The Effect of Lysulin on Glycemic Control and Advanced Glycation in Inadequately Controlled Type 2 Diabetes Mellitus: A Double blinded Placebo Controlled Study

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PROTOCOL SYNOPSIS

Protocol Title:	The Effect of Lysulin on Glycemic Control and Advanced Glycation in Inadequately Controlled Type 2 Diabetes Mellitus: A Double blinded Placebo Controlled Study
Site Numbers & Names:	Phoenix VA Health Care System
Research Hypothesis:	Dietary supplement (Lysulin™) reduces blood glucose, hemoglobin A1c and Advanced Glycation Endproducts in individuals with inadequately controlled type 2 diabetes.
Study Schema: Drugs / Doses / Length of Treatment)	Lysulin 3,330 mg/d (two 555mg tablets TID) x 12 weeks
Study Objectives: • **Primary:** • **Secondary:**	The primary objective is to determine whether 12 weeks of treatment with Lysulin, compared to placebo, causes a reduction from baseline in the plasma levels of glucose, hemoglobin A1c (HbA1c) and Advanced Glycation End products (AGEs) in patients with inadequately controlled type 2 diabetes mellitus. Secondary objectives include determining whether 12 weeks of treatment with Lysulin increases beta-cell function as measured by plasma Cpeptide levels.
Study Design	This is a randomized, prospective, double-blind study. Randomization to Lysulin and placebo will be in a 1:1 fashion.
Accrual Goal: (Total number of subjects)	60 subjects for the successful completion of 56 subjects
Accrual Rate: (Number of subjects expected per month)	15 subjects/month
Correlative Studies: (PK/PD, etc.)	N/A
Inclusion Criteria:	1. Signed written informed consent 2. Able to communicate meaningfully with the investigator and legally competent to provide informed written consent 3. Men and women, ages 21-75 years 4. Diabetes, with HbA1c ≥ 7.5 % and < 10% within 6 weeks of screening 5. Stable dose of insulin (<20% change in units) 6 weeks prior to enrollment
Exclusion Criteria:	**1) Sex and Reproductive Status** a) Women of child-bearing potential who are **unwilling or unable** to use an acceptable method to avoid pregnancy for the entire study period. b) Women who are pregnant or breastfeeding

	2) Target Disease Exceptions a) Gastrointestinal disease (including gastrectomy, chronic pancreatitis, bariatric surgery and gastroparesis) b) Hepatic disease (ALT, AST >2.5 times the upper limit of normal) c) Kidney disease (GFR ≤50 mL/min) d) Hypertension (blood pressure >160/95 mmHg) at screening for the mean of three consecutive readings performed in a sitting position after a 5-minute resting period. If treatment for hypertension has recently
	been initiated, subjects must be clinically stable for 4 weeks prior to screening. e) Cardiac disease (myocardial infarction within past 6 months, clinically significant arrhythmia, unstable angina, uncontrolled congestive heart failure, or coronary artery bypass surgery within 1 year or expected to require coronary bypass surgery within 12 months of study entry) **3) Medical History and Concurrent Diseases** a) Type 1 diabetes mellitus b) Active malignancy other than basal cell or squamous cell skin cancer (cancers in remission or stable and not receiving active therapy permitted) c) Significant clinical allergic rhinitis or asthma, regularly requiring inhaled corticosteroids and/or antihistamines d) Chronic inflammatory disease (e.g. Rheumatoid arthritis) **4) Additional Laboratory Test Findings** a) Hemoglobin <12 g/dl in men, <11 g/dl in women **5) Allergies and Adverse Drug Reactions** History of a serious hypersensitivity reaction to compounds containing lysine, such as anaphylaxis, angioedema, or exfoliative skin conditions. **6) Prohibited Treatments and/or Therapies** a) Regular use of supplements containing lysine, zinc or vitamin C for the last 3 months b) Current or expected treatment with any of the following medications at screening: systemic glucocorticoids (for >2 weeks), antineoplastic agents, transplant medications, drugs for weight loss or anti-retroviral medications c) Treatment with antihistamines or inhaled corticosteroids within 3 months prior to screening d) Start or change of hormonal replacement therapy within 3 months prior to screening **7) Other Exclusion Criteria** a) Prisoners, or subjects who are involuntarily incarcerated b) Subjects who are compulsorily detained for treatment of either a psychiatric or physical (e.g., infectious disease) illness c) Currently abusing alcohol or drugs, or have a history of alcohol or drug abuse that in the investigator's opinion could cause the subject to be non-compliant; or have a general history of non-compliance with medications d) Any acute febrile illness within 2 weeks of screening with a temperature ≥ 100°F

Criteria for Evaluation: Efficacy, safety, stopping rules, etc.)	Study participation will be stopped for adverse effects at the discretion of the study investigator. Subjects may stop study participation at any time and for any reason. Enrollment may be stopped due to futility or efficacy by interim analysis.
Statistical analysis:	A linear mixed effects model will be used to evaluate treatment-induced endpoints. The models will be fit for sequential values of the response variable (including the baseline measurement).
Statistical power:	54 participants will be required to complete the study to detect a of 0.7% points reduction in HbA1c and 19 mg/dl reduction in fasting plasma glucose levels with 80% power at the alpha level of 0.05. This corresponds to SDs for HbA1c 0.9% points and fasting glucose 25 mg/dl.

BACKGROUND AND SIGNIFICANCE

Type 2 diabetes mellitus (T2DM) is a progressive disease and is associated with long term microvascular complications such as retinopathy, nephropathy, and neuropathy as well as cardiovascular morbidity and mortality. There is direct link between the levels of glycemia and the development of diabetes complications[1,2]. Glucose lowering trials conducted in type 1 diabetes (T1D) and in new onset type 2 diabetes (T2D) patients supported the concept that improving glucose control was an effective method to reduce diabetes microvascular complications[3,4]. Although individually these trials did not show statistically significant reductions in cardiovascular disease (CVD), observational follow-up of these trials suggested improved glycemic control reduces cardiovascular outcomes and may reduce mortality[5,6]. In contrast intensive glycemic control for up to 6 years has at best only modestly reduced the development of CVD and renal disease in people with longstanding, inadequately controlled T2D[7-11]. To achieve adequate glycemic control in patients with chronic T2D frequently requires combination of diabetes agents at increasing dosage resulting in increasing risk of side effects, including hypoglycemia, weight gain, edema and changes in blood lipid profile, that may counteract the benefactor effects of glucose lowering. Another explanation for the modest and slow benefit of glucose lowering on diabetes complication, is that chronic hyperglycemia has built up a legacy of vascular and kidney injury which is not easily or rapidly resolved[12]. This may in part be due to increased tissue levels of advanced glycation end-products (AGEs) that formed by the non-enzymatic glycosylation of reducing sugars to amino-groups of proteins, lipoproteins or nucleic acids[13,14]. Multiple clinical and epidemiological studies indicate an association between AGEs/glycoxidation products diabetes complications[15-24]. We have recently demonstrated that several plasma “free” AGEs from a panel measured by LC-MS predicted long-term progression of coronary and carotid atherosclerosis in a subset of participants of the Veterans Affairs Diabetes Trial (VADT)[25]. Increases in several AGEs

also appeared to predict incident CVD events in the same VADT subset as well as in a small subcohort of the Action to Control Cardiovascular Risk in Diabetes (ACCORD)[26].

Given the importance of chronic hyperglycemia and AGEs in the development of diabetes complications, there has been substantial interest in treatments that in addition to improving glucose control may also rapidly AGE levels. Lysulin™ is a nutraceutical tablet which contains the essential amino acid lysine, a micronutrient zinc and vitamin C as the active ingredients, together with other standard excipients (Lysulin Inc, San Diego, CA) (www.lysulin.com). When supplemented separately, all 3 ingredients reduced blood glucose in both animal models as well as humans with T2D[27-30]. The mechanisms of action appear to include improvement of both major mechanisms of T2D, insulin sensitivity and insulin secretion[27-30]. Importantly, lysine also reduced AGE formation[31,32]. To support the glucose-lowering efficacy of Lysulin, preliminary single-arm study in patients with T2D reported a significant, on average 10% (or 0.9 percent point) reduction in HbA1c after 12 weeks of treatment with 3.3 g daily Lysulin™. Given its minimal toxic profile due to natural character of all ingrediencies, the addition of Lysulin™ to current diabetes agents may improve glycemia in parallel with reducing dosage and variety of diabetes medications resulting in lower risk of side effects.

HYPOTHESIS

Administration of Lysulin™ for 12 weeks will improve glycemic control in T2D patients with inadequate glycemic control. Lysulin™ may also reduce AGE levels and increase beta-cell function.

STUDY APPROACH

Trial Overview: This will be a 3-month, randomized, placebo-controlled, double-blinded trial that tests the effects of Lysulin™ on glycemic control and AGEs levels in blood, and renal function in T2D patients, when added to the usual care for glycemic control in a standard primary care setting.

Trial Design: 60 patients with Type 2 DM with inadequate glycemic control will be randomly allocated in blocks for 3 months to Lysulin™ (1,110 mg TID, i.e. 3.3 g/day) or matching placebo. Fasting plasma glucose and HbA1c will be measured at baseline and then at week 6 and week 12 (visit windows at week 6 and 12 visits will be -7/+3 days). Plasma AGEs, creatinine and Cpeptide levels will be determined at baseline and at the end of the study. Lipid levels will be measured at baseline, week 6 and at the end of study.

Inclusion criteria: Age 21-75 years, Type 2 diabetes mellitus, HbA_{1c} ≥7.5 % and <10.0% within 6 weeks of the screening visit treated non-pharmacologically with diet or stable doses of oral antihyperglycemic agents with or without insulin, stable body weight (< 5% change in last 3 months). Patients on insulin therapy will to be on a stable dose, i.e. < 20% variation in insulin units, 6 weeks prior to the study.

Exclusion criteria: Type 1 DM, current or recent use of supplements containing lysine, zinc or vitamin C, uncontrolled hypertension (blood pressure ≥160/90 mmHg), kidney disease (serum creatinine GFR ≤50 mL/min, major illness, severe gastrointestinal disease, pregnancy, liver function tests > 2.5 times normal values in the past 3 months, currently abusing alcohol or drugs, or have a history of alcohol or drug abuse that in the investigator's opinion could cause the subject to be non-compliant; or have a general history of non-compliance with medications.

Study Visits Schedule: All visits will be performed in the Clinical Research Center (CRC) at the Phoenix VA. Completion of the entire study will involve up 3-4 visits to the CRC. After initial visit for obtaining of informed consent and screening, qualified patients will undergo a baseline testing (on the same day as screening visit or on a separate day), including blood collection for baseline measurements, and will be randomized to treatment (Lysulin™ or matching placebo). Study medication will be dispensed to the subject in a double-blinded fashion by Phoenix VA research pharmacist. Subjects will be instructed to take two tablets orally with breakfast, lunch and dinner. They will return to the CRC for clinical evaluation and blood draws after 6 and 12 weeks (final visit). Subjects will be instructed to not make major lifestyle modifications, including changes to their diet or exercise, during their study participation. At least one phone call contact will take place between interim visits to ensure medication is being taken appropriately and to help address any study related questions. At each visit a comprehensive assessment of side effects, medication compliance/competency and concurrent medical history will be obtained and entered into the database. Additional brief visits may occur as needed to ensure participant comfort/competency with treatment.

Study endpoints:

Primary: Fasting plasma glucose and HbA1c concentrations

Secondary: Fasting plasma AGEs, C-peptide concentrations and lipid measurements

Recruitment Approach: Participants will be recruited utilizing VA electronic medical records to identify potential candidates meeting inclusion criteria, as well as from the Phoenix VA primary care, specialty and women's clinics, community diabetes clinics, ads in VA newsletters and flyers,

permission to be contacted forms, on hold message, social media, clinic list, BISL, list of A1c results from lab, and through VINCI.

Diabetes care during the study: The research team will provide diet and exercise guidance at baseline and review at subsequent study visits, and monitor side effects, medication changes or general health issues of all subjects. We will notify relevant PCP providers of study activity to avoid unnecessary diabetes medication changes during the study. Addition of diabetes agents will be discouraged, but of course supported if clinically indicated. However, general and diabetes healthcare and routine lab tests will be conducted by the PCP as warranted. With hypoglycemia, excessive hyperglycemia or other adverse events - we will work with the participant and/or primary care team to evaluate/change contributory diet or behavior and/or reduce/modify other diabetes medications as appropriate.

Biochemical analyses: Plasma glucose, HbA1c, creatinine and all safety screening will be measured at the VAMC clinical laboratory using established automated methods. Plasma Cpeptide levels will be measured by commercial ELISA kit in the VAMC research laboratory. AGEs will be measured by LC-MS/MS utilizing internal stable heavy isotope substituted standards (Preventage Healthcare technology) if initial data analysis indicates Lysulin had a rapid and favorable effect on HbA1c values.

Data Handling: All participants will be given non-identifying study IDs and all case report forms and samples are labeled with this information. The code for these IDs is stored in locked files in the locked office of the project manager. Only approved VA research employees will have access to study samples and data.

Statistical Analysis Plan: Univariate numerical and graphical procedures will be used to explore distributional characteristics and dependence structure of measured variables. Data transformations (e.g., logarithm) may be used to improve distributional characteristics and/or to improve linearity of relationships between response and covariates. A linear mixed effects model will be used to evaluate treatment-induced endpoints (glucose, HbA1c, AGEs, C-peptide). This approach allows the inclusion of potential covariates, and explicitly accounts for repeated measurements on each patient (and patient-to-patient variability). We will fit models to all sequential values of the response variable (including the baseline measurement), rather than computing a change score. This structure allows statistically efficient evaluation of treatment effects[33] and allows modeling of differential treatment effects for patients with varying initial glycemic control.

Power Calculation: 54 participants will be required to complete the study to detect a of 0.7% points reduction in HbA1c and 19 mg/dl reduction in fasting plasma glucose levels with 80% power at the alpha level of 0.05. This was based on SDs for HbA1c=0.9% points and fasting glucose=25 mg/dl from our previous study in patients with > 5 years duration of type 2 diabetes [34].

Interim analysis: Using a 2-stage group sequential trial design, an interim analysis will be performed by an independent statistician after completing first 28 participants, i.e. 14 per group. The sample size for interim analyses as well as the boundary values for the treatment effect were computed by SEQDESIGN procedure of SAS v9.4. The SEQTEST procedure will compare the test statistic with the boundary values at the interim stage so that the study can be stopped to reject or accept the null hypothesis. If the interim analysis will indicate futility, the enrollment of new patients will be discontinued. All active participants will complete the study as planned.

Ethical considerations: The study is testing a potential benefit of a diabetes supplement and is not providing a direct clinical benefit to the research subject. Participation in the study will be voluntary and won't affect standard of care. The VA has very strict research guidelines regarding clinical research. All protocols receive IRB, safety, scientific and privacy committee reviews and approval before being initiated. Audits of all studies are conducted by the compliance officer on annual basis: these include consent audits; case report form audits and other study conduct activity. All studies and their adverse events are also reviewed annually by the IRB. The study will be conducted in accordance with the Declaration of Helsinki, all local and national VA policies, and adhere to ICH GCP guidelines.

Payment Schedule: Study participants will be reimbursed $15 for screening visit and $25 for each completed study visit for up $115 for the whole study.

Reporting of Adverse Events: There is a VA policy for reporting serious adverse events, unexpected/unanticipated adverse events, and standard adverse events. Depending on the category of the event, it is reported within 5 days or annually to the IRB.

The sponsor-investigator will be responsible for reporting of all serious adverse events (SAE), unexpected/unanticipated adverse events, and standard adverse events to the appropriate authority and independent ethics committee/institutional review boards based upon federal regulations and local/IRB policies.

If the SAE or problem is UNANTICIPATED (i.e., it reflects a RISK that is NEW or GREATER than previously known) then this would be reported more rapidly and within the required local expedited

policy time frame but if the AE or problem was ANTICIPATED, this would be reported in accordance to the local policy (e.g., which may be an AE reported at continuing review).

Adverse Event Definitions

Adverse Event (AE): An AE is any undesirable medical event occurring to a subject in a clinical trial, if related to the trial product(s). This includes events reported from the first trial related activity after the subject has signed the informed consent and until post treatment follow-up period as defined in the protocol. The following should not be recorded as AEs, if recorded as medical history/concomitant illness on the CRF at screening:

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the subject has signed the informed consent
- Pre-existing conditions found because of screening procedures

Clinical Laboratory Adverse Event: A clinical laboratory AE is any clinical laboratory abnormality regarded as clinically significant i.e. an abnormality that suggests a disease and/or organ toxicity and is of a severity, which requires active management, (i.e. change of dose, discontinuation of trial product, more frequent follow-up or diagnostic investigation).

Serious Adverse Event (SAE): A serious AE is an experience that at any dose results in any of the following:

- Death
- A life-threatening* experience
- In-patient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening*, or required hospitalization may be considered an SAE when, based upon appropriate medical judgement, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Unanticipated (Unexpected) Adverse events: The terms "unanticipated" and "unexpected" refer to an event or problem in VA research that is new or greater than previously known in terms of nature, severity, or frequency, given the procedures described in protocol-related documents and the characteristics of the study population.

Serious unanticipated problems involving risks to subjects or others include:

(1) Interruptions of subject enrollments or other research activities due to concerns about the safety, rights, or welfare of human research subjects, research staff, or others.

(2) Any work-related injury to personnel involved in human research, or any research-related injury to any other person, that requires more than minor medical intervention (i.e., basic first aid), requires extended surveillance of the affected individual(s), or leads to serious complications or death.

Serious Adverse Drug Reaction (SADR): An adverse drug reaction (ADR) is an adverse event for which a causal relationship (Possible/Probable relation) between the study drug and the occurrence of the event is suspected. The ADR should be classified as serious if it meets one or more of the seriousness criteria.

Non-Serious Adverse Event: A non-serious AE is any AE which does not fulfil the definition of an SAE.

Severity Assessment Definitions:

- Mild: Transient symptoms, no interference with the subject's daily activities
- Moderate: Marked symptoms, moderate interference with the subject's daily activities
- Severe: Considerable interference with the subject's daily activities, unacceptable

Related AE or a Related Problem: A "related" AE or a "related" problem in VA research is an AE or problem that may reasonably be regarded as caused by, or probably caused by, the research.

Collection, Recording and Reporting of Adverse Events: All events meeting the definition of an adverse event must be collected and reported from the first trial related activity after the subject has signed the informed consent and until the end of the post-treatment follow-up period as stated in the protocol.

Follow-up of Adverse Events: During and following a subject's participation in a clinical trial, the sponsor-investigator and institution will provide adequate medical care to the study subject for

any study-related adverse events, including clinically significant laboratory values related to the study as per local VA policy.

All adverse events classified as serious or severe or possibly/probably related to the trial product must be followed until the subject has recovered, is recovering, or the condition has reached a new stable condition, and all queries have been resolved according to local VA policy.

Pregnancy: Study subjects will be instructed to notify the sponsor-investigator immediately if they become pregnant. Pregnancy complications should be recorded as adverse event(s). If the infant has a congenital anomaly/birth defect this must be reported and followed up as a serious adverse event.

Overdose: An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

Timeline: We anticipate completion of study about 8 months after recruiting the first patient.

Implications and Future Directions: If successful, this study may provide support for adding Lysulin or comparable supplements as add-on treatment in T2D. However, definitive confirmation of these findings and a more thorough exploration of the relevant signaling events will be needed and could be effectively pursued using animal and cell models. Future long-lasting RCT will be needed to establish whether these types of supplements provide long-term improvement of glycemic control and reduce the risk of diabetes complications.

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