

Combination of Linagliptin, metformin and lifestyle to prevent type 2 diabetes  
in high risk patients with prediabetes

**PRELLIM Project** (*Diabetes Prevention with Lifestyle, Linagliptin and Metformin*)

Research Protocol

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## Introduction

Type 2 diabetes (T2D) is a worldwide health problem, reaching estimated prevalence around 18 % in countries like Mexico, and an unstoppable increase in the number of new cases, having a big economic impact due mainly to micro and macrovascular complications, and being the first cause of mortality in Mexico (1, 2)

Physiopathology of hyperglycemia is complex and multifactorial in T2D. It is known that insulin resistance (IR) is one of the first observed abnormalities, leading to a decrease in the glucose uptake by muscle, increase in hepatic glucose output and lipolysis; different factors have been involved in IR (3-5). IR stimulates insulin secretion from the pancreatic  $\beta$  cells, causing hyperinsulinemia to compensate IR and maintain normal glucose levels; however, if IR persists,  $\beta$  cell can be damaged by different factors; incretin defect, oxidative stress, and amyloid deposition (3, 6-8), causing a reduction on  $\beta$ -cell function and mass up to 50-80 % (3, 4, 6, 9-12). Together with pancreatic  $\beta$ -cell dysfunction,  $\alpha$ -cell dysfunction and hyperglucagonemia have been also involved in the development of hyperglycemia (6, 13-16); glucagon is one of the main regulators of hepatic glucose production, and together with the reduction on incretin hormones (GLP-1, GIP) and amyloid deposition are physiopathological defects that contribute to pancreatic islet dysfunction and hyperglycemia (17-21).

Prediabetes is a condition with intermediate hyperglycemia, and based on the American Diabetes Association criteria(22) can be defined as: i) impaired fasting glucose (IFG): FPG  $\geq 5.5$ mmol/L [ $\geq 100$ mg/dl]; ii) impaired glucose tolerance (IGT): 2 h plasma glucose  $\geq 7.8$ mmol/L [140 - 199mg/dl]; and iii) IFG + IGT: FPG  $\geq 5.5$ mmol/L [ $\geq 100$ mg/dl] and 2 h plasma glucose  $\geq 7.8$ mmol/L [140 - 199mg/dl]. Most of the physiopathological abnormalities observed in T2D, have been already reported in prediabetes with a different severity (23), IR (24), pancreatic  $\beta$ -cell dysfunction (10, 11, 25, 26), pancreatic  $\alpha$ -cells dysfunction and relative hyperglucagonemia (13, 15, 25), incretin defect, although it has been questioned whether incretin defect is a cause(27) or consequence of hyperglycemia in T2D (28). It has been previously shown that clinical and physiopathological characteristics are different between these three categories of prediabetes. IFG mainly presents hepatic insulin resistance and mild impairment on first phase insulin secretion ( $\beta$ -cell dysfunction), IGT is more related to muscle

insulin resistance and first and second phase insulin secretion ( $\beta$ -cell dysfunction), while the combination of IFG+IGT present marked pancreatic  $\beta$ -cell dysfunction and whole body IR, with the greatest risk to develop T2D.

Prevalence of prediabetes varies between 9.7 to 56.8 %; in Mexico it has been reported to be around 30 % (29). Eventually, most of the patients with prediabetes (70-80 %) will develop T2D during their life, and of note, they have a higher risk and may already present micro and macrovascular complications (30).

Pancreatic  $\beta$ -cell function has been reported as one of the main factors involved in the progression from prediabetes to T2D in observational studies as well as in clinical trials (31-33)

Different pharmacologic and non-pharmacologic strategies have been used to reduce the progression risk from prediabetes to diabetes and prevent T2D. Lifestyle modification programs, based on physical activity and nutrition, have proved to be a useful strategy, reducing the risk up to 58 % in patients with IGT, in China and Finland (34) (35). The Diabetes Prevention Program Study (DPP) was designed to compare the risk reduction between an intensive lifestyle program vs placebo and metformin (36); DPP by the first time evaluated the effect of metformin, an insulin sensitizer that mainly reduces hepatic glucose production(37); this study showed also a risk reduction of 58 % in the lifestyle group, and only 31 % in the metformin group, in comparison to the placebo group; however, the main limitation of the lifestyle programs is the adherence to the program, and that besides of that, there is a progression from prediabetes to T2D and eventually most of the patients will develop T2D in the long term follow-up, since in the DPP Outcome Study(38) the cumulative T2D incidence at 15 years of follow-up was 55, 56 and 62 % in the lifestyle, metformin and placebo group, respectively.

Other kind of drugs have been also used to prevent T2D; rosiglitazone in the DREAM study showed a 62 % risk reduction in patients with IGT to develop T2D (39), in the ACT-NOW Study, Pioglitazone showed a 72 % risk reduction in patients with IGT, but was associated with significant weight gain and edema, perhaps due to the used dose(40).

Few studies have been performed to evaluate the effect of incretin drugs to prevent T2D. If there is a reduction in the incretin effect in prediabetes, there is two ways of improving it, with GLP-1 receptor agonist or GLP-1 analogues, or with the use of a DPP-IV inhibitor, that eventually will increase endogenous GLP-1 levels two or three-fold. Most of the previous studies have been done with GLP-1 RA or agonist. Exenatide, a GLP-1 receptor agonist, after 24-weeks, in patients with obesity and prediabetes, together with a lifestyle program showed reductions in calorie intake and glucose normalization, but without a difference in fasting, 2h glucose and T2D incidence, but with a mean body weight reduction of around 4.0 kg(41). Liraglutide 1.8 mg/d, also in patients with prediabetes or early T2D (<12 months), was compared to a lifestyle modification program in a small sample of patients (n=40), achieving in both groups a weight reduction of around 7.0 kg, and a slightly better improvement in 2h glucose and  $\beta$ -cell function in the liraglutide group(42); also in patients with prediabetes, liraglutide showed to improve glucose values during OGTT and  $\beta$ -cell function during 14 weeks of treatment(43). At doses of 3.0 mg/d, liraglutide together with diet and exercise has shown a reduction in the incidence of T2D (HR 0.21 95% CI 0.13-0.34, p <0.0001), and improvement in glucose during OGTT in patients with overweight-obesity and prediabetes during a 3 year study (44).

Endogenous GLP-1 is inactivated in 1-4 minutes by the DPP-IV, and with DPP-IV inhibitors the incretin effect gets improved; the use of DPP-IV inhibitors in prediabetes has been less explored, since there are few studies with short duration and small sample size; in an 8-week study, sitagliptin showed no effect in patients with IFG(45), but in patients with IGT it was reported a reduction in glucose levels as well as an improvement in insulin and glucagon levels during OGTT(46). In a 12-week study in patients with overweight and prediabetes, sitagliptin improved also glucose profile and  $\beta$ -cell function during OGTT (47). Saxagliptin in a 24-week study (n = 5-7 per group), showed similar changes than metformin in fasting glucose and HbA1c and a slightly better improvement in 2 h glucose levels in patients with IFG or IGT(48). Linagliptin has been used only in 12-week study (n=8 per group) in comparison to metformin in overweight-obese patients with IGT, finding mainly a reduction in 2h glucose with linagliptin (49).

Linagliptin is a potent and selective DPP-IV inhibitor with a long half-life to be used once daily; it is the only DPP-IV inhibitor cleared mainly by the liver (>80 %) and by consequence

it can be used without dose adjustment in patients with high risk or any kind of renal dysfunction (50); besides this, linagliptin has shown in recent clinical trials a safe profile regarding renal and cardiovascular function (51), and has proven efficacy in T2D treatment as a monotherapy or combined therapy (52)

In human islets from non-diabetic and diabetic donors, linagliptine has shown to better improve insulin secretion,  $\beta$ -cell function and  $\alpha$ -cell function in non-diabetic islets, suggesting that this kind of drugs could have a better effect at very early stages of the disease, like prediabetes (53).

Few studies have tried to evaluate integral and realistic interventions to prevent T2D, focusing these therapies to the group of patients with prediabetes at the highest risk to develop T2D, combining oral and practical medications with a realistic and real life feasible lifestyle program, and moreover, no study has deeply evaluated the physiopathological effects of such therapies.

The goal of this work was to evaluate the effect of linagliptin + metformin plus a lifestyle program vs metformin plus a lifestyle program on glucose metabolism, pancreatic  $\beta$ -cell function and T2D incidence in Mexican patients with IGT + IFG during 18 months.

## **Methods**

### ***Study design and participants***

The PRELLIM project (Diabetes Prevention with Linagliptin, Lifestyle and Metformin) is a single center double-blind randomized clinical trial performed in patients with IGT and at least two T2D risk factors. Patients will be selected from a metabolic cohort study performed at the Metabolic Research Laboratory in the University of Guanajuato, México, as part of the University Cohort Project CARE-In-DEEP Study (Cardiometabolic Risk Evaluation and Interdisciplinary Diabetes Education and Early Prevention). For this particular study, patients will be screened with anthropometrical, physical activity, nutritional, biochemical and metabolic evaluation, including oral glucose tolerance test and hyperglycemic clamp. Patients are eligible for enrollment in the study according to the following criteria: *i*) impaired glucose tolerance (2 h glucose 140-199mg/dl [7.8 to 11.0 mmol per liter]) during a single oral glucose

tolerance test, *ii*) age between 18 – 65 years, and *iii*) at least two T2D risk factors according to the American Diabetes Association; exclusion criteria: i) treatment with drugs affecting glucose levels during the previous 3 months, ii) previous pathological conditions affecting glucose metabolism or body weight (thyroid disease, Cushing’s syndrome, Acromegaly); iii) excessive alcohol intake (acute or chronic), iv) fasting plasma triglyceride >400mg/dl, v) pregnancy, vi) Systolic blood pressure >180mmHg or diastolic blood pressure >105mmHg (subjects could be re-screened after hypertension treatment). Written informed consent will be obtained from all participants and the study was approved by the Ethical Council at the Hospital Regional de Alta Especialidad del Bajío.

## **Procedures**

***Anthropometrical and body composition measurements.*** Weight will be measured while participants are barefoot and wearing minimal clothing. Height is obtained while the participants are standing barefoot with their shoulders in a normal position. BMI (kg/m<sup>2</sup>) is obtained from standardized measurements of weight and height and is computed as a ratio of weight (kg):height squared (m<sup>2</sup>). Waist circumference will be measured at the high point of the iliac crest at the end of normal expiration to the nearest 0.1 cm. Body composition will be assessed with electrical bioimpedance through a Tanita Scale SC-240. All measurements will be performed by personal trained to use standardized procedures and reproducibility is evaluated, resulting in concordance coefficients between 0.88 and 0.94. Weight will be recorded at least every two months, while electrical bioimpedance every 6 months.

***Nutritional and Physical Activity Evaluation.*** A semi-quantitative food frequency questionnaire (FFQ) previously validated (54), will be applied to evaluate dietary intake. This questionnaire included data regarding the consumption of 116 food items. For each food, a commonly used portion size (e.g. 1 slice of bread or 1 cup of coffee) is specified on the FFQ and participants reported their frequency of consumption of each specific food over the previous year. Participants chose from 10 possible responses, ranging from “never” to “6 or more times per day.” For our analysis, the reported frequency for each food item is converted into a daily intake. Total energy intake is computed by summing the energy intakes from all foods. The PA level of participants is assessed using a self-administered questionnaire that is

verified when the patient assist for the metabolic evaluation. The questionnaire has a validated Spanish translation which has been adapted for use in the Mexican population (55). The questionnaire is self-administered and estimates the minutes devoted to the practice of different recreational physical activities during a typical week in the last year (including walking, running, cycling, aerobics, dancing, and swimming as well as playing football, volleyball, basketball, tennis, fronton, baseball, softball, and squash, among other activities). Each item includes time intervals that allow participants to detail the exact number of minutes or hours they dedicate to each form of recreational PA, as well as the intensity of each PA (light, moderate, vigorous). The total duration of each recreational PA is expressed in minutes per day. We calculated the number of hours per week devoted to each activity, which were then multiplied by the intensity of each activity, defined as multiples of the metabolic equivalent (MET) of sitting quietly. We used the Compendium of Physical Activities to assign METs to each activity (56). We then add the average weekly energy expenditure attributable to each activity to derive the total MET-hours per week. Energy intake and physical activity will be evaluated every 6 months.

### **Metabolic evaluation.**

*Oral glucose tolerance test (OGTT):* All subjects will be admitted to the Metabolic Research Laboratory of the Department of Medicine and Nutrition, Division of Health Sciences at the University of Guanajuato the day of the study between 7 and 8 AM, and a catheter will be placed into an antecubital vein for all blood withdrawal. Subjects will not be allowed to eat or drink anything after 10 PM on the night before, until the study is completed. After the intravenous catheter is placed and the first blood sample is drawn, the patients ingest 75 grams of glucose. Serum samples for glucose and insulin measurement will be drawn at -15, and 0 minutes and every 30 minutes thereafter for two hours. Prediabetes is diagnosed based on the OGTT; and only patients with IGT (2 h glucose 140-199 mg/dl), and ideally together, although not exclusively, with IFG (FG 100-125mg/dl) will be included in this study. Patients with newly diagnosed T2D will be excluded from the study.

*Hyperglycemic clamp.* All patients will be invited to the hyperglycemic clamp, with the goal to have at least 10 to 20 % in both groups at 0, 6 and 12 months. Subjects are admitted to the

Metabolic Research Laboratory at University of Guanajuato on the day of the study at 7:00 AM. A catheter is placed in the antecubital vein for glucose infusion, and a second catheter is placed into a vein on the dorsum of the other hand and the hand will be inserted into a heated (65°C) box. At 8 AM, following the collection of two baseline blood samples (-30 and 0 minutes), a single step hyperglycemic clamp will be performed. At the start of the hyperglycemic clamp (time zero), the plasma glucose concentration is acutely raised (glucose bolus with D50 %) and maintained at 125 mg/dl above baseline (i.e., approximately 225 mg/dl) for 120 minutes, with a continuous D10% glucose infusion. Serum glucose will be measured every 3 minutes during the first 15 minutes, and every 5 minutes thereafter; insulin will be measured every 3 minutes during the first 15 minutes and every 15 minutes thereafter; C-peptide will be measured every 3 minutes during the first 15 minutes and at 30, 60, 90 and 120 minutes. Plasma glucagon is measured at 0, 15, 30, 60 and 120 minutes. The hyperglycemic clamp will be performed at a 5-7 day interval after OGTT.

**Measurements.** Glucose will be measured with an Analox glucoanalyzer GM9 (Analox Instruments) and by colorimetric glucose oxidase (Vitros 5600; Ortho Clinical Diagnostics). Lipid levels are measured by dry chemistry with colorimetric method (Vitros 5600; Ortho Clinical Diagnostics). Insulin and C-peptide will be measured by chemiluminiscent immunometric assay (IMMULITE 2000 Immunoassay system, Siemens), and Glucagon by Quantikine ELISA Immunoassay (R&D Systems Inc, Minneapolis, USA) with an intra-assay CV of 2.7 – 3.6 %. HbA1c will be determined using high-performance liquid chromatography with a DS-5 Analyzer (Drew Scientific, Inc. Miami, FL, USA). Renal function will be evaluated only at 12 and 24 months of follow-up with the 24 h creatinine clearance and the albumin urinary excretion.

**Calculations.** The incremental and the AUC for glucose and insulin during the OGTT are calculated according to the trapezoidal rule. Insulin secretion is calculated dividing the  $AUC_{insulin_{0-120}}$  by the  $AUC_{glucose_{0-120}}$  during the OGTT. The insulin secretion/insulin resistance (IS/IR) index (disposition index = DI) during OGTT is calculated as  $(AUC_{insulin_{0-120}} / AUC_{glucose_{0-120}}) * \text{Matsuda index}$  (57). Insulin sensitivity during OGTT is calculated from the Matsuda index (58), and at fasting with the homeostasis model assessment

(HOMA-IR). From the hyperglycemic clamp the incremental and AUC for glucose, insulin and C-peptide are also calculated according to the trapezoidal rule. Different measurements of pancreatic  $\beta$ -cell function will be derived from the hyperglycemic clamp: DI will be obtained as a product of insulin sensitivity (Matsuda index) and the incremental AUC for insulin and C-peptide from 0 to 15 and from 0 to 120 minutes.

*Randomization and masking.* Patients will be randomly assigned in a 1:1 ratio to receive linagliptin/metformin 2.5/850mg every 12 h + lifestyle modification program, or metformin 850mg every 12 h + lifestyle modification program during 24 months. Randomization will be performed using an electronic random numbers table, ensuring to have a similar number of patients per group. Participants and investigators involved in the patients follow-up and outcome measurements will be masked to treatment allocation during the entire study.

*Interventions.* i) Linagliptin + metformin + lifestyle: Patients allocated to this group will start linagliptin/metformin pills of 2.5/850mg once daily during the first month, and after that the dose was increased to 2.5/850mg twice daily from the second month until the end of the study (24 months). ii) Metformin + lifestyle: Patients on this group will start metformin pills of 850mg once daily during the first month, and increased to 850mg twice daily thereafter until the end of the study (24 months). Both groups will receive the same lifestyle implementation program based on a prescribed diet every two months seeking to reduce their body weight at least by 5-7 %, adjusting their energy requirements based on their weight every two months, and composed from 55-60 % of carbohydrates, 25-30 % fat, and 10-15 % proteins. If the patient is sedentary, he or she will be advised to start with 45 min/week of mild to moderate exercise with a specific activity according to patients preference; patients will be counseled to increase the duration and frequency or intensity of exercise every two weeks until reaching 150min/week of moderate activity or 75 min/week of intense activity. If the patient was already physically active, it will be recommended to continue like this and vary its exercise routines.

*Follow-up visits and outcome.* Patients will have a follow-up visit every month during the first 12 months, and every 2 months thereafter. Every appointment is about 30-45 minutes;

medications tolerance and side effects will be recorded in every patients' visit. Nutritional and physical activity assessment according to the patient's weight will be performed by a Nutritionist every month from 0 to 12 months, and every 2 months thereafter. Monthly adherence to medications will be evaluated by pill counting; nutritional adherence and energy intake will be evaluated every 6 months by a food frequency questionnaire, and physical activity at 0 and 12 months. Every 6 months patients will have an OGTT. Primary objective is to evaluate at 6, 12, 18 and 24 months glucose profile during OGTT, insulin secretion and pancreatic  $\beta$ -cell function by the OGTT DI and the hyperglycemic clamp, and T2D incidence and regression to normoglycemia; T2D will be diagnosed and confirmed by at least two consecutive measurements of the same criteria: glycated haemoglobin  $\geq 6.5\%$ , fasting glucose  $\geq 126\text{mg/dl}$ , or 2 h glucose  $\geq 200\text{ mg/dl}$ . In a subgroup of patients, hyperglycemic clamp will be performed at 0, 6 and 12 months to complement the measurements of insulin secretion and pancreatic  $\beta$ -cell function (basal  $n = 19$ , 6 months  $n = 25$ , 12 months  $n = 32$ ; total 76 hyperglycemic clamps). Secondary endpoints include changes in body weight, insulin sensitivity and glucagon levels.

### **Statistical analysis**

The sample size calculation was based on the fact that we expected an improvement of at least 50 % on the pancreatic  $\beta$ -cell function and glucose profile during the OGTT in patients with the combined treatment and at least an improvement of 20% on the metformin group, based on previous reports about glucose levels and pancreatic  $\beta$ -cell function in prediabetic patients before and after treatment (40, 59); in order to be able to detect a minimum difference of 30 % between groups and assuming an alpha error of 5% and a beta error of 20%, we require approximately 41 patients per group.

The primary analysis is the comparison of the change in glucose profile during OGTT as well as measurements of pancreatic  $\beta$ -cell function during OGTT and hyperglycemic clamp. Intergroup comparisons between absolute values and delta values will be performed with a t test for independent groups; intra-group comparisons will be performed using a paired t-test. Non-numerical variables will be compared between groups with the chi squared test. Relative risk and hazard ratio will be calculated to evaluate the TD2 risk in the groups of treatment.

The Log-rank (Mantel-Cox) test will be used to compare the incidence of T2D in the study groups.

Statistical analyses and graphics will be performed using SPSS Version 21.0 (SPSS Inc) and GraphPad Prism 5.0. Statistical significance is considered when p value is less than 0.05.

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