Presence of diabetic nephropathy requiring dialysis.
7. Presence of active proliferative retinopathy requiring surgery within 6 months.
8. Pregnancy or lactation (female subject of reproductive age must be on contraception).
9. Active cardiovascular disease:

a. Recent angina (less than five years)

b. Recent myocardial infarction (less than five years)

c. Congestive heart failure

10. Active psychiatric disorder or previous history of psychosis.

11. Unable to understand or provide consent.

12. Previously documented hypersensitivity to insulin.

13. History of hypoglycemia unawareness.

14. Ongoing involvement in another investigational drug trial.

### 4.8 Treatments administered

Subjects received either study drug (Regular Insulin, Novo Nordisk, Mississauga, Ontario, Canada) or saline placebo. Study drug or placebo consisted of intranasal delivery via an electronic atomizer (Kurve Technology, Inc., Lynnwood, WA). Dose escalation occurred every two weeks starting at 20 IU twice daily, 40 IU twice daily and a maximum of 80 IU twice daily for a total of six weeks of treatment. Subjects in the placebo group received a volume of normal saline identical to those in the active intervention group without difference in taste, smell or appearance.

### 4.9 Study drug dose escalation

Dose escalation occurred starting at 20 IU twice daily, to 40 IU twice daily and a maximum of 80 IU twice daily. Three dose escalations occurred every two weeks for a total of six weeks study drug treatment duration.

### 4.10 Initial dose administration and observation

At the week five baseline visit of the study, the research team reviewed the logbooks and ensured that no severe hypoglycemic events had occurred. Subjects received instruction on the proper use of the electronic atomizer device. Education regarding hypoglycemia (precipitating factors, signs and symptoms and treatment) was reinforced. Subjects were provided pre-filled glucagon syringes and education on their use in case of worrying symptoms of hypoglycemia. Subjects then self-administered the first dose of either study drug (Regular Insulin 20 IU) or placebo. Each dose was self-administered until the electronic atomizer reservoir was empty and an obvious difference in the sound of the device was observed by the subject. Serial finger prick glucose measurements were then taken every 15 minutes for two hours. Subjects successfully completing the initial administration without hypoglycemia were discharged home with the electronic atomizer and appropriate amount of insulin/normal saline placebo to administer 20 IU twice daily, 30 minutes after the completion of breakfast (between 6am and 10am) and dinner (between 4pm and 8pm), for two weeks.

### 4.11 Dose escalation

Subjects returned for week seven and week nine visits where the dose of insulin was

increased to 40 IU twice daily and 80 IU twice daily respectively. At each visit the subject underwent review of hypoglycemic event frequency and severity and, upon approval of the study physician, proceeded to self-administer the next dose. Serial finger prick glucose measurements were performed as at the week five baseline visit.

**4.12 Dose adjustments**

Subjects not developing mild or severe hypoglycemia during the supervised initial dose administration or severe hypoglycemia after tolerating the initial supervised dose administration continued the planned dose escalation schedule without dose adjustments.

In subjects developing mild or severe hypoglycemia during the supervised initial dose administration the study drug dosage was reduced to the previous dose tolerated for two weeks and supervised initial dose administration occurred for the subsequent visits.

If a subject developed severe hypoglycemia after tolerating the initial supervised dose administration the study drug dosage would be reduced to the previous dose tolerated for two weeks. If the severe hypoglycemia event occurs while on the initial lowest dose the subject would be removed from the study.

**4.13 Study treatment discontinuation criteria**

Study treatment discontinuation would occur if: (1) mild hypoglycemia frequency increased to greater than 30% over the baseline phase; or (2) if a severe hypoglycemic episode occurred in the chronic administration phase of each dose.

Adverse events were monitored by the unblinded study physician. To account for specific reasons for severe hypoglycemia, all severe hypoglycemic episode(s) were reviewed on a case by

case basis by the unblinded study physician to determine subject continuation on study treatment.

### 4.14 Removal of subjects from therapy or assessments

Subjects were removed from further participation from the study at anytime at the discretion of the principal investigator. Indications for removal may have included inappropriate use of study drug or lack of co-operation with the study team.

### 4.15 Blinding

Subjects were blinded to whether they were receiving intranasal insulin or saline. The study physicians and research nurse were blinded to whether the subjects were in the active treatment or control groups. Unblinding would only occur in situations where the attending physician for a patient believed that it was urgently needed due to serious adverse effects potentially related to the treatment. The unblinded study physician (Dr. D. Zochodne) monitored adverse effects and rates of hypoglycemia throughout the study via weekly updates from the study nurse. The research pharmacy was not blinded at any point in the study and provided treatment allocations to the unblinded study physician.

### 4.16 Randomization and concealment

Subjects were randomized by a computer random generation program with study physicians blinded to patient randomization status. Randomization occurred with a 2:1 ratio of study drug to placebo (eight subjects receiving intranasal insulin and four receiving normal saline). No block randomization occurred and study personnel were blinded to the randomization at all times allowing full concealment.

### 4.17 Treatment compliance

Subjects recorded fingerprick blood glucose measurements six times daily in the logbook. Boxes are available in the logbook at two timepoints in each day indicating the times the doses of study drug or placebo was self-administered. Subjects were instructed that filling in the time of the dose administration boxes is mandatory and essential for study result interpretation.

### 4.18 Concomitant medications

Subjects were to remain on all prior medications including subcutaneous insulin injection regimen without adjustments unless required for systemic glycemic control by the subject's family physician or endocrinologist. Changes in doses and medications (including insulin) were reviewed at each study visit and documented.

### 4.19 Concomitant interventions

Subjects were instructed to not initiate additional therapeutic interventions for neuropathy, neuropathy-related pain, or management of diabetes for the duration of the study unless their safety was at risk. Initiation of concomitant intervention was described as a potential reason for withdrawal from the study at the discretion of the principal investigator.

### 4.20 Primary outcome measure

Hypoglycemia monitoring: Subjects recorded blood glucose measurements six times daily and any occurrence of hypoglycemic symptoms or measurements in the provided logbook.

Subjects recorded any associated symptoms into the logbook. Mild hypoglycemic events were measured as events per subject week. Severe hypoglycemic events were measured as a binary variable of having occurred or not.

Outcome measure 1: Occurrence of acute hypoglycemia post-intranasal administration of insulin.

Criteria for non-safety 1: The proportion of subjects developing hypoglycemia (at a given dose) within 120 minutes of administration exceeding 30% at the completion of the study

Outcome measure 2: Number of hypoglycemic events during treatment with intranasal administration of insulin.

Criteria for non-safety 2: Increase in mild hypoglycemic events by 30% in treatment phase (within a given dose interval) or the occurrence of one severe hypoglycemic event.

**4.21 Secondary outcome measures**

Outcome measure 1: Treatment Satisfaction Questionnaire for Medication (TSQM)

Criteria for non-tolerance 1: TSQM score less than 6/10

Outcome measure 2: Adverse effects

Criteria for non-tolerance 2: Qualitative imbalance in the occurrence of adverse effects between treatment and placebo groups using Medical Adverse Effects Dictionary for Regulatory Activities (MedDRA) terms.

Outcome measure 3: The Utah Early Neuropathy Scale (UENS)

Criteria for preliminary evidence of an effect (slowing of progression of DPN) 3: A difference of 4 points between the insulin and saline placebo groups comparing UENS at end of study versus beginning of study (change score)

Outcome measure 4: Corneal confocal microscopy (CCM) nerve fiber branch length and density

Criteria for preliminary evidence of an effect (slowing of progression of DPN) 4: A difference between CCM measures in the treatment versus placebo group (qualitative or quantitative change between end of study versus beginning of study (change score).

Outcome measure 5: Clinical neurophysiology (nerve conductions studies)

Criteria for preliminary evidence of an effect (slowing of progression of DPN) 5: A difference between sensory nerve conductions (sural sensory and radial sensory) in the treatment versus placebo group (qualitative or quantitative change between end of study versus beginning of study (change score).

Outcome measure 6: Visual Analog (VAS) pain scale

Criteria for preliminary evidence of an effect (slowing of progression of DPN) 6: A difference between VAS pain questionnaire score in the treatment versus placebo group (qualitative or quantitative change between end of study versus beginning of study (change score).

### 4.21.1 *Treatment Satisfaction Questionnaire for Medication (TSQM; Appendix B)*

The TSQM was used to assess the overall global impression of the treatment by subjects. The TSQM is a general measure of treatment satisfaction initially derived from an extensive literature review resulting in 55 initial questions. Patient focus groups (n=31) identified the most

relevant questions (n=14) that were then validated in a chronic disease cohort (n=567) demonstrating construct validity for individual satisfaction with medication effectiveness, side effects, and convenience. Reliability (high reliability if it produces similar results under consistent conditions) was assessed through internal consistency estimates which were were good (Cronbach's Alpha 0.85-0.87) across the three constructs. (118)

4.21.2 *Medical Adverse Effects Dictionary for Regulatory Activities (MedDRA) terms*

MedDRA terms were encoded at each visit and are a current international standard for clinical trial adverse event monitoring and reporting and were established and are governed and endorsed by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Adverse effect frequency severity was recorded as well as action taken and outcome. Attribution was applied as the previously described categories.

4.21.3 *Utah Early Neuropathy Scale (UENS)*

The UENS was performed to quantify clinical severity of DPN. The scale was performed by a neuromuscular neurologist at baseline (week 5) and end of treatment visits (week 11) as per the published protocol and using comparable equipment to replicate the physical examination (119). The UENS was developed for the purpose of developing a scale sensitive enough to detect relatively small changes in neuropathy on clinical grounds. As most neuropathies are sensory-predominant the authors were concerned that the existing neuropathy clinical rating scales (i.e. Total Neuropathy Score (120), Michigan Diabetes Neuropathy Scale (MDNS) (121)) were weighted too heavy for motor aspects and were not sensitive to small changes in sensory signs

and symptoms and in particular to quantify small-fiber loss in early neuropathy. The UENS was derived through an iterative process with examination of reliability and sensitivity. The UENS was validated through the enrollment of 206 subjects who underwent clinical examinations, nerve conduction studies, skin biopsy for intraepidermal nerve fiber density determination, and quantitative sensory testing. UENS scores were validated against the gold standard of reporting clinical symptoms confirmed by abnormalities on at least two of: electrophysiology, quantitative sensory testing and/or epidermal nerve fiber density. Strong diagnostic test characteristics including inter-rater reliability (94%), correlation to the MDNS (correlation coefficient 0.758) and a diagnostic sensitivity of 92% higher than other existing scales (i.e. MDNS 62%) with a receiver operating curve (ROC) area under the curve (AUC) of 0.88. (122)

4.21.4 *Corneal confocal microscopy (CCM)*

CCM was used to measure nerve fiber branch length (CNFBL) and density (CNFBD) as surrogate markers of DPN severity. CCM is a non-invasive method to measure nerve fibers within the cornea that has the highest density of small pain fibers (C fibers) within the human body. Diabetes has a predilection for small pain fibers and most often affects them early in the course making CCM an optimal technique for early diagnosis and monitoring of progression (123). CCM was compared to intraepidermal skin biopsy (intraepidermal nerve fiber density (IENFD)) as well as the gold standard of clinical assessment, quantitative sensory testing, autonomic function testing, and nerve conduction studies. A strong correlation was observed between CNFD and IENFD. A correlation was not observed between CNFL and IENFL nor with CNFL and severity of neuropathy (124). Overall, inter-rater reliability of CCM is 0.95, and a specificity of 93%, sensitivity of 91% for a diagnosis of neuropathy (125).

4.21.5 *Nerve conduction studies*

Nerve conduction studies were performed to assess severity of DPN and included measurements of sural and radial sensory nerve action potential and conduction velocities (126, 127). Nerve conduction studies are relatively insensitive for early DPN as they do not assess small fibers. The sensory potentials measured are exclusively large fibers that are often but not always affected in DPN. Test characteristics for sensory conduction velocities and amplitudes vary considerably between reports but are in the range of 59-68% (sensitivity) and 70% (specificity) in one recent study (128).

4.21.6 *Visual analog (VAS) pain scale*

The VAS pain scale was completed to severity of neuropathic pain (129). The VAS Pain scale is unvalidated but serves as a general marker of pain severity that is not specific to neuropathic pain.

**4.22 Feasibility outcome measures**

Feasibility outcome measure 1: Number of subjects recruited into the study per month.

Criteria for non-feasibility 1: 1) Recruitment of less than 50% of subjects expected (6 subjects) at study completion (1 year from study initiation); 2) Greater than twice the expected time interval to recruitment of 12 subjects (1 year)

Feasibility outcome measure 2: Screen pass rate proportion

Criteria for non-feasibility 2: The proportion of screen failures exceeding 60% at the completion of the study

Feasibility outcome measure 3: Proportion of study intervention or placebo administrations completed

Criteria for non-feasibility 3: The overall percentage of overall administrations not completed greater than 50%.

4.22.1 *Additional feasibility outcome measures*

Proportion of study visits attended

Proportion of study procedures completed

Number of protocol deviations per subject

Proportion of subjects completing all study visits to study exit (including subjects discontinuing treatment.

**4.23 Safety and tolerability assessments**

At each study visit subjects reported any adverse effects or hypoglycemic episodes. Any new diagnoses or symptoms or exacerbation of previously described symptoms or conditions were recorded. Adverse events were recorded using Medical Dictionary for Regulatory Activities (MedDRA) terminology and tabulated by body system, maximal severity, medication attributability, and dosage for each treatment group.

### 4.24 Hypoglycemia

Hypoglycemia is defined by: 1) the development of autonomic or neuroglycopenic symptoms (Table 4.1); 2) a low plasma glucose (PG) level (<4.0 mmol/L for patients treated with insulin or an insulin secretagogue); and 3) symptoms responding to the administration of carbohydrate in the absence of a blood glucose measurement. The severity of hypoglycemia is defined by clinical manifestations (Table 4.2) (117).

For the purpose of this study, severity of hypoglycemia was dichotomized into mild and severe (Table 4.3). Hypoglycemia was defined as "mild" if subjects experience autonomic symptoms and the individual is able to self-treat or the subjects experience autonomic and neuroglycopenic symptoms and are able to self-treat.

**Table 4.1** Symptoms of hypoglycemia

| *Neurogenic (autonomic)* | *Neuroglycopenic* |
|---|---|
| Trembling | Difficulty concentrating |
| Palpitations | Confusion |
| Sweating | Weakness |
| Anxiety | Drowsiness |
| Hunger | Vision changes |
| Nausea | Difficulty speaking |
| Tingling | Headache |
| | Dizziness |

**Table 4.2** Canadian Diabetes Guidelines Definitions: Severity of hypoglycemia

| |
|---|
| **Mild:** Autonomic symptoms are present. The individual is able to self-treat. |
| **Moderate:** Autonomic and neuroglycopenic symptoms are present. The individual is able to self-treat. |
| **Severe:** Individual requires assistance of another person. Unconsciousness may occur. PG is typically <2.8 mmol/L. |

**Table 4.3** Study Definitions: Severity of hypoglycemia

| |
|---|
| **Mild:** Autonomic symptoms are present and the subject is able to self-treat. **OR** Autonomic and neuroglycopenic symptoms are present the subject is able to self-treat. |
| **Severe:** Individual requires assistance of another person. Unconsciousness may occur. PG is typically <2.8 mmol/L. |

Hypoglycemia was defined as "severe" if subjects are unable to treat themselves, exhibit neurological symptoms, and had a measured blood glucose <2.8 mmol/l or, if not measured, the clinical manifestations are reversed by oral carbohydrate, subcutaneous glucagon, or intravenous glucose.

Hypoglycemia unawareness was defined as occurring when the threshold for the development of autonomic warning symptoms is close to or lower than the threshold for the neuroglycopenic symptoms; for example, the first signs of hypoglycemia will usually be

confusion or loss of consciousness (117).

### 4.25 Sample size and analysis

Sample size was based on previous Phase II studies of intranasal administration of insulin in subjects with DM1 (21). Twelve subjects were recruited for this pilot RCT. No sample size calculation was performed as this pilot study examined the preliminary safety of intranasal administration and feasibility for a larger intranasal insulin study. As described in the *Dose-finding safety study methodology section* it is common for dose-finding safety studies to not use a sample size calculation to determine the sample size but rather a decision based on previous similar studies and the risk profile of the intervention.

### 4.26 Statistical analysis

*Baseline characteristics*: Baseline characteristics described using means with 95% confidence intervals. Calculated means and medians were comparable and means were used for tests of proportions. Comparisons between treatment and placebo groups were made using unpaired t-tests of means and two-sample tests of proportions. *Chronic administration*: Frequency of mHG events was compared by calculating a change score of the raw number of mHG ((number of mHG per two week interval at given dose) – (number of mHG in baseline interval)) and then calculating a mean (with 95% CI) per group for each dose level. A percent change from baseline was also calculated (number of mHG per two week interval at given dose/number of mHG in baseline interval)*100%. Mean percent change from baseline (with 95% CI) per group was also calculated for each dose level. *Acute administration*: Means with 95% CI were calculated by group and dose for each time point. Unpaired t-tests were performed at each time point for the two independent groups. Lowest blood glucose measurement per group and

dose was also plotted to assess for any evidence of hypoglycemia (<4.0 mmol/L). *UENS*: Change scores were calculated by subtracting the week 11 score from the week five score in each patient then calculating a mean with 95% CI per group. A change in score of two or more points was considered clinically significant. An unpaired t-test of means was performed comparing mean change in UENS between insulin and placebo groups. Nerve conduction studies: NCS were performed in the clinical neurophysiology laboratory (Foothills Medical Centre, Calgary, AB) by one of two neuromuscular neurologists blinded to group allocation and the result of previous nerve conductions. These were performed using standard procedures including ensuring appropriate limb temperature using an infrared thermometer. Warm water immersion was used to elevate limb temperature below 33.0$^{o}$C in the hand and 30.0$^{o}$C in the foot. Cleaning of the skin was performed with an alcohol wipe, attachment of surface electrodes and accompanying wires. Electrical stimulation of motor and sensory nerves were performed an a standard manner (130). Mean change in conduction velocity were calculated as change score and a mean with 95% CI was calculated per group. An unpaired t-test of means was performed to assess for significance between groups.

Intention to treat analysis was used with all subjects receiving at least one dose of insulin or placebo included in the final analysis within their allocated group. Calculation of means, 95% confidence intervals and performance of t-tests used Stata/IC 11.2 for Mac (StataCorp LP, College Station, TX). Graphs were generated using Excel for Mac version 14.3.0 (Microsoft Corporation, Redmond, WA).

### 4.27 Trial approvals and registration

This study has been approved by the University of Calgary Conjoint Health Research Ethics Board (Ethics ID: E-22861). A letter of no objection and medical device approval for the ViaNase Electronic Atomizer were obtained from Health Canada. This study was registered with clinicaltrials.gov (NCT01469559) prior to initiation of recruitment.

# Chapter 5: Results

## 5.1 Baseline characteristics

The baseline characteristics of randomized subjects are summarized in Table 5.1. Subjects in the placebo group had a higher glycated hemoglobin (10.8 versus 7.9 mmol/L; p=.003). There was a trend to longer duration of diabetes, shorter duration of DPN, and high pain scores in the placebo group. Despite the small sample size the UENS and nerve fiber length on corneal confocal microscopy were remarkably similar.

**Figure 5.1** CONSORT flow diagram illustrating the flow of subjects through the trial.

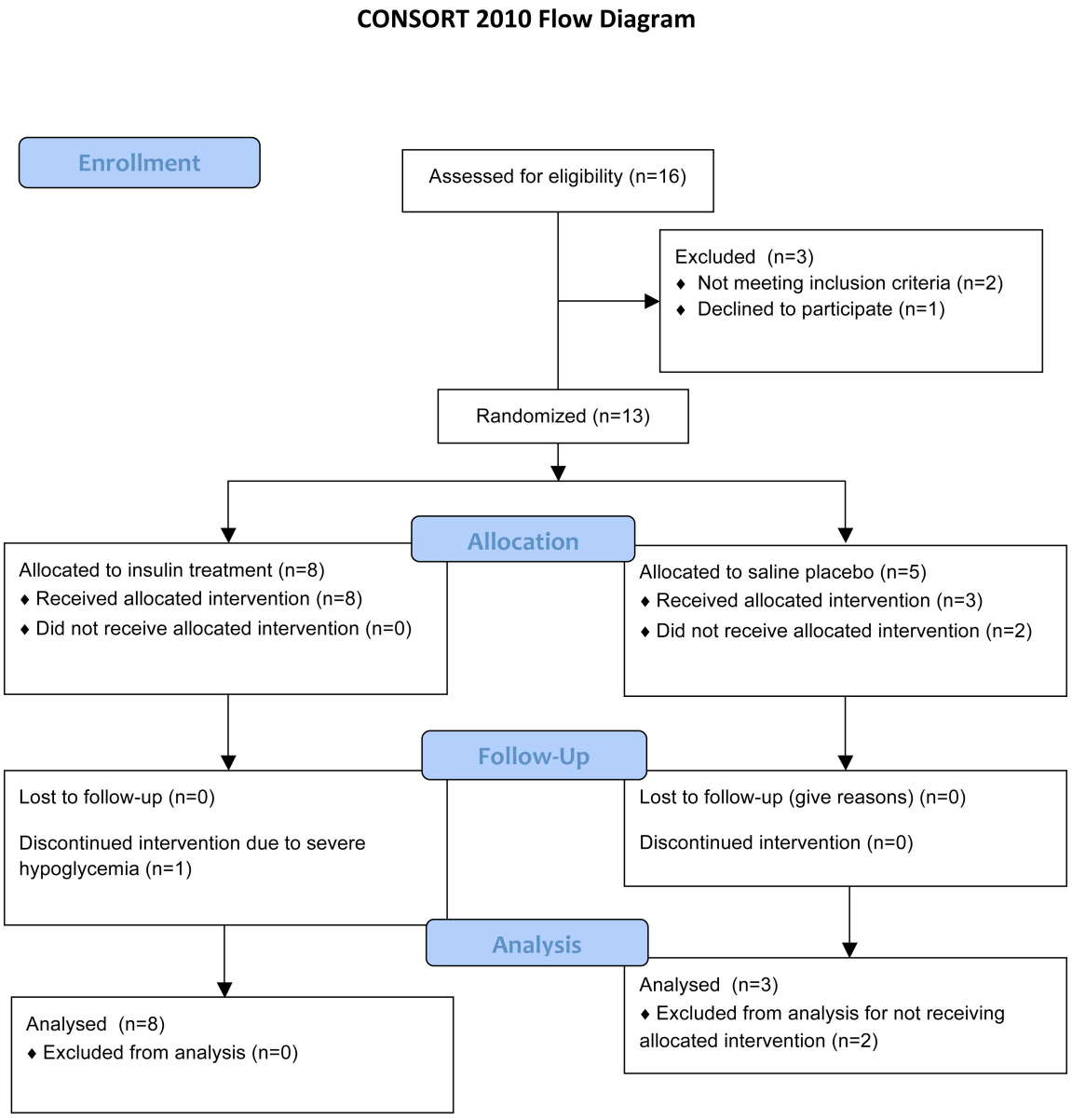


**Table 5.1** Clinical characteristics of randomized subjects

| Characteristic | Treatment (n=8) | | Placebo (n=3) | | p-value |
|---|---|---|---|---|---|
| | Mean | 95% CI | Mean | 95% CI | |
| Age (years) | 51.6 | 43.9, 59.4 | 44 | 11.6, 76.5 | 0.29* |
| Female | 62.5% | | 66.7% | | 0.91$^{\Phi}$ |
| Duration of Diabetes (Years) | 24.5 | 10.1, 38.9 | 30.3 | -15.9, 76.6 | 0.53* |
| Duration of Diabetic Polyneuropathy (Years) | 8 | 1.0, 15.0 | 3 | -2.0, 8.0 | 0.35* |
| Glycated Hemoglobin (%) | 7.9 | 7.1, 8.6 | 10.8 | 4.0, 14.6 | **0.003*** |
| Corneal Nerve Fiber Density (nerves/mm2) | 12.5 | 8.3, 16.7 | 13.2 | 5.9, 20.5 | 0.83* |
| Corneal Nerve Fiber Length (mm/mm2) | 31520 | 20179, 42169 | 32444 | 14543, 50345 | 0.88* |
| Visual Analog Scale Pain score | 48.4 | 23.1, 73.6 | 60 | -71.4, 191.4 | 0.65* |
| Utah Early Neuropathy Score | 14.8 | 10.1, 19.4 | 14.7 | -1.9, 31.2 | 0.98* |
| Sural Nerve Conduction Velocity (m/s) | 25.9 | 7.5, 44.3 | 28.3 | -32.7, 89.4 | 0.88* |
| Retinopathy | 50.0% | | 33.3% | | 0.62$^{\Phi}$ |
| Nephropathy | 25.0% | | 66.7% | | 0.20$^{\Phi}$ |

*denotes two-sample unpaired ttest of means; $^{\Phi}$denotes two-sample test of proportions

## 5.2 Dosing error and analysis

Following completion of the study by the first four subjects it was discovered that the insulin

dispensed was underdosed by 20%. Therefore, two subjects allocated to insulin had received 32 IU/d, 64 IU/d, and 128 IU/d in place of 40 IU/d, 80 IU/d and 160 IU/d respectively. Because our primary outcome was hypoglycemia, the 32 IU/d dose was excluded. For analyses, we created two groups by merging patients receiving 64 IU/d and 40IU/d in one group, and those receiving 128 IU/d and 80 IU/d in another group. We favored adverse effects versus a favorable outcome

**5.3 Chronic Administration**

Subjects received intranasal insulin over two week intervals with review of hypoglycemic event frequency and severity between intervals at doses of 40 IU/d, 80 IU/d and 160 IU/d. The mean raw numbers of hypoglycemic events per interval were compared at each dose to the run-in baseline interval and a mean percent change was calculated.

Comparison of the raw number of mHG events between dose intervals were available in all subjects who had received at least one dose of insulin or placebo and are summarized in Table 5.2. No appreciable difference was observed in mean raw numbers of mHG events or mean percent change in mHG between insulin and placebo groups any dose. The mean change in raw numbers of mHG is a more appropriate reflection of the degree of change in mHG frequency since in two subjects they had zero mHG at baseline preventing mean percent change calculations, and one subject had 1 mHG event in the baseline resulting in a large mean percentage change for even one extra mHG event. In the 80 IU/d group there was little change from baseline in the insulin group and a -74.0% mean reduction from baseline in the placebo group.

**Table 5.2** Changes in frequency of mild hypoglycemic events from baseline to end of each dosing interval

| | **Mean change in raw number of mild hypoglycemic events from baseline** | **95% CI** | **Range** | **Mean percent change in number of mild hypoglycemic events from baseline** | **95% CI** | **Range** | **Number** | **Subject-weeks** |
|---|---|---|---|---|---|---|---|---|
| 20 units twice daily | | | | | | | | |
| Treatment | -0.6 | -3.5, 2.3 | -4, 1 | -12.0 | -65.9, 41.9 | -100, 100 | 8 | 16 |
| Placebo | -2.3 | -9.9, 5.2 | -4, 1 | -4.2 | -228.8, 220.5 | -50, 100 | 3 | 6 |
| | | | | | | | | |
| 40 units twice daily | | | | | | | | |
| Treatment | -0.3 | -3.9, 3.2 | -7, 7 | 6.6 | -54.3, 67.5 | -100, 100 | 9 | 18 |
| Placebo | -3.3 | -6.0, -0.5 | -5, 0 | -74.0 | -109.2, -38.7 | -100, -50 | 4 | 8 |
| | | | | | | | | |
| 80 units twice daily | | | | | | | | |
| Treatment | -1.2 | -6.0, 3.6 | -5, 4 | -16.7 | -115.9, 82.6 | -100, 100 | 5 | 10 |
| Placebo | 3 | - , - | -5 | 300 | - , - | 300 | 1 | 2 |

## 5.4 Elimination of the 160 IU/d dose

The 160/d IU dose was eliminated from the study following a subject who developed an unexpected sHG event requiring administration of glucagon by the spouse. The event was reviewed with the unblinded study physician who advised discontinuation of the study medication for the subject and elimination of the dose for the remaining four subjects (three

insulin, one placebo). The elimination of the 160 IU/d dose resulted in one subject receiving insulin remain on the 80 IU/d dose for four weeks instead of two.

### 5.5 Acute administration

Insulin 20 IU was administered to eight subjects and saline placebo to four. Data is summarized as mean group blood glucose measurements in Table 5.3. No difference in glycemia was observed between insulin and placebo groups at any timepoint between 15 and 120 minutes post-administration (Figure 5.2). Insulin 40 IU was administered to nine subjects and saline placebo to three (Figure 5.3; data summarized in Table 5.4). One subject received insulin 40 IU twice due to discontinuation of the 80 IU dose. Insulin 80 IU was administered to five subjects and saline placebo to three with differences in glycemia were observed (Figure 5.4; data summarized in Table 5.5). No symptoms of hypoglycemia were reported with any dose. There were no occurrences of blood glucose below 4.0 mmol/L to suggest asymptomatic hypoglycemia.

**Figure 5.2** Acute glycemia post-intranasal administration (20 IU)

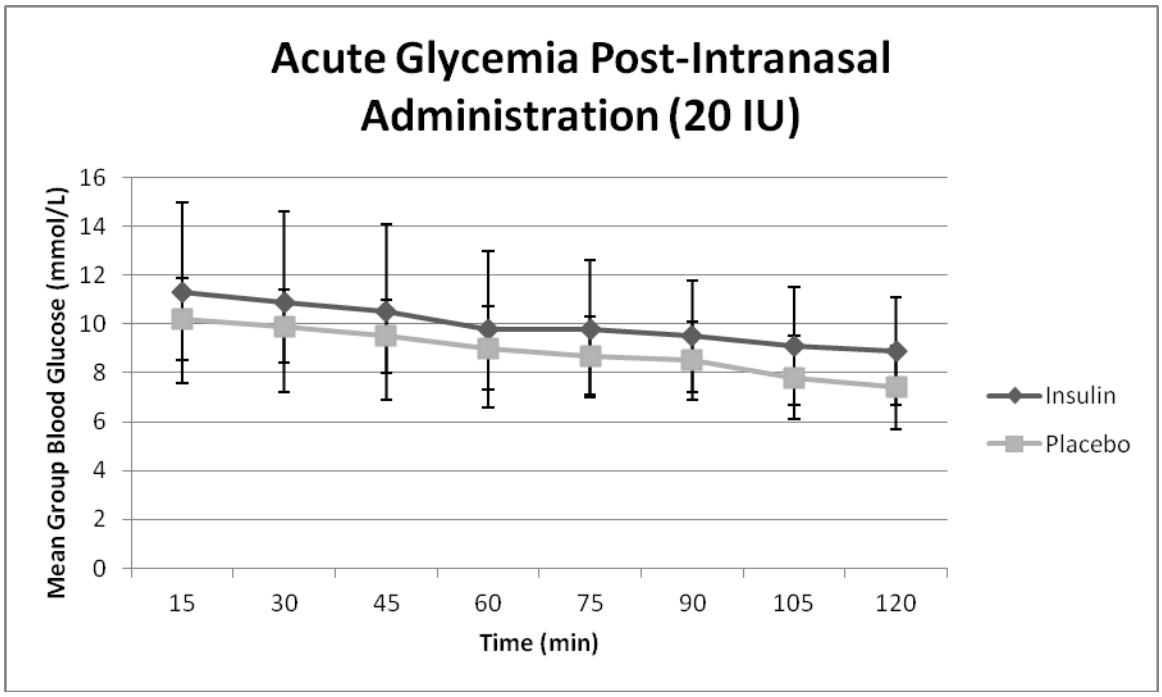



**Table 5.3** Group mean glucose (mmol/L) post-initial intranasal administration of insulin 20 IU

| **20 IU Acute Administration** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time (min) | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 |
| Insulin (mmol\l) | 11.3 | 10.9 | 10.5 | 9.8 | 9.8 | 9.5 | 9.1 | 8.9 |
| Insulin SD (mmol\l) | 3.7 | 3.7 | 3.6 | 3.2 | 2.8 | 2.3 | 2.4 | 2.2 |
| n | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Placebo (mmol\l) | 10.2 | 9.9 | 9.5 | 9 | 8.7 | 8.5 | 7.8 | 7.4 |
| Placebo SD (mmol\l) | 1.7 | 1.5 | 1.5 | 1.7 | 1.6 | 1.6 | 1.7 | 1.7 |
| n | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

**Figure 5.3** Acute glycemia post-intranasal administration (40 IU)

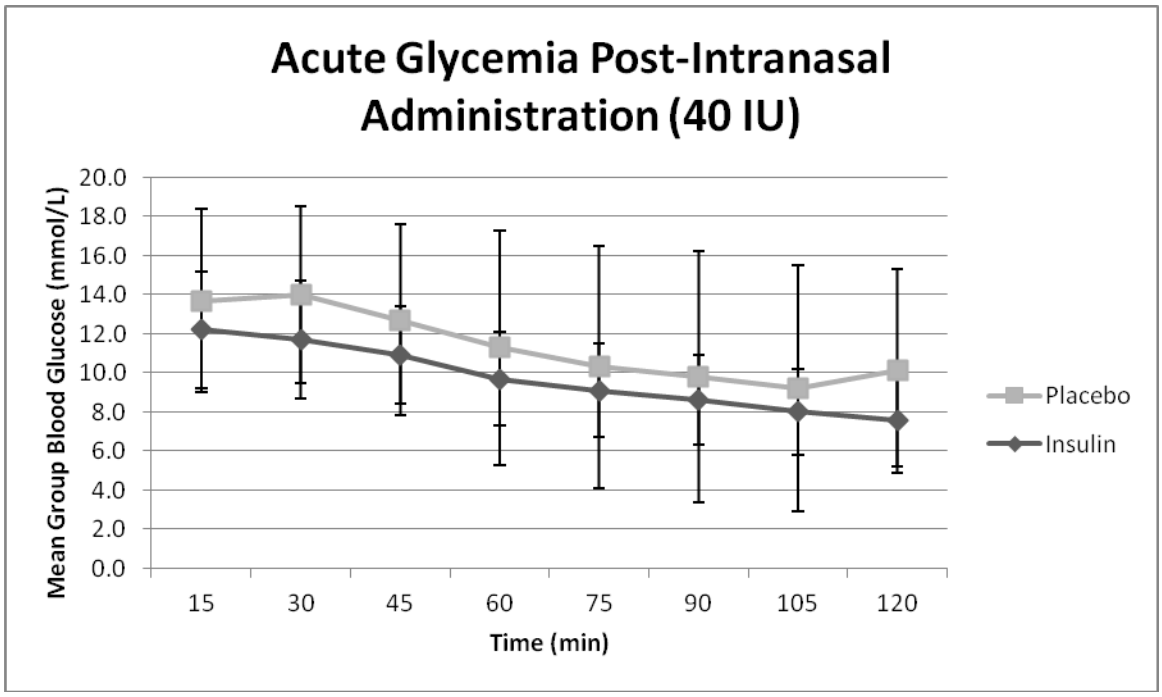



**Table 5.4** Group mean glucose (mmol/L) post-initial intranasal administration of insulin 40 IU

| **40 IU Acute Administration** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time (min) | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 |
| Insulin (mmol\l) | 12.2 | 11.7 | 10.9 | 9.7 | 9.1 | 8.6 | 8.0 | 7.6 |
| Insulin SD (mmol\l) | 3.0 | 3.0 | 2.5 | 2.4 | 2.4 | 2.3 | 2.2 | 2.4 |
| n | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Placebo (mmol\l) | 13.7 | 14.0 | 12.7 | 11.3 | 10.3 | 9.8 | 9.2 | 10.1 |
| Placebo SD (mmol\l) | 4.7 | 4.5 | 4.9 | 6.0 | 6.2 | 6.4 | 6.3 | 5.2 |
| n | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

**Figure 5.4** Acute glycemia post-intranasal administration (80 IU)

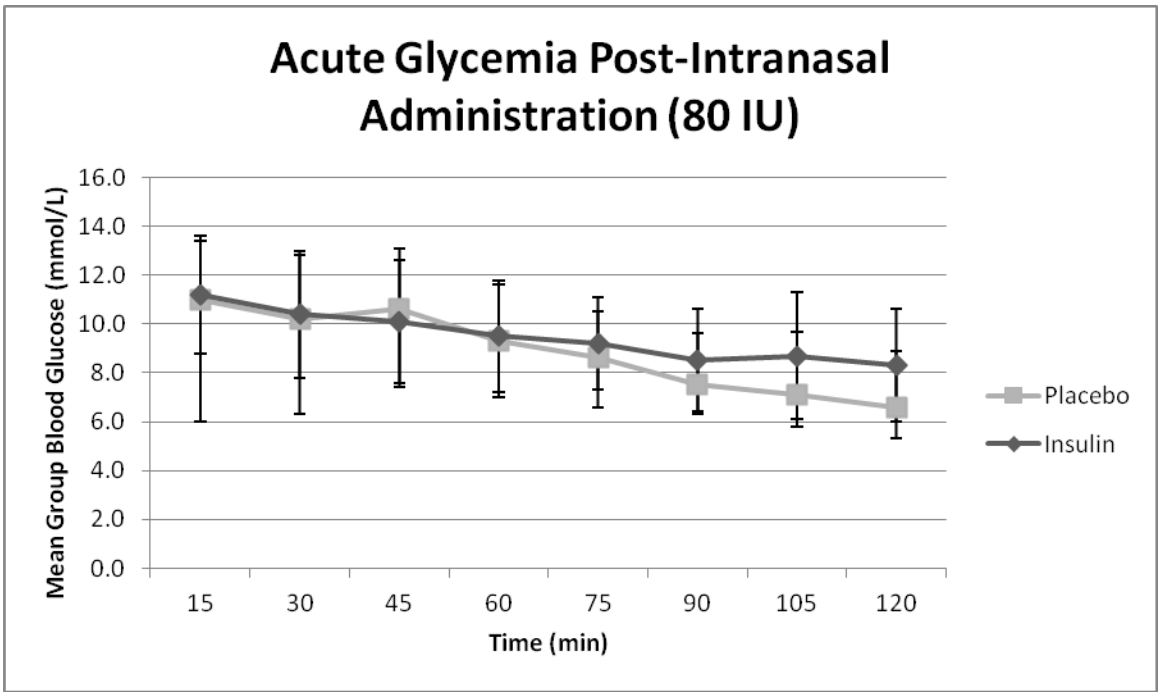



**Table 5.5** Group mean glucose (mmol/L) post-initial intranasal administration of insulin 80 IU

| **80 IU Acute Administration** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| **Time** | **15** | **30** | **45** | **60** | **75** | **90** | **105** | **120** |
| Insulin | 11.2 | 10.4 | 10.1 | 9.5 | 9.2 | 8.5 | 8.7 | 8.3 |
| Insulin SD | 2.4 | 2.6 | 2.5 | 2.3 | 1.9 | 2.1 | 2.6 | 2.3 |
| n | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Placebo | 11.0 | 10.2 | 10.6 | 9.3 | 8.6 | 7.5 | 7.1 | 6.6 |
| Placebo SD | 5.0 | 3.9 | 3.2 | 2.3 | 2.0 | 1.2 | 1.3 | 1.3 |
| n | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

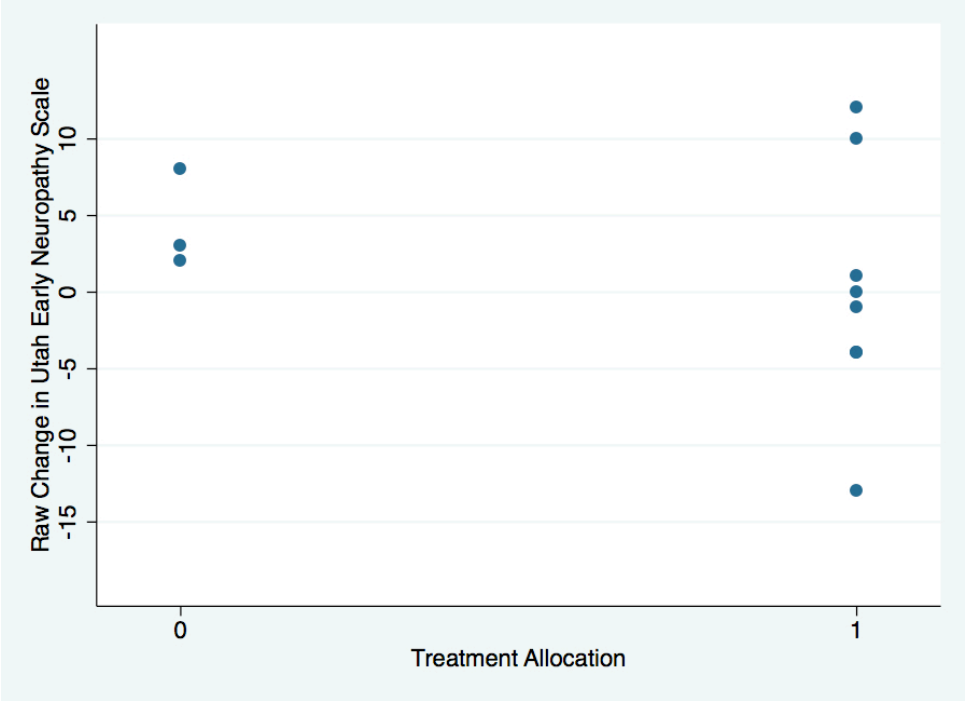
## 5.6 Changes in measures of DPN

Subjects in the insulin group received a total of 44 subject weeks on treatment whereas subjects in the placebo group received saline for a total of 16 subject weeks. Outcome measures were compared between the end of treatment visit (week 11) and the pre-treatment baseline visit (week 5).

### *5.6.1 Utah Early Neuropathy Scale (UENS)*

All three placebo subjects demonstrated worsened neuropathy. In the insulin group 3/8 subjects demonstrated mild improvement, 3/8 no change and 2/8 worsened neuropathy. The mean change in UENS score was 0.1 (95% CI: -6.6, 6.8; n=8) in the insulin group and 4.3 (95% CI: -3.7, 12.3; n=3) in the placebo group (NS; p=.41). The data is summarized in Figure 5.5.

**Figure 5.5** Scatterplot of raw change in UENS from baseline to study completion




*increase in UENS means worsening of neuropathy; Allocation: 1=treatment; 0=placebo.

*5.6.2 Nerve conduction studies*

Mean change in sensory nerve conductions of the sural nerve demonstrated a weak trend in improvement in conduction velocity by 11.6 (95% CI -2.3, 25.5) m/s in the treatment group compared to 2.7 (95% CI -3.1, 8.4) that was not significant (p=.39) with very wide CI due to small sample size. Mean change in sensory nerve conductions of the radial nerve demonstrated a smaller mean deterioration in the treatment group of -0.4 (95% CI -6.9, 6.1) m/s compared to the placebo group -6.2 (95% CI -33.5, 21.0) m/s that was not significant (p=.34).

*5.6.3 Corneal confocal microscopy (CCM)*

A small trend was observed with a more positive mean change in corneal nerve fiber length (NFL) in the treatment group (3790 mm/mm$^2$ (95% CI -1830, 9410)) than the placebo

group (1158 mm/mm$^2$ (95% CI -11481, 13797) but with very wide confidence intervals due to the small sample size (Figure 5.6). There was no difference in mean change in nerve fiber density between the two groups (treatment: 0.81 nerves/mm$^2$ (95% CI -1.21, 2.82); placebo 1.38 nerves/mm$^2$ (95% CI -4.94, 7.70).

**Figure 5.6** Boxplot of raw change in corneal nerve fiber length from baseline to study completion


*decrease in length means worsening of neuropathy; Allocation: 1=treatment; 0=placebo.

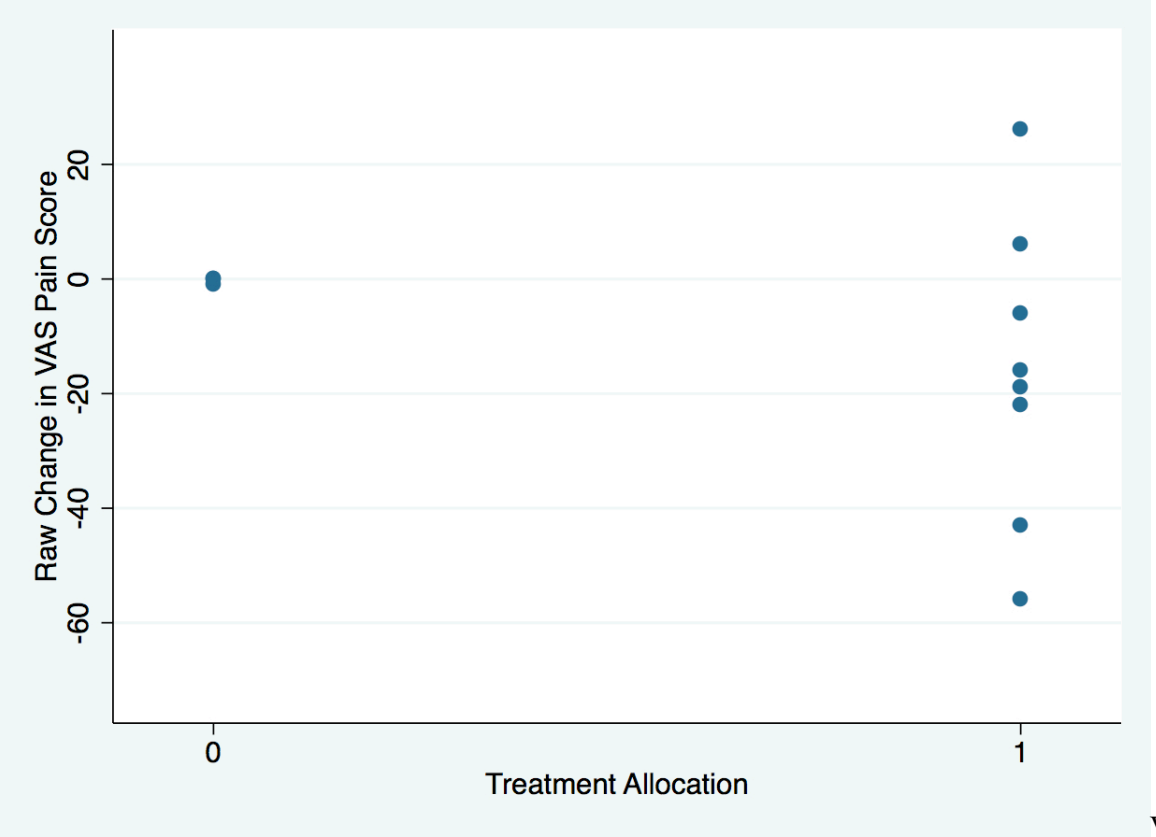
### *5.6.4 Treatment satisfaction*

There was no difference in treatment satisfaction as measured by the TSQM (Treatment score 8.3 (95% CI 6.0, 10.5); Placebo score 8.7 (95% CI 4.9, 12.5); p=.807).

### *5.6.5 Visual analog scale pain score*

A trend to greater reduction in pain was observed in the treatment group (-16.3 95% CI -38.0, 5.5) compared to the placebo group (-0.3 95% CI -1.8, 1.1) that was not significant (p=.33). Data are summarizing in Figure 5.7.

**Figure 5.7** Scatterplot of raw change in VAS Pain score from baseline to study completion




VAS: Visual Assessment Pain Scale; increase in score means worsening of neuropathy; Allocation: 1=treatment; 0=placebo.

### 5.7 Concomitant Insulin Use in Randomized Subjects

Any changes in concomitant insulin use regimen were documented. Subject insulin regimens and changes are summarized in Table 5.6. Only one subject reduced their insulin dose (Insulin Glargine from 21 IU at baseline to 18 IU in mid-study). This subject was in the insulin treatment arm.

**Table 5.6** Subcutaneous insulin regimens in randomized subjects and changes during the study interval

| | Concomitant insulin | Change in dosage during study | Allocation |
|---|---|---|---|
| 10003 | Lantus 7 IU*<br>Humalog 15 IU | No changes | Treatment |
| 10005 | Levenir 20 IU<br>Novorapid 9 IU | No changes | Placebo |
| 10006 | Humalog 15 IU<br>Lantus 10 IU | No changes | Placebo |
| 10007 | Lantus 10 IU<br>Humalog 15 IU | No changes | Treatment |
| 10008 | Humalog 36 IU | No changes | Treatment |
| 10010 | Levemir 18 IU<br>Novorapid 6 IU | No changes | Treatment |
| 10011 | Novorapid 9 IU<br>Levemir 16 IU | No changes | Treatment |
| 10012 | Humulin N 33 IU<br>Humalog 18 IU | No changes | Placebo |
| 10013 | Humalog 45 IU<br>Lantus 54 IU<br>Metformin 250 mg BID | Metformin titrated up to 1000 mg BID | Placebo |
| 10014 | Novorapid 36 IU<br>Glargine 21 IU | Glargine reduced to 18 IU | Treatment |
| 10015 | Levemir 40 IU<br>Humalog 30 IU | No changes | Placebo |
| 10016 | Lantus 16 IU<br>Humalog 11 IU | No changes | Treatment |

** IU represents international units per day*

### 5.8 Adverse effects

Adverse effects are reported in two tables and divided into any occurrence (Table 5.7) and those being categorized as possibly, probably or definitely related to intranasal insulin or placebo (Table 5.8).

Several adverse effects were observed most notably an unusual odor, epistaxis and rhinorrhea. These were observed with similar frequency between treatment and placebo groups. There was no comparator not receiving intranasal administration and thus it is not possible to determine whether the presence of these adverse effects was more than would be expected without intranasal administration but it is possible. Fatigue, vomiting, achiness and diarrhea, attributed to the study drug, were observed in one subject in the treatment group. Importantly the vomiting and diarrhea resolved while on still on intranasal insulin.

Overall, the adverse effects were limited and deemed tolerable by the subjects as supported by the findings of the treatment satisfaction questionnaire.

**Table 5.7 Adverse events (by any occurrence)**

| | Treatment | | | Placebo | | |
|---|---|---|---|---|---|---|
| | Reported | Out of | Percent | Reported | Out of | Percent |
| Headaches | 2 | 8 | 25.0% | 2 | 5 | 40.0% |
| Altered smell sensation | 2 | 8 | 25.0% | 1 | 5 | 20.0% |
| Epistaxis | 1 | 8 | 12.5% | 1 | 5 | 20.0% |
| Rhinorrhea | 1 | 8 | 12.5% | 1 | 5 | 20.0% |
| Nasal congestion and inflammations | 2 | 8 | 25.0% | 0 | 0 | 0.0% |
| Fatigue | 2 | 8 | 25.0% | 0 | 0 | 0.0% |
| Vomiting | 1 | 8 | 12.5% | 0 | 0 | 0.0% |
| Hypoglycemia (severe) | 1 | 8 | 12.5% | 0 | 0 | 0.0% |
| Myalgia | 2 | 8 | 25.0% | 0 | 0 | 0.0% |
| Diarrhea | 2 | 8 | 25.0% | 0 | 0 | 0.0% |
| Nausea | 2 | 8 | 25.0% | 0 | 0 | 0.0% |
| Lightheadedness | 2 | 8 | 25.0% | 0 | 0 | 0.0% |
| Anxiety symptoms | 0 | 0 | 0.0% | 1 | 5 | 20.0% |
| Skin infection | 0 | 0 | 0.0% | 1 | 5 | 20.0% |
| Carpal tunnel syndrome (exacerbation) | 1 | 8 | 12.5% | 1 | 5 | 20.0% |
| Skin irritation | 1 | 8 | 12.5% | 1 | 5 | 20.0% |
| Chills | 1 | 8 | 12.5% | 0 | 0 | 0.0% |
| Corneal abrasion | 1 | 8 | 12.5% | 0 | 0 | 0.0% |
| Abnormal weight gain | 1 | 8 | 12.5% | 0 | 0 | 0.0% |
| Decreased appetite | 1 | 8 | 12.5% | 0 | 0 | 0.0% |
| Increased appetite | 1 | 8 | 12.5% | 0 | 0 | 0.0% |
| Knee pain | 1 | 8 | 12.5% | 0 | 0 | 0.0% |
| Hypothyroidism | 1 | 8 | 12.5% | 0 | 0 | 0.0% |
| Ulcer (gastric) | 1 | 8 | 12.5% | 0 | 0 | 0.0% |
| Nasopharyngitis | 1 | 8 | 12.5% | 0 | 0 | 0.0% |
| Blurred vision | 1 | 8 | 12.5% | 0 | 0 | 0.0% |
| Abdominal pain | 1 | 8 | 12.5% | 0 | 0 | 0.0% |

**Table 5.**8 Adverse events (meeting attribution criteria possible and up)

| | Treatment | | | Placebo | | |
|---|---|---|---|---|---|---|
| | Reported | Out of | Percent | Reported | Out of | Percent |
| Headache | 2 | 8 | 25.0% | 0 | 0 | 0.0% |
| Altered smell sensation | 2 | 8 | 25.0% | 1 | 5 | 20.0% |
| Epistaxis | 1 | 8 | 12.5% | 1 | 5 | 20.0% |
| Rhinorrhea | 1 | 8 | 12.5% | 1 | 5 | 20.0% |
| Fatigue | 1 | 8 | 12.5% | | | |
| Vomiting | 1 | 8 | 12.5% | | | |
| Hypoglycemia (severe) | 1 | 8 | 12.5% | | | |
| Myalgia | 1 | 8 | 12.5% | | | |
| Diarrhea | 1 | 8 | 12.5% | | | |

## 5.8 Blinding

At the final study visit subjects, the study nurse and physicians were asked whether they thought that the subject was allocated to insulin or placebo. The proportion of correct responses [subjects (50%), study nurse (67%), and study physicians (67% each)] suggests that blinding was intact since subjects had a 67% chance of being in the insulin group.

## 5.9 Feasibility

### *5.9.1 Willingness of subjects to participate*

To determine the feasibility of a larger study we examined the willingness of participants to participate. We estimated that it would take six months to recruit 12 subjects into the study at an average recruitment rate of two subjects/month. Our criteria for non-feasibility were: 1) Recruitment of less than 50% of subjects expected (6 subjects) at study completion (one year from study initiation); 2) Greater than twice the expected time interval to recruitment of 12

subjects (one year). During this study twelve subjects were recruited over six months at an average rate of two subjects per month. Non-feasibility criteria 1 and 2 were not met.

### *5.9.2 Screen pass proportion*

We also examined the probability of consented participants to successfully pass screening into the study. Our non-feasibility criteria was: 1) The proportion of screen failures exceeding 60% at the completion of the study. 16 subjects were screened. 13 of 16 (81.3%) subjects met all inclusion and no exclusion criteria. One withdrew prior to randomization. 12 were randomized. The non-feasibility criterion was not met.

### *5.9.3 Compliance with intranasal dose administration*

Our non-feasibility criterion for the overall percentage of overall administrations not completed was: greater than 50%. Overall compliance with intranasal dose administration was excellent (99.8% in the treatment and 100.0% in the placebo groups) and the non-feasibility criterion was not met.

Additional review of feasibility factors revealed that all study visits were attended. Eleven of 12 randomized subjects completed all study visits to study exit.

All study procedures were completed for all 11 subjects completing the study. One subject withdrew after randomization due to inability to reliably attend all remaining study visits. Sixteen subjects provided signed informed consent and attended the screening visit. Four subjects were screen failures. As mentioned, one subject withdrew shortly after randomization. Review of adherence to protocol revealed 37 protocol deviations. 29/37 were a result of scheduling of NCS, CCM and study visits outside of protocol defined windows. These deviations

were exclusively a result of lack of availability of rooms at the Foothills Medical Centre clinical neurophysiology laboratory and the Vision Clinic at Alberta Children's Hospital. Holiday closures at the study centre (TRW Heritage Medical Research Clinic) also contributed. 4/37 protocol deviations were a result of inadvertent underdosing of subjects as described elsewhere. The remaining 2/37 protocol deviations were the result subject unavailability resulting in study visits one to two days beyond the protocol schedule window and 2/37 were the result of the physical examination at the time of the screening visit being deferred to the randomization visit due to lack of investigator time to complete the full visit.

# Chapter Six: General Discussion

## 6.1. The study

We successfully performed a pilot RCT of 12 subjects DM1 and DPN receiving intranasal insulin or saline placebo. The primary outcome measure examined safety of the study treatment through monitoring of hypoglycemia. The finding that a dose of 160 IU/day resulted in one severe hypoglycemic event requires consideration that the 160 IU/day dose may be too high for subjects with DM1 concomitantly administering their routine subcutaneous insulin. There was no significant or obvious difference between insulin and placebo groups in the 40 and 80 IU/day groups suggesting these are safe doses to use in future studies of safety and efficacy.

## 6.2 Higher glycated hemoglobin in the placebo group at baseline

The baseline characteristics of the two groups were very similar, although a significant difference in glycated hemoglobin levels was observed with higher glycated hemoglobin (10.0 %; 95% CI: 8.00, 11.96) in the placebo group compared to the treatment group (7.9 %; 95% CI: 7.09, 8.63). Glycated hemoglobin is a chronic measure of glycemia that is associated with glycemic control. The higher level in the placebo group suggests an imbalance with poorer glycemic control in that group compared to the treatment group. While this study was neither designed nor powered to compare outcome measures of efficacy between groups, examination of the results for trends may be helpful when interpreted with understanding of the study limitations. However, given the imbalance of glycated hemoglobin and thereby glycemic control it would be expected that DPN in the placebo group would progress at a faster rate than in the treatment group.

## 6.3 Other imbalances in baseline characteristics

Trends in imbalances between the insulin and placebo groups were also observed when comparing baseline characteristics. Pain was likely to be more severe in the placebo group. The impact of these findings on the overall interpretation of the study is limited. Safety and tolerability are unlikely to be affected by these imbalances. The measures of efficacy would be more susceptible to resulting bias and may, to some extent, explain the trends in benefit of the insulin over placebo on these measures.

## 6.4 Results of outcome measure assessments

UENS assessments demonstrated no change in the insulin group and a small reduction in the placebo group consistent with mild worsening of neuropathy. The difference between groups was small and non-significant. Given the small sample size these findings are difficult to interpret with certainty. However, at best, if accurate, the slower progression in decrease of the UENS may be related to the poorer glycemic control and more severe neuropathy (as discussed above) in the placebo group rather than a true slowing of progression due to treatment with intranasal insulin. Given the limitations of the study the difference in change in UENS is in an interesting observation that requires further study in a properly powered study. Given the imbalance in glycemic control, it is debatable what the "effect size" should be used for future study sample size calculations. On one hand the effect size is potentially explainable by a confounding factor (differences in glycemic control between groups). On the other hand a four point change on the UENS is likely clinically significant and may represent a useful minimally clinically important difference but for this purpose it would require further study.

Providing further support to the slower progression of neuropathy in the insulin group on UENS is the complimentary observation in the nerve conduction studies performed. A weak trend was observed in the mean change in sensory nerve conductions of the sural nerve improvement in conduction velocity and radial nerve sensory nerve conduction velocity with relative preservation or improvement in the insulin group compared to placebo. Corneal confocal microscopy (CCM) demonstrated no convincing trends. A trend was also seen in the VAS Pain score with potentially greater reduction in pain in the treatment group (-16.3 95% CI -38.0, 5.5) compared to the placebo group (-0.3 95% CI -1.8, 1.1). Overall, the outcome measures of efficacy revealed a weak trend favoring the insulin group but confounded by poorer glycemic control at baseline in the placebo group.

### 6.5 Lack of statistical power

The small sample size of subjects in this RCT precludes conclusive interpretation of hypothesis testing (i.e the t-tests performed). “The power of a statistical test is the probability that the test will reject the null hypothesis when the null hypothesis is false (i.e. the probability of not committing a Type II error, hence the probability of confirming the alternative hypothesis when the alternative hypothesis is true)” (131). Using a calculation to estimate the power of this sample size focusing on the UENS results with mu1=0.1, mu2=4.3, sigma (the combined standard deviation of the change in UENS from baseline to end of study of both groups), and a sample size of eight in the insulin group, the power to detect a significant difference is only 22%. When performing the same calculation for the placebo group (n=4) the calculated power is 13%. The statistical analyses provided are thus only for consideration but are not sufficiently powerful to support any specific conclusions.

## 6.6 Adverse effects

Adverse effects were comparable between groups. Expected adverse effects included altered smell sensation, epistaxis and rhinorrhea were present in both groups. One previous study reported headache and rhinorrhea as adverse events in a study of intranasal insulin for mild cognitive impairment (107). Due to small sample sizes in both studies, it is difficult to compare frequency of the adverse events. Unexpected adverse effects that were more common in the insulin group in our study included headache, fatigue, vomiting, myalgia and diarrhea. These occurred in the minority of subjects and their relationship to insulin remains unclear. Future studies should specifically monitor these adverse effects to improve our understanding of their possible relationship to intranasal insulin.

### *6.6.1 Nasopharyngeal adverse effects*

A previous randomized, placebo-controlled, double-blinded, cross-over study examined intranasal administration of regular insulin (60 IU once daily) without absorption-enhancing adjuvants in 20 normal subjects described 11 subjects reporting adverse symptoms equally in the treatment and placebo portions of the study. Two subjects complained of an unpleasant odour and/or taste after the use of the spray. Nine subjects also felt modest nasal irritation that persisted for a few seconds to a few minutes. One subject developed spontaneous nasal bleeding once during insulin therapy that resolved quickly. Two subjects reported that they had once had blood in mucus when blowing their nose, one during insulin therapy and the other during the placebo period (108).

In our study, 2/8 of subjects (25.0%) receiving insulin and 1/5 of subjects receiving saline (20.0%) also reported altered smell sensation supporting the previous findings that this adverse effect is present in both groups and is likely related to the saline vehicle rather than insulin. Epistaxis occurred in 1/8 of subjects (12.5%) receiving insulin and 1/5 of subjects (20.0%) receiving saline again suggesting a relationship between intranasal administration of saline vehicle rather than insulin itself. Importantly, these adverse effects were considered mild by subjects with both groups scoring high on the 10-point TSQM and with no statistical difference between groups (Treatment score 8.3 (95% CI 6.0, 10.5); Placebo score 8.7 (95% CI 4.9, 12.5); p=.807). Overall, nasopharyngeal adverse effects in our study were mild and well tolerated. Close monitoring of altered smell and epistaxis should be included in future studies as they are consistently observed adverse effects in studies of intranasal administration.

## 6.7 The design

This study was designed as a pilot RCT study that included outcome measures of safety, tolerability, efficacy and feasibility. We included placebo-control, randomization and double-blinding in this study to ensure consistent interpretation of symptoms and signs and their attribution to the study medication. In retrospect the inclusion of these rigorous design elements greatly assisted in the interpretation of the final data.

There were many instances throughout the trial where subjects reported symptoms possibly consistent with hypoglycemia. Placebo-control, randomization and blinding allowed for more objective interpretation of symptoms and attribution to the study medication. Importantly, the blinding was maintained throughout the study as evidenced by the assessment of blinding analysis that revealed intact blinding for subjects, study nurse and study physicians.

## 6.8 Are 40 IU/d and 80 IU/d too low?

This study has demonstrated the safety and tolerability of intranasal administration of insulin doses of 40 IU/d and 80 IU/d. The highest dose tested (160 IU/d) resulted in a single severe hypoglycemic event. This is significant since only subjects with no history of severe hypoglycemic events were included in this study. This pilot RCT cannot confirm that that the 160 IU/day dose is unsafe and will result in an increased risk of severe hypoglycemia in future studies but this finding should be considered to ensure sufficient safety precautions are included in future studies if this dose is included. Further determination of which dose is ideally suited for future study should consider previous studies of cerebrospinal fluid penetration of intranasally administered insulin.

In one Phase I study of 36 normal subjects CSF insulin expressed as area under curve (AUC) with 80 minutes of intranasal insulin administration (40 IU) was significantly elevated in comparison to sterile water placebo intranasal administration (102). Unfortunately, no other dose of insulin was tested.

Given the CSF penetrance data for the 40 IU dose and lack of available of data for the 20 IU dose future studies should consider testing the 40 IU twice daily (80 IU/day) dose for efficacy. Four subjects incorrectly received a reduced dose of approximately 60 IU twice daily and did not experience an increase in mild hypoglycemic events or any severe hypoglycemic events but the sample size is too small to draw conclusions about that dose so they were analyzed with the 80 IU/day group.

## 6.9 Study feasibility and study visit windows

We used several questions with accompanying criteria to assess the feasibility of the study entry criteria and protocol that may be considered when designing future studies of intranasal insulin for DPN. Analysis revealed that this study did not meet any non-feasibility criteria including recruitment rate, screen pass proportion, and compliance with intranasal dose administration. Additional review of factors with no associated criteria demonstrated that all study visits were attended, however, a relatively high number of protocol deviations occurred. The majority (29/37) of protocol deviations occurred as a result of scheduling difficulties at the three study clinics (Foothills Medical Centre Clinical Neurophysiology Laboratory, Alberta Children's Hospital Vision Centre, and Treatment, Research and Wellness (TRW) Heritage Medical Research Clinic). Looking forward to future studies it is clear that the schedules visit windows should be increased. The visit window for this study was ± 3 days which should likely be increased to at least ± 5 days for future studies. Such an increase window would have captured all visits within protocol.

## 6.10 Strengths of the study

This the first study examining safety and tolerability of intranasal insulin in human subjects with DM1. The robust design of the study included placebo-control and randomization. Double-blinding, which on review was successful, was also used. These robust elements of study design support the interpretation of symptoms during the study and their appropriate attribution to the study treatment or placebo. They further support the validity of the outcome measures. Feasibility was examined in a structure manner and the findings support further studies of intranasal insulin for DPN.

## 6.11 Limitations of the study

This study employed a small sample size to address the primary objective of safety and tolerability. The small sample size limited the risk of severe adverse effects to a small, well screened study population but resulted in imbalances in the baseline characteristics of the two groups and insufficient power to interpret the results of the measures of efficacy. The possible small benefit of intranasal insulin for DPN observed in this study must be taken with strong caution and cannot result in any specific conclusions about efficacy. The occurrence of one severe hypoglycemic event at the 160 IU/day dose requires further study to confirm this finding due to this study's small sample size.

## 6.12 Implications of the current study

The current study supports the safety and tolerability of intranasal insulin for the treatment of DPN in subjects with DM1 and a history of good glycemic control, a stable subcutaneous insulin regimen and no recent severe hypoglycemic reactions. The current study also demonstrates excellent compliance to the intervention and supports the feasibility of a larger study from the practical perspectives of recruitment and application of outcome measures. These findings are important, as there is no currently available disease modifying therapy to prevent or slow the progression of DPN beyond effective glycemic control. The findings of this study require further investigation.

## 6.13 Future studies

This study has provided data supporting safety, tolerability and treatment satisfaction of this treatment. Weak trends have been observed that may represent preliminary evidence of benefit of intranasal insulin for DPN but due to imbalances between groups at baseline and lack of power further study is required before any conclusions can be drawn. The adverse effect and

nasopharyngeal reaction profile appears to be consistent with previous studies in normal subjects. This study has also demonstrated the feasibility of studying intranasal insulin in DM1 subjects with DPN.

The next steps require investigation of intranasal insulin in a larger number of subjects to confirm safety and tolerability as well as to further investigate a potential therapeutic effect on DPN. The next study should also include a population of subjects with DM1 and DPN with more variable glycemic control and potentially, a history of more recent severe hypoglycemic events in order to improve the generalizability of the study findings to a higher proportion of the DM1 patient community.

The results support progression to a larger Phase II RCT designed to study the efficacy of intranasal insulin for DPN and to assess safety and tolerability of the intervention in a study population with higher risk of hypoglycemia at baseline. A larger Phase II RCT will provide further safety, tolerability and efficacy data that may justify a Phase III RCT that is powered with sufficient sample size to rigorously assess efficacy and includes a study population that is highly generalizable to the source population of DM1 patients with DPN.

The next study would maintain the design elements of randomization and blinding to enable mitigation of biases and confounders. The study would measure the change in the UENS scores between the initial and the 12 month follow-up visit within each group. Based on expert opinion, the minimal clinically important difference is of 4 points on the UENS scale. In a previous study, the standard deviation (SD) for the UENS score in a cohort of patients with neuropathy was found to be 6.1. (119) Assuming equal variance the change from baseline to follow-up in the control and intervention groups and a conservative correlation coefficient of 0.5 between the first and the second measurement, at a significance level of 5%, to achieve a power

of 90%, in order to detect a change of 4 points UENS score between groups, we would need 49 patients in each group (calculation performed using the formula: $2N = (4(Z_\alpha + Z_\beta)_2\sigma_2)/(\delta_2)$). A review of the clinical trial literature for treatments for DPN revealed varying dropout proportions. The largest dropout proportion occurred in a 7-month trial of alpha-lipoic acid in DPN, where 25% of subjects dropped out. (67) Applying this conservative dropout proportion the number needed to be able to achieve the predicted power will be 62 per group.

A finding of clinically meaningful efficacy in a Phase III RCT would have profound implications on the impact of DPN on patients, health care systems and society.

**6.14 Conclusions**

This first-in-human pilot RCT has provided preliminary data to support the safety and tolerability of intranasal insulin for the treatment of DPN in persons with DM1. Some interesting trends in measures of efficacy were observed that require further study and measures of feasibility demonstrated that a larger study could be performed.

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## Appendix A: The Utah Early Neuropathy Scale (UENS)

Visit Type: 6 dfseq UNS

CRU-021-IIDPN Plate #016

Subject ID #: 7 ptid — 8 ptinit Visit Date: 9 visdate

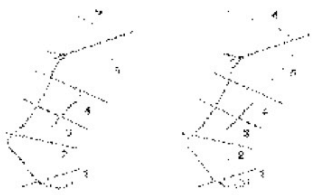
*Center# Patient # Patient Initials* *dd mmm yyyy*

**The Utah Early Neuropathy Scale**

**Motor Examination**
Normal
Weak
Great toe extension: 10 UEN Left 11 UEN Right
Total both sides (out of 4): 12 UEN

**Pin Sensation**
Normal
For each segment with reduced sensation: 13 UEN Left 14 UEN Right
For each segment with absent sensation: 15 UEN Left 16 UEN Right
Total both sides (out of 24): 17 UEN

Segments for pin sensation reporting



**Allodynia/Hyperesthesia**
Normal
If present in toes or foot: 18 UEN Left 19 UEN Right
Total both sides (out of 2): 20 UEN

**Large Fiber Sensation**
Normal
Diminished
Absent
Great toe vibration: 21 UEN Left 22 UEN Right
Time: 23 UEN s 24 UEN s
Great toe joint position: 25 UEN Left 26 UEN Right
Total both sides (out of 8): 27 UEN

**Deep Tendon Reflexes**
Normal
Diminished
Absent
Ankle: 28 UEN Left 29 UEN Right
Total both sides (out of 4): 30 UEN

**Total Score (out of 42):** 31 UEN

**Investigator Signature:** 32 InvSig **Date:** 33 datadate

*dd mmm yyyy*

version date: 19 January 2012

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Study # 21 Plate # 16 Fri Aug 3 12:19:22 2012

## Appendix B: Treatment Satisfaction Questionnaire for Medication

Final Items for the Treatment Satisfaction Questionnaire for Medication (TSQM)[++]

| Item # | TSQM Item |
|---|---|
| 1* | How satisfied or dissatisfied are you with the ability of the medication to prevent or treat your condition? |
| 2* | How satisfied or dissatisfied are you with the way the medication relieves your symptoms? |
| 3* | How satisfied or dissatisfied are you with the amount of time it takes the medication to start working? |
| 4** | As a result of taking this medication, do you currently experience any side effects at all? |
| 5 | How bothersome are the side effects of the medication you take to treat your condition? |
| 6 | To what extent do the side effects interfere with your physical health and ability to function (i.e., strength, energy levels, etc.)? |
| 7 | To what extent do the side effects interfere with your mental function (i.e., ability to think clearly, stay awake, etc.)? |
| 8 | To what degree have medication side effects affected your overall satisfaction with the medication? |
| 9 | How easy or difficult is it to use the medication in its current form? |
| 10 | How easy or difficult is it to plan when you will use the medication each time? |
| 11 | How convenient or inconvenient is it to take the medication as instructed? |
| 12 | Overall, how confident are you that taking this medication is a good thing for you? |
| 13 | How certain are you that the good things about your medication outweigh the bad things? |
| 14* | Taking all things into account, how satisfied or dissatisfied are you with this medication? |

(Atkinson MJ, Sinha A, Hass AL, Colman SS, Kumar RN, Brod M, Rowland CR. Validation of a general measure of treatment satisfaction, the Treatment Satisfaction Questionnaire for Medication (TSQM), using a national panel study of chronic disease. Health Qual Life Outcomes. 2004; 2: 12)

## Appendix C: Schedule of Events

| Activity | Screening Visit Day 1 | Initial Visit Wk 3 | Baseline Visit Wk 5 | Treatment Period | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Wk 6 | Wk 7 | Wk 8 | Wk 9 | Wk 10 | Wk 11 |
| Visit | 1 | 2 | 3 | T1[a] | 5 | T2[a] | 7 | T3[a] | 9 |
| Written Informed Consent | X | | | | | | | | |
| Inclusion/Exclusion Review | X | | X | | | | | | |
| Medical History/ Demographics | X | | | | | | | | |
| Vital Signs/ Weight/Height[1] | X | | X | | X | | X | | X |
| Physical Examination | X | | | | | | | | |
| Neurological Examination | X | | | | | | | | |
| Screening Bloodwork performed | X | | | | | | | | |
| Review of Screening Bloodwork | | X | | | | | | | |
| Hypoglycemia Education and Provision of Blood Glucose Monitoring Supplies | | X | | | | | | | |
| Blood Glucose Record Review for Run-In Hypoglycemic Events | | | X | | | | | | |
| Randomization | | | X | | | | | | |
| Monitored Initial Intranasal Administration of Insulin (Serial Fingerprick Glucose Measurements q15 minutes for 120 minutes) | | | X | | X | | X | | |
| | | | | | | | | | |
| Insulin Dose Escalation | | | | | X | | X | | |
| Safety Bloodwork | | | | | X | | X | | X |
| Dispense Study Medication | | | X | | X | X | X | | |
| Drug Accountability/ Compliance | | | | X | X | X | X | X | X |
| Utah Early Neuropathy Scale Treatment Satisfaction Questionnaire | | | X | | X | | X | | X |
| Nerve Conduction Studies | | | X | | | | | | X |
| Corneal Confocal Microscopy | | | X | | | | | | X |
| VAS Pain Score | | | X | | X | | X | | X |
| Adverse Event Review | | | X | X | X | X | X | X | X |