Research Protocol

# “Effect of quadruple therapy on pancreatic islet function, insulin resistance and cardiovascular function in patients with mixed prediabetes and obesity: Randomized Clinical Trial”

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October 2019

## EPIDEMIOLOGICAL IMPACT OF TYPE 2 DIABETES

The type 2, Diabetes mellitus (DM2) is one of the most common endocrine diseases in the general population, and in Mexico it's the leading cause of death. DM2 is considered as a syndrome with an altered intermediate metabolism caused by inadequate insulin secretion, resistance to the effect of the same or to the combination of both, which consequently cause both fasting and postprandial hyperglycemia [1]. The risk factors for DM2 are: overweight and obesity, first-degree relatives with DM2, ≥ 45 years of age, women with a history of macrosomic products (> 4kg) and / or with a history of gestational diabetes, women with a history of polycystic ovaries , arterial hypertension ≥140 / 90mmhg, dyslipidemias (HDL cholesterol ≤40mg /dl, triglycerides ≥250mg/dl, and cardiovascular disease.

It's estimated that some 11.7 Mexicans will be DM2 for 2025, with means an increase of 207.8% by occupying 7th place with more cases worldwide (2). The National Health Survey conducted ,in 2000, a prevalence of DM 2 of 8.2%, reporting that this increase in prevalence is partly due to the higher number of patients with DM2 under 40 years old, and the high prevalence of risk factors contributing to the development of chronic complications is what explains the leading cause of death (3). However, the 2016 halfway, National Health and Nutrition Survey (ENSANUT) reported an increase in overall prevalence of DM2 of 9.4% (4); this survey was found to be an significant increase in the number of people < 40 years of age with DM2

In countries like Mexico, DM2 is a pathology that has marked an epidemiological transition. It is currently one of the most important public health problems reaching epidemic al characteristics. The impact of this pathology is very important since more than 180 thousand new cases are registered annually and cause around 36 thousand deaths. The annual cost of diabetes for Mexicans is estimated at 430 million dollars per year, 15 million for metabolic control, 85 million for additional health services and 330 million for indirect costs. These figures represent an approximate cost of $ 45 per person per year and this expense corresponds to three quarters of the entire budget granted for Health [6].

Complications of DM2 may include microvascular: retinopathy, neuropathy and nephropathy, or macrovascular heart disease, cerebral vascular disease and peripheral vascular disease. DM2 is the leading cause of end-stage kidney disease in the world, the leading cause of non-traumatic amputations and the leading cause of blindness. On the other hand, cardiovascular disease is the leading cause of death over 75% of patients with diabetes, as they have a high risk of coronary artery disease, with a higher incidence of ischemic events and death after an acute infarction of the myocardium; in the absence of ischemic heart disease, the risk of developing it is the same as those without diabetes with a backdrop of ischemic heart disease. This implies that prevention measures for patients with asymptomatic diabetes should be similar to secondary prevention measures for non-diabetic patients with ischemic heart disease [7]. Prediabetes has also been associated with increased cardiovascular morbidity, so it is suggested that endotheliopathy also starts early stages of the disease, also affecting renal function [8-10].

## PHYSIOPATHOLOGY OF DIABETES TYPE 2

There is a complex interrelationship between the different components involved in the process of energy production, consumption and storage. Endogenous glucose production is given by 85% by the liver, and baseline consumption is approximately 2mg/kg/min, with the main consumer being the brain during fasting, with 50% uptake of hepatic glucose production, and muscle during the postprandial period with up to 80% uptake of glucose ingested. After food intake, there is a rapid increase in insulin secretion, stimulated in part by the secretion of incretins at the intestinal level to subsequently decrease a little and have a new elevation after 30 minutes. This insulin increase occurs along with suppression in glucagon secretion, which together aim to decrease hepatic glucose production, and increase peripheral glucose uptake, mainly at muscle level [11].

The pathogenesis of DM2 is multifactorial and complex. The knowledge established for the time being indicates a strong environmental influence, without an important contribution of the genetic factor [12].

The two most important factors that contribute to the pathophysiology of DM2, and for which it is known as a dual disease, are insulin resistance and beta cell dysfunction. The sequence of pathophysiological events seems to indicate that the first thing to appear is insulin resistance, conditioned by several factors such as lack of physical activity, the westernized diet high in simple carbohydrates and fatty acids ethnic group and perhaps a genetic influence so far not determined [13].

Several pathophysiological mechanisms have been described in insulin resistance. At the liver level it has been observed that there is a decrease in the number and activity of insulin receptors, decrease in glucose uptake due to a decrease in the number and activity of glucotransporters and, by the condition of some enzymes in the glycolytic pathway that secondaryly prevent the ingress of glucose, as well as an effect of free fatty acids at this level interfering with insulin signaling. On the other hand, at the muscle level this insulin resistance is also very important, because this is the place where the greatest uptake of insulin-dependent glucose (80%). Here there has also been a decrease in glucotransporters (in this case GLUT4), accumulation of triglycerides and excess free fatty acids that compete with glucose as an energy substrate and increase malonilCoA levels that have the ability to inhibit carnitinepalmitotransferase type 1, an important enzyme for beta oxidation of fatty acids. In addition, both at the liver, muscle and mainly adipose level, an interesting interrelationship has been found between different cytokines, such as tumor necrosis factor alpha (TNFa) that conditions insulin resistance by causing phosphorylation of intracellular part of the insulin receptor and insulin receptor substrate 1 (IRS-1), and its counterpart, the adiponectin which is the only cytokine protective against insulin resistance.

Due to this insulin resistance, at the peripheral level the glucose (muscular) uptake decreases and at the hepatic level it is not able to suppress glucose production, therefore, as a compensatory mechanism the beta cell increases insulin secretion and thanksAt this hyperinsulinemia, equilibrium is achieved and euglycemia is maintained, so hyperinsulinemia is an indirect marker of insulin resistance [11].

However, over time and the persistence of insulin resistance and the increase of some toxic factors for the beta cell (glucose and lipids), it begins to deplete and decrease insulin secretion, so that ability to maintain suppressed hepatic glucose production and fasting hyperglycemia appears (Figure 1), until we reach diagnostic levels of DM2 where we have a 50-80% reduction in the functional capacity and volume of beta cells [11, 14].

**DISFUNCTION OF PANCREATIC ISLOTS**

Interestingly, once insulin resistance exists, the conditions whether or not beta cell dysfunction and pancreatic islets occur as a functional unit. ¿what are the pathophysiological mechanisms that lead the beta cell to inability to maintain normoglycemia and glucose normotolerance? There are different factors such as: non-well established genetic factors, alteration in the cell cycle regeneration-apoptosis with a decrease of the first and increase of the last, a toxic effect of the same glucose (glucotoxicity), prolonged exposure free fatty acids [16], deficiency or resistance to the effect of incretins [17], oxidative stress and increased production of superoxide at the beta cell level [18, 19], and a factor identified for many decades but perhaps undervalued, abnormalities in the secretion of amiline or IAPP (islet amyloid polypeptide), conditioning the formation and deposit of amyloid at the level of pancreatic islets as well as a dysfunctional remodeling of them [20-23].

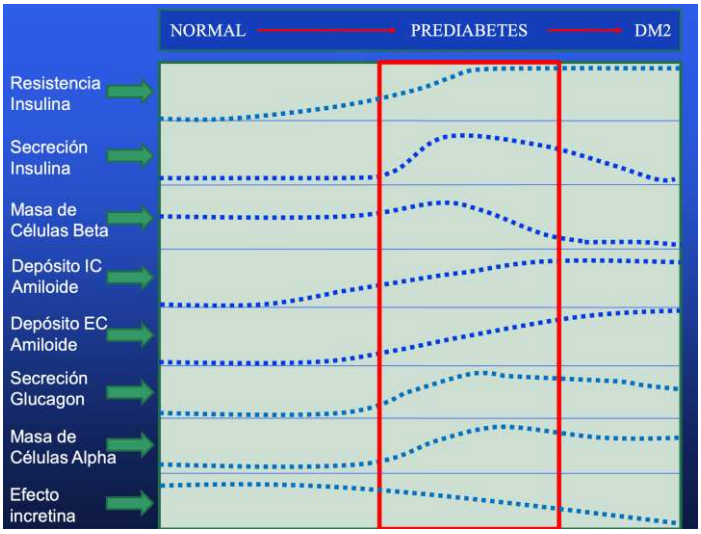


Figure 1. Natural history of type 2 diabetes mellitus. The sequence of pathophysiological events from normoglycemia to DM2 is observed, highlighting how most of them begin their appearance from prediabetes. CI: intracellular, EC: extracellular. (Guardado-Mendoza, R, unpublished) (Fig. 1) [15]

Thus, if we try to summarize the main pathophysiological alterations that are present in patients with DM2, these would be:

- Insulin resistance at the muscle level [23-25]
- Insulin resistance at the liver level [23]
- Pancreatic beta cell dysfunction with consequent reduction in insulin secretion [14, 20, 23, 24, 26-28]
- Pancreatic alpha cell dysfunction expressed as non-suppression of glucagon secretion to hyperglycemia [20, 29-32]
- Decreased incretin effect [33-35]
- Abnormalities in amylin secretion and amyloid deposits [36-41]

From the above, we can point out that there are two pathophysiological phenomena in the progression of the disease: insulin resistance and pancreatic islet dysfunction. These pathophysiological alterations, as already mentioned, occur progressively during the course of the disease, so it is important to know the physiological alterations and risk factors associated and involved with each of them that allow us to focus timely prevention and diagnosis strategies on stages prior to the onset of DM2 and thus be able to impact on the onset of chronic complications and morbidity in these patients.

## PREEDIABETES

Since the natural history of the disease is a continuous spectrum, and as such, DM2 is only a limited part of that spectrum, a stage known as prediabetes was identified, which is identified with fasting glucose levels between 100 and 125mg/dL , and is known as altered fasting glucose, or with glucose levels between 140-199mg/dL at 2 hours after an oral charge of 75g glucose, known as glucose intolerance; complications, both macro and microvascular[42], have been reported in both entities. Altered fasting glucose and glucose intolerance identify different populations, as fasting glucose reflects the rate of hepatic glucose secretion and to varying degree the secretion of insulin by the pancreas, since one of its effects the main is to inhibit hepatic glucose production; Incontrast, glucose intolerance, evaluates the rate of tissue uptake of glucose (in muscle) and the ability of the pancreas to compensate for this defect. In patients with prediabetes, the presence of pathophysiological phenomena similar to those occurring in DM2 but in less severity [42] has been reported; patients with prediabetes have insulin muscle and hepatic resistance [23, 43-45], pancreatic beta cell dysfunction [23, 26, 27, 44, 46], pancreatic alpha cell dysfunction with the consequent absolute or relative hyperglucagonemia [29, 31, 46], reduction of the incretin effect [35, 47]; adipose tissue is an active endocrine organ that secretes several adipctocins (leptin, adiponectin, resistine, visfatin) and cytokines (TNF alfa, IL-1, IL-6) that are also involved in insulin resistance and inflammation present in subjects with obesity and prediabetes [48, 49]. The pathophysiology of prediabetes can be summarized as follows: subjects with altered fasting glucose have predominantly resistance to insulin at the liver level and decreased the first phase of insulin secretion, while patients with glucose intolerance they have insulin resistance at the peripheral level, specifically at the muscle level, and deterioration in the first and second phases of insulin secretion [50, 51].

The prevalence of prediabetes in different populations is as follows: glucose intolerance, 6.3 to 20.3%, impaired fasting glucose from 9.7% to more than 30%, impaired fasting

glucose with glucose intolerance from 31 to 56.8% [52]. In Mexico, data from the ENSANUT 2006 show a prevalence of impaired fasting glucose greater than 30% [53].
The transition from prediabetes (GAA and ITG) to DM2 can take a long time, however, the existing information indicates that the majority of these patients (70-80%) will develop DM2 at some time in their life; The natural history of these entities is variable, some studies have reported that after a follow-up of 3-5 years, 25% may progress to DM2, 50% persist with previous alteration, and 25% may return to normoglycemia and normotolerance to glucose People with GAA + ITG and who are also overweight and other risk factors for DM2 are the most likely to present this disease progression, and factors such as reduced insulin secretion and severe insulin resistance, serve as markers for Identify these types of patients. These patients have a slightly elevated cardiovascular risk (relative risk 1.1 - 1.4), which rises up to 2 to 4 times more with the progression to DM2 [54], in the same way, in prevalence of patients with prediabetes a prevalence has been observed of 8% retinopathy which rises to 13% as it progresses to DM2 [55].
Considering again the continuous spectrum of the disease, we can realize that there is another stage that also belongs to a pre-diabetic state, this is when there is only insulin resistance without fasting or postprandial hyperglycemia, which in fact is the initial stage of disease, and that commonly occurs in patients with obesity [25].

A chronological sequence has been proposed in the appearance of the different types of prediabetes [25, 45], which would be: i) isolated insulin resistance without fasting hyperglycemia or postprandial hyperglycemia, ii) isolated GAA, iii) isolated ITG, and iv) GAA + ITG, for the appearance of these 4 states the presence of insulin resistance (in liver, muscle and adipose tissue) and the dysfunction of the pancreatic beta cell are key; It is clear, by definition, that the diagnosis of the last three is made with the measurement of fasting glucose and the performance of the oral glucose tolerance curve (OGTT) with an oral load of 75 g of glucose, on the other On the other hand, for the diagnosis of the first (isolated insulin resistance), there are several methods. One of the most used methods in epidemiological studies is the insulin resistance homeostasis model (HOMA-IR) which is obtained by fasting glucose and insulin by means of the following formula: HOMA-IR = (Plasma insulin insulin fasting in mU / l / Fasting plasma

glucose in mmol / l) /22.5; As already mentioned, fasting glucose is determined by the rate of hepatic glucose production, and fasting insulin is the main regulator of hepatic glucose production, so the product of both represents insulin resistance mainly at the liver level Said insulin resistance correlates 70-80% with insulin resistance at the peripheral level; In patients ranging from normoglycemia, prediabetes and even DM2, HOMA-IR has a correlation ranging from 0.6 to 0.88 with other more sensitive methods, such as the hyperinsulinemic-euglycemic clamp, considered as the gold standard for identifying resistance to insulin [56-62]. However, the cut-off point for HOMA-IR from which insulin resistance is detected has been variable in different studies, some have reported cut-off points of 2.7 [63], others of 2.6 with a specificity of up to 87% to identify insulin resistance [64,65], and it is generally accepted that a HOMA-IR greater than 3.0 is associated with insulin resistance.

**TREATMENT OF PREDIABETES**

Different pharmacological and non-pharmacological strategies have been used to try to prevent the progression of prediabetes to DM2. In general, there are two types of preventive strategies, those that are carried out at the population level and whose objective is to avoid the presence of modifiable risk factors, and that which is carried out in high-risk population, such as patients with GAA , ITG and gestational diabetes, this strategy aims to improve insulin resistance and pancreatic beta cell dysfunction.

The Da Qing IGT and Diabetes Study (1997) was one of the first clinical trials to evaluate the effectiveness of lifestyle modifications to prevent DM2; It included 530 patients over 25 years of age, all of them with ITG documented by OGTT, the study groups were: 1) Group treated with a diet of 25-30 kcal / kg formed by 55-65% carbohydrates, 10-15% protein, and 25-30% fat, with individual counseling sessions (on food intake) weekly at the beginning, then monthly for the next 3 months and then every 3 months; 2) Exercise: in this group of patients the purpose was to spend 1 or 2 U of exercise per day, with advice on the type of exercise and time, depending on the state of health; 3) Combined group with diet and exercise, combined advice of the diet and exercise group to improve lifestyle and 4) Control group. The average follow-up was 6 years, and in the group treated with diet a reduction in the risk of DM2 of 33% was

achieved, in the group treated only with exercise a 47% reduction in risk was achieved, and in the group treated with both, a reduction of 38% [69].

Another study based on lifestyle modifications, is the one conducted in Finland: Finnish Diabetes Prevention Study Group (2003), was a simple blind clinical trial that included 522 patients with ITG and at least one risk factor for DM2; There were two study groups: 1) Control group that was given oral and written information on diet and exercise with annual visits, and 2) Intervention group, which performed 30 min / day of physical activity in order to lose 5% of body weight, a reduced fat diet (<30% of total energy consumed) and increased fiber consumption were recommended, and follow-ups were every 2 months the first year and then every 3 months. The average follow-up of this study was 3.2 years, and the results showed that in the intervention group a reduction of the risk of DM2 of 58% was achieved, however, it is worth mentioning that not all patients in the intervention group achieved The therapeutic objectives, that it was necessary to intervene 22 patients with ITG to prevent a case of DM2 and that at the end of the study the risk reduction was becoming smaller due to the lack of attachment to the intervention [70].

The most representative study on the impact of lifestyle modifications on the prevention of DM2 is the Diabetes Prevention Program (DPP); This study was a randomized clinical trial with an average follow-up of 2.8 years, which included 3,234 patients with BMI> 24 and GAA and / or ITG that were randomized in 3 study groups: 1) Metformin 850 mg daily treated during the first month and then 850 mg twice daily plus routine recommendations (orally and in writing) annually on lifestyle, 2) placebo-treated group plus routine lifestyle recommendations, and 3) group treated with an intensive program for modify the lifestyle, which consisted of 150 min of physical activity per week and reduce the intake of fat and calories in the diet, in order to lose 7% of body weight. This group had 16 personalized sessions during the first 24 weeks, and then every month. It is worth mentioning that in this study, only 50% of the participants in the intervention group to modify the lifestyle managed to lose 7% of body weight within 24 weeks of initiation, and only 38% maintained it until the end of study; 74% maintained the required physical activity 24 weeks after the study and only 58% until the end of the study; at 24 weeks of the study, the intervention group achieved a reduction in energy

intake of 450 kcal / day compared to approximately 250 kcal / day in the other two groups. The results showed that with intensive changes in lifestyle, the risk of DM2 was reduced by 58%, and with metformin by 31%; even so, the cumulative incidence of DM2 in 3 years was 14.4% in the intervention group, 21.7% in the metformin group, and 28.9 in the placebo group [71]. However, the long-term results (15 years) this intervention showed that the effect of the treatment is lost over time and that eventually the majority of patients will develop DM2.

In addition to metformin, other antidiabetic medications have been used: acarbose in a cohort of 1429 patients with ITG showed a reduction in DM2 risk of 25% with a necessary number to treat of 11 to avoid a case of DM2, however, more of the fourth part of patients did not complete the follow-up due to the side effects of the medication [72]; rosiglitazone in the DREAM study that included 5269 patients with GAA and / or ITG randomized to 4-8mg of rosiglitazone or placebo, at an average follow-up of 3 years, the rosiglitazone-treated group showed a DM2 risk reduction of 62% [73 ]; The ACT NOW study that used pioglitazone 45mg daily in subjects with GAA and / or ITG shows a risk reduction of DM2 of about 80% compared to placebo [74].

Currently, the pharmacological interventions used in patients with DM2, are directed towards pathophysiological alterations, that is, much emphasis has been made recently so that the treatment is implemented with a pathophysiology-based approach. In prediabetes, if we try to prevent the progression of the disease, it is clear that the options that may have a major 11petite1111 each of the pathophysiological alterations are those that could have the greatest effect to prevent the progression of the disease [26, 27, 43, 46, 75]; Within these pharmacological interventions, those that could be more effective, due to the pharmacological profile, are:

• **METFORMIN**: Metformin is the most widely used antidiabetic drug in the world. Although its main effect is exerted at the liver level, it also has an effect on other tissues such as muscle, adipose and 11petite111111 tissue, 11petite its effects are related to the control of insulin resistance and / or 11petite111111al1111ia. At the liver level, metformin activates an enzyme known as AMP kinase (AMPK), which causes an

inhibition of lipogenic and gluconeogenic enzymes, decreasing lipolysis, increasing fatty acid oxidation, reducing hepatic glucose production (12petite121212al1212i), increasing Hepatic glucose uptake and glycolysis, all this is because it increases the sensitivity to the effect of insulin. At the muscle level, metformin also activates AMPK, thus improving insulin signaling and therefore muscle glucose uptake, among other effects such as: improvement in inflammation, reduction of 12 petite 12 12 12 a and increase in acid oxidation. Fatty At the 12petite12 adipose tissue, metformin reduces lipolysis and also due to the activation of AMPK modulates the secretion of adipocytokines, reducing gluco and lipotoxicity, which leads to increased insulin signaling and therefore glucose uptake by visceral fat, reduction in 12petite121212a and increase in the oxidation of fatty acids [76]. Through all these mechanisms, metformin reduces fasting plasma glucose and 12petite121212al1212ia; as monotherapy it manages to reduce up to 1.5% HbA1c; It is the only medication approved for use in people with prediabetes, it is associated with weight reduction in a variable percentage, as monotherapy is very unlikely to cause hypoglycemia; Metformin doses range from 500 to 2550mg / day, and its main side effects are: 12 petite, flatulence and intestinal distress so it is recommended to start with low doses and increase until reaching the desired 12pet; Although theoretically it is associated with the presence of lactic acidosis, it seems that the risk was greater with fenformin (former biguanide that was taken off the market just because it was associated with lactic acidosis) [76]. It is contraindicated in people with renal insufficiency, defined by a serum creatinine> 1.5mg / dl or a urine creatinine clearance of 24hrs <30ml / min, advanced chronic liver disease, acute infection that conditions predisposition to acidosis, and in people with major alcoholism [77]. The disadvantage of metformin is that it has been observed in long-term studies in prediabetes (15 years of follow-up) that the effect is almost equated with placebo and that eventually the majority of treated patients 12pet develop DM2.

• **PIOGLITAZONE**: Pioglitazone belongs to a group of medications known as thiazolidinediones; This type of medicine activates the γ peroxisome proliferating receptor (PPARγ), by binding to this receptor they form a heterodimer together with the 12 petite 12 12 12 al 12 receptor and other molecules, through this mechanism they regulate the 12 petite 12 12 of several genes that affect the oxidation of fatty acids,

increases the transcription of the gene encoding gluco-transporter 4 (Glut4), affects the production of cytokines (increasing the secretion of adiponectin and reducing that of TNF 13peti), and improves 13petite131313al function; It is through these mechanisms that pioglitazone increases insulin sensitivity in muscle, adipose tissue and liver [78]. Pioglitazone is the only drug of this family that persists in the market as an antidiabetic, its doses range from 15 to 45mg / day, previously troglitazone was taken off the market for hepatotoxicity, and recently rosiglitazone has been heavily questioned for its association with events cardiovascular Pioglitazone 13petite to reduce between 0.5 and 1.4% glycosylated Hb in patients with DM2, and has shown great utility in delaying the progression of prediabetes to DM2, at 13petite doses [74], something that had been previously reported with the use of rosiglitazone [73]. Unlike rosiglitazone, pioglitazone has shown a better cardiovascular profile, since in addition to reducing fasting and postprandial glucose levels, it reduces insulin levels and improves tissue sensitivity to it, it has also shown a Beneficial effect on the lipid profile by reducing levels of free fatty acids and triglycerides, and slightly increasing HDL levels [79]. For all the above, it is not surprising that pioglitazone has an important role in the prevention of DM2, however, the only study conducted in this regard is ACT NOW, whose results have reported that it reduces the risk of progressing to DM2, in patients with GAA and / or ITG, up to 72% when 13petite doses of the drug are used; However, the 13petite using 13petite doses of pioglitazone is frequently associated with the appearance of side effects, which are: weight gain (0.5 – 4kg) mainly due to increased subcutaneous fat, reduced visceral fat and fluid retention ( edema), therefore it is contraindicated in patients with heart failure mainly functional class 3 and 4 of the New York Heart Association; It is also associated with anemia, which may be due to dilution due to fluid retention; reduction of bone mineral density; hepatotoxicity, so it is contraindicated in patients with liver enzyme values (AST and ALT) 2.5 times above the normal level [79].

• INCRETINES: As already mentioned in the pathophysiology section, patients with DM2 and prediabetes have dysfunction of the pancreatic islets (beta cell dysfunction with consequent reduction in insulin secretion, 13peti cell dysfunction with increased production of 13petite13, and amyloid deposits) and this is a crucial 13petit for the progression of prediabetes disease to DM2, so that medications that can improve the functionality of this islet, increasing insulin secretion and reducing 13petite13, could

have an important 14petite1414 the disease. There are two incretins, GLP-1 and GIP, of these two, the first is the one that has been reduced in patients with DM2 and with prediabetes [34, 35, 47, 80] and both are produced and released by intestinal cells. The pharmacological effects of incretins are: increased insulin secretion, decreased 14petite14 secretion, delayed gastric emptying, reduced 14petite which leads to weight reduction. In general there are two ways to increase the incretin effect, by applying incretin analogs, which are injectable, and by inhibiting the enzyme that degrades the endogenous GLP-1, which is dipeptidyl peptidase 4. When found to be reduced the incretin effect in patients with DM2 and with prediabetes, and knowing that the effect of incretinomimetic drugs improves the function of the pancreatic islet and could have some effect on the peripheral action of insulin [81-84], it is interesting to know the impact that They could have DM2 prevention, especially when combined with insulin sensitizers. There are few studies that evaluate the effect of incretins in people with prediabetes; With GLP-1 analogues, a study randomized 38 patients with GAA or ITG to receive exenatide 10 μg every 12 hrs subcutaneously for 24 weeks, finding that in addition to the weight-reducing effect, a large percentage of patients with prediabetes treated with exenatide reverted to normal (77% vs. 56% for the placebo group) [85]. In another randomized clinical trial, the effect of liraglutide, another analog of GLP-1, on glucose metabolism, insulin resistance and insulin secretion in 24 patients treated with liraglutide and 27 with placebo, of which 10 and 11 were compared , respectively, had impaired fasting glucose + glucose intolerance; a 29% reduction in peripheral insulin resistance was achieved and a 21% increase in insulin secretion [86]. In another study, 22 patients with GAA were randomized to receive 100 mg of sitagliptin, DPP-IV inhibitor, for 8 weeks reporting no effect on glucose metabolism, although reports of some clinical cases mention an important effect on glucose in patients with prediabetes. Other non-antidiabetic medications have also been used and have shown variable results on the prevention of DM2 (valsartan, statins, orlistat, fibrates, estrogens, etc.) [87].

## DIFFERENCES BETWEEN DPP-IV INHIBITORS

### Potency and Efficacy to inhibit DPP-IV enzyme

It is important to mention that all DPP-IV inhibitors are reversible competitive inhibitors and it is difficult to compare their effects when analyzing individual studies since the experimental conditions may be different; however, there is a study in which inhibitors were compared under identical conditions showing similar efficacy (maximum effect) to inhibit DPP-IV in vitro, although there were differences in potency, the most potent being linagliptin [88] .

Half life

As for the half-life, vildagliptin and saxagliptin [89] have shorter half-lives, however, linagliptin [90] and sitagliptin [91] have a longer duration of effect, which gives the latter two the ability to inhibit the DPP-IV enzyme for 24 hours at > 80%; although it is worth mentioning that in the original study that evaluated the half-life and potency of sitagliptin to inhibit DPP-IV was performed in healthy volunteers [91], unlike the original study in which the half-life and potency of linagliptin was evaluated for inhibit DPP-IV that was performed in patients with type 2 diabetes mellitus (DM2) [90], a scenario in which medications are usually used. However, it is important to mention that the activity in these assays is evaluated ex vivo and is generally not corrected by the dilution of the sample, so the actual inhibition of DPP-IV is expected to be even greater 15oc the measurement.

Selectivity and elimination route

The selectivity of the different DPP-IV inhibitors by this enzyme has been evaluated in in vitro studies and it has been reported that both linagliptin [88] and sitagliptin [92] are the ones that have the highest selectivity for the DPP-IV enzyme; linagliptin has a selectivity > 10,000 for DPP-IV 15oc for DPP-8/9 compared to sitagliptin which is >5550; This is important because the inhibition of these two DPP-8/9 enzymes is what theoretically has been thought to be associated with lymphocyte activity inhibition side effects, although this effect has not been observed in the clinic, since these 2 Enzymes are found at the 15oce15celular level [93-95]. On the other hand, linagliptin only has lower selectivity on the fibroblast activation protein α (FAPα) which is a protein that is not found in adult tissue so the implications of this data are even lower, since the inhibitors of DPP-IV are only indicated in adults. Sitagliptin, vildagliptin and saxagliptin are

eliminated in more 16oc 80% by the renal route, however, linagliptin is eliminated in more 16oc 80% by bile route, so it can be used in patients with any degree of renal insufficiency without 16oce adjustment and without losing the pharmacological effect. [96].

## CLINICAL ASPECTS OF DPP-IV INHIBITORS

### Clinical efficacy

Regarding the clinical efficacy and the ability of different DPP-IV inhibitors to reduce HbA1c, fasting glucose and postprandial glucose, various meta-analyzes and clinical studies have shown similar efficacy, achieving a reduction in HbA1c of between 0.5 - 1.0% (≈0.8%), with greater reductions when baseline values are higher [93-95, 97, 98].

### Side effects and safety

So far, no greater cup of side effects have been found with DPP-IV inhibitors compared to control groups, and likewise, no differences in side effects have been reported between the various DPP-IV inhibitors [93, 94, 97, 98]; In addition, its safety regarding the risk of pancreatitis and pancreatic cancer has been validated by the FDA and the European Medicines Agency recently [99].

TABLE 1. Differential characteristics between DPP-IV inhibitors

| **NAME** | **POWER (IC50)** | **DOSE** | **EFFECTIVENESS TO INHIBIT THE DPP-IV x 24h** | **MIDDLE LIFE (h)** | **ELIMINATION** | **SELECTIVITY** | **USE IN RENAL FAILURE** | **CLINICAL EFFECTIVENESS ON HBA1C** |
|---|---|---|---|---|---|---|---|---|
| Linagliptina [90] | 1Nm | 5mg /24h | > 80 % | 10 – 40 | Bile > 80 % | Alta | Recommended | 0.5 – 1.0 % |
| Sitagliptina [91] | 19nM | 100 mg/ 24h | > 80 % | 8 – 24 | Renal > 80 % | Alta | Not recommended or adjust dose | 0.5 – 1.0 % |
| Vildaglip | 62nM | 50m | > 80 % | 1.5 – | Renal > | Media | Not | 0.5 – 1.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| tina [89] | | g/12 h | | 4.5 | 80 % | | recomme nded | % |
| Saxagli ptina | 50nM | 5mg /24h | > 70 % | 2 – 4 | Renal > 80 % | Media | Not recomme nded or adjust dose | 0.5 – 1.0 % |

- **LINAGLIPTINE**

The mechanism of action of linagliptin is to inhibit the DPP-IV enzyme (dipeptidyl peptidase type IV); This enzyme (DPP-IV) has the biological effect of inactivating the glucagon-like peptide type 1 (GLP-1), so being inhibited by linagliptin this favors that the levels of endogenous GLP-1 rise up to 3.2 times by above the previous values (same that are reduced in patients with type 2 diabetes mellitus, which partly explains that these patients have a reduction in the incretin effect), which determines the biological effects of GLP-1 as stimulation of insulin secretion by pancreatic beta cells and inhibition of glucagon secretion by pancreatic alpha cells. This effect on the stimulation in insulin secretion is totally dependent on glucose levels, so it does not cause hypoglycemia. Linagliptin has a half-life of 12 h so it can be used every 12 or 24 h, it is eliminated by bile in more than 70% so it can be used in patients with kidney disease without dose adjustment, it offers a potential for achieve a sustained inhibition of more than 90% in DPP-IV for 24 h and is highly selective to inhibit DPP-IV compared to other enzymes such as DPP-8 and DPP-9, their doses range from 2.5 to 5mg / day . Linagliptin is indicated in the treatment of patients with uncontrolled type 2 diabetes mellitus, which does not cover control goals such as: glycosylated Hb less than 7%, fasting glucose less than 110mg / dl and postprandial glucose less than 140mg / dl. It can be used as a combination therapy with metformin, sulfonylureas, thiazolidinediones and insulins, either as double or triple therapies.

A randomized clinical trial was conducted in the Mexican population comparing the effect of linagliptin with metformin in subjects with glucose intolerance. Sixteen subjects with glucose intolerance, overweight or obesity were included. They were randomized into 2 groups, one group was treated with metformin and another with linagliptin, both for 3 months. The group that was treated with linagliptin obtained a significant decrease in glucose at 120 min. [110]

## OTHER THERAPIES WITH POTENTIAL CARDIOPROTECTOR IN PREDIABETES

- **SGLT2 inhibitors (glucosuric)**

In addition to metformin, incretins and pioglitazone, it has been proposed that other recent therapies may also be useful in the treatment of the disease in early stages. These types of drugs include glucosurics, or inhibitors of the renal glucose transporter SGLT2. Under normal conditions, thanks to SGLT2 (97%) and SGLT1 (3%), all glucose that is filtered by the renal glomerulus is reabsorbed into the renal proximal tubules, so there should be no glycosuria; The glomerulus is capable, in a healthy person, of filtering about 140-160 g of glucose per day. There is a clinical disorder known as familial renal glycosuria, in which there is a mutation of SGLT2 that conditions glycosuria of up to 60-120 g per day, without these patients having any negative metabolic, hemodynamic or renal consequences. As part of the therapeutic innovations, SGLT2 inhibitors, or glucosurics, were developed, which are drugs that inhibit the activity of SGLT2 by 30-50%, thus reducing renal reabsorption of glucose and thereby favoring glycosuria. These types of medicines have been used for several years in the world and have revolutionized the treatment of DM2, which is what they are indicated for, by basing its effect on renal glucose elimination and not on the dependence of sensitivity to Insulin or insulin secretion. It has been shown that the patient with DM2 has a greater renal reabsorption of glucose in part due to the high regulation of SGLT2 by hyperglycemia [100]. There are few previous studies evaluating the usefulness of glucosurics in prediabetes; there is a report in patients with obesity, and mostly with prediabetes, who were treated with Dapagliflozin and an improvement in body weight, blood pressure and hyperglycemia frequency was observed [101, 102]; recently, another work was published with Empagliflozin, in patients with impaired fasting glucose in which the treatment for a few weeks substantially improved the function of pancreatic beta cells and therefore glucose metabolism [103]. Of this type of medication, Empagliflozin is particularly interesting, a drug that entered Mexico since 2015.

Empagliflozin has become an interesting drug because it is the only antihyperglycemic drug so far that has proven useful in reducing cardiovascular morbidity and mortality in

patients with DM2. and high cardiovascular risk [100, 104-107], and its main pharmacological characteristics are described below [100]:

- Mechanism of action: Empagliflozin inhibits approximately 30-50% of the activity of SGLT2, reducing renal reabsorption of glucose and favoring significant decreases in plasma glucose and glycosylated hemoglobin. Indirectly, it can improve insulin resistance and insulin secretion.
- **Dose**: 10, 12.5 or 25 mg every 24 hours; It can be consumed at any time of the day, with or without food.
- **Current indications**: Type 2 diabetes to improve metabolic control in patients with DM2 and a history of cardiovascular events. Its indication in patients with heart failure without DM2 is under evaluation.
- **Other beneficial effects:** Empagliflozin has been shown to significantly reduce cardiovascular morbidity and mortality and may also function as a nephroprotective, making it the only drug indicated in patients with DM2 and previous cardiovascular events to reduce the risk of a new cardiovascular event
- **Contraindications:** Patients with Type 1 Diabetes, patients hospitalized for severe glucose decompensation (diabetic ketoacidosis); There is no consensus on its use in children under 18 years of age, pregnant and breastfeeding, so it is not recommended. It is not indicated if there is significant renal involvement (creatinine clearance less than 30ml / min).
- **Side effects:** There is no risk of hypoglycemia, it can occur only when associated with insulin or sulfonylureas. A slightly higher frequency of genital infections has been observed in women, so if it is present, it is suggested to discontinue the medication.

A study conducted by Abdul-Goni et al. (2017) in which they used empagliflozin to reduce fasting plasma glucose and improve the function of beta cells in subjects with impaired fasting glucose. Sixteen subjects were studied, of which 8 had normal glucose levels and 8 had impaired fasting glucose. They were randomized into 2 groups, both received empagliflozin 25mg / d for 2 weeks, subjects with impaired fasting glucose had a significant decrease in fasting glucose and an improvement in the function of beta cells measured by the insulin availability index . [111].

A study conducted by DeFronzo et al. (2015), studied the safety and efficacy of the empagliflozin / linagliptin combination as therapy in subjects with DM2 not controlled with metformin. They were randomized to a combination of empagliflozin 25 mg / linagliptin 5 mg (n = 137), empagliflozin 10 mg / linagliptin 5 mg (n = 136), empagliflozin 25 mg (n = 141), empagliflozin 10 mg (n = 140 ), or linagliptin 5 mg (n = 132) as a complement to metformin (> 1,500 mg / day) for 52 weeks. The primary end point was the change from the start in HbA1c at week 24. At week 24 the reductions in HbA1c were significantly greater for empagliflozin 25 mg / linagliptin 5 mg (-1.19%). The efficacy remained until week 52. The proportion of subjects with adverse events (gastroenteritis, hypertension, headache, diarrhea and constipation) during the 52 weeks was similar in all groups, without hypoglycemia as adverse events [112].

The study conducted by Kaku K. et al. (2019) evaluated the efficacy and safety of empagliflozin and linagliptin fixed dose combinations in Japanese patients with poorly controlled type 2 diabetes mellitus (HbA1c ≥7.5 ≤10.5%). They were randomized into 4 treatment groups: 1) empagliflozin 10mg / placebo, 2) empagliflozin 25mg / placebo, 3) empagliflozin 10mg / linagliptin 5mg and 4) empagliflozin 25mg / linagliptin 5mg. The change from baseline HbA1c at week 24 was greater (P <.0001) with Empagliflozin / Linagliptin than with Empagliflozin / Placebo (Empagliflozin 10mg / Linagliptin 5mg: −0.94% vs. −0.12%; adjusted mean difference, - 0.82%; Empagliflozin 25mg / Linagliptin 5mg: −0.91% vs. −0.33%; adjusted mean difference, −0.59%). During periods of 24 and 52 weeks, higher proportions of patients reached HbA1c <7.0% and greater decreases in fasting blood glucose were observed with Empagliflozin / Linagliptin compared to Empagliflozin / Placebo. These results support the combination of a fixed dose Empagliflozin / Linagliptin as a potential option for Japanese patients with DM2 who require combination therapy. [113]

Lingvay I. (2017) conducted a non-systematic review of the literature focusing on a fixed-dose combination of SGLT2 inhibitors and DPP-4 inhibitors available in the United States. SGLT2 inhibitors and DPP-4 inhibitors have complementary mechanisms of action that address several of the underlying pathophysiological abnormalities present in DM2 without overlapping toxicities. Trials of cardiovascular outcomes reported to date

support the safety of the DPP-4 class and suggest possible cardioprotective effects for SGLT2 inhibitors, at least based on the first reported study, which used empagliflozin. Recently, clinical evidence shows that SGLT2 inhibitor / DPP-4 inhibitor therapy is an effective combination for treatment with DM2, which provides reductions in glycosylated hemoglobin (HbA1c) from 1.1% to 1.5% and weight reductions of approximately 2 kg when added to metformin. [114]

## RESEARCH QUESTION

What is the effect of quadruple therapy (linagliptin + empagliflozin + metformin + lifestyle modifications) compared to standard therapy (metformin + lifestyle modifications) on pancreatic islet function, insulin resistance and cardiovascular function in patients with mixed prediabetes and obesity?

## JUSTIFICATION

DM2 is a chronic disease that has reached global epidemic proportions due to the growing number of patients in all countries; It has become the disease that causes more chronic and acute complications to patients, consuming a large percentage of health spending in countries such as Mexico, generates a large number of disabilities at productive ages and is one of the main causes of cardiovascular morbidity and mortality.

The best strategies will be those that are aimed at early stages of the disease, because it will involve raising awareness in the population and having more impact on the stability and / or reversibility of pathophysiological alterations, which will ultimately result in a lower incidence of DM2.

Some studies have been carried out on prevention of DM2 based on lifestyle modifications, some of them have used strategies with a very narrow and intensive follow-up, which limits the durability of adherence to the intervention and hinders the practical applicability of the measures , have not compared the effect of some of the medications that have so far shown more efficacy for this purpose and in some of these

studies the doses used are frequently associated with the occurrence of side effects; of these studies, none have been carried out in Mexico, so if the combination of drugs with additive pathophysiological impact plus cardiovascular protection is used in early stages, better results can be obtained and with greater impact on the natural history of the disease with a view to a long-term follow-up that yields measures that may have an applicability in clinical practice, with the purpose of contributing to the control of this global epidemic that is surpassing health systems, especially in developing countries such as Mexico.

The combination of medications with different mechanisms of action, in low doses with lifestyle modifications could be a useful strategy not only to prevent DM2, but also to prevent macro and microvascular complications early.

## OBJECTIVES

**Goal:**

To assess the effect of quadruple therapy (linagliptin + empagliflozin + metformin + lifestyle modifications) compared to standard therapy (metformin + lifestyle modifications) on pancreatic islet function, insulin resistance and cardiovascular function in patients with mixed prediabetes and obesity.

**Specific goals:**

1. Identify patients with glucose intolerance and obesity who may enter the study.
2. To assess the effect at 12 months with treatment with metformin 1700 mg / d and lifestyle modifications on insulin resistance, pancreatic islet function, and cardiovascular function in a group of patients with mixed prediabetes and obesity.
3. To assess the effect at 12 months of treatment with the combination of linagliptin 2.5 mg / metformin 850 mg every 24 h + Empagliflozin 12.5 mg / metformin 850 mg every 24 h and lifestyle changes on insulin resistance, the function of Pancreatic islet and cardiovascular function in a group of patients with mixed prediabetes and obesity.

4. Evaluate and compare cardiovascular risk markers (TNFa, IL6, hsPCR) before and after treatments in the 2 study groups.
5. Compare insulin resistance and pancreatic islet function between the study groups at the end of the intervention.
6. Evaluate and compare glucose metabolism using OGTT at the beginning and end of treatment in the study groups.

**Secondary objectives**

- Evaluate the percentage of patients who recovered glucose normotolerance after the intervention in each of the study groups.
- Evaluate and compare the changes in body composition, through body composition analysis by DEXA, in the two study groups at the beginning and at the end of the intervention.
- Evaluate therapeutic adherence in the study groups.

## HYPOTHESIS

Quadruple therapy (linagliptin + empagliflozin + metformin + lifestyle modifications) has a greater effect on pancreatic islet function, insulin resistance and cardiovascular function compared to standard therapy (metformin + lifestyle modifications) in patients with mixed prediabetes and obesity.

## MATERIAL AND METHODS

**Type of study:** Single Center Parallel Clinical trial, randomized, double blind

**Study universe:** Mexican patients with mixed prediabetes and obesity.

**Study population:** Mexican patients with mixed prediabetes and obesity, from the State of Guanajuato.

**Location:** Regional High Specialty Hospital of Bajío and Division of Health Sciences of the University of Guanajuato, León, Gto.

**Type of sampling:** Non-probabilistic, of consecutive cases.

**Sample size:** The sample size calculation was performed based on the formula to compare proportions. We expect that quadruple therapy (linagliptin + empagliflozin + metformin + lifestyle modifications), improve at least 75% the outcome variables (insulin resistance, pancreatic islet function and cardiovascular function), at 6 months of initiated the intervention, as long as the standard therapy (metformin + lifestyle modifications) achieves only 30% [69-71, 73], so that the study is designed to detect a minimum difference of 45% between the study groups with a type I (alpha) error of 0.05 and a sample power of 80%, and according to the following formula [108]:

$$n = \frac{(Z\alpha/2+Z\beta)^2\, \bar{p}(1-\bar{p})(r+1)}{(d)^2 r}$$

Where,

$(Z\alpha / 2 + Z\beta)\, 2 = 7.9$

p1 = proportion of individuals in the best treatment who did not achieve improvement = 0.25

p2 = proportion of individuals in the worst treatment who did not achieve improvement = 0.70

r = ratio of individuals in the treatment groups = 1

d = difference expected to be found = 0.45

n = 14 + 20% of losses = 17 patients per group

## SELECTION CRITERIA

**Inclusion Criteria:**

- Patients with prediabetes, defined for the existence impaired glucose tolerance (glucose between 140 and 199 mg/dL at the 2 hours of the Oral Tolerance Glucose Test (OGTT) with impaired fasting glucose (fasting glucose between 100 and 125 mg/dL)
- Patients who accept to participate in the study and sign the informed consent letter.

**Exclusion Criteria:**

- Patients with diagnosed Type 2 Diabetes previously or detected during the OGTT
- Patients in actual treatment or during the last 3 months with metformin, pioglitazone or another antidiabetic drug, includin insulin
- Serum creatinine > 1.6 mg/dL
- Hypertriglyceridemia very high (>500 mg/dL)
- Pregnant women
- Altered arterial hypertension (Systolic >180 mmHg or Diastolic >105 mmHg)
- Excessive alcohol intake, acute or chronic
- Medications or medical conditions that affect glucose homeostasis (thiazides, beta blockers, glucocorticoids for systemic use, weight-reducing drugs or anorexigenics, Cushing´s syndrome, thyrotoxicosis

## VARIABLES

### INDEPENDENT VARIABLE

**1) Intervention**

a. Measurement level: Qualitative nominal

b. Indicator:

1. A for the group receiving quadruple therapy (linagliptin + empagliflozin + metformin + lifestyle modifications)

2. B for the group receiving standard therapy (metformin + lifestyle modifications)

c. Conceptual definition: Therapeutic strategy whose purpose is to avoid alterations in glucose metabolism and progression to DM2, and reduce cardiovascular risk.

d. Operational Definition:

1. When patients receive 1 tablet of linagliptin 2.5mg / metformin 850mg every 24 hours + 1 tablet of empagliflozin 12.5mg / metformin 850mg every 24 hours + lifestyle modifications for 12 months

2. When patients receive 1 tablet every 12 hours of metformin 850 mg + lifestyle modifications for 12 months

### DEPENDENT VARIABLES

**1) Improvement in glucose metabolism (regression of hyperglycemia and glucose intolerance)**

a. Type of variable and level of measurement: Qualitative dichotomous nominal
b. Indicator: Yes or No
c. Conceptual definition: The ability of therapeutic interventions to reverse fasting hyperglycemia and glucose intolerance.
d. Operational definition: It will be considered as YES when the fasting glucose level at 12 months after the start of the intervention is <100mg / dl and the glucose at 2h during the OGTT is <140mg / dl, and as NO when at 12 months after the start of the intervention, fasting glucose levels are ≥100mg / dl and / or at 2h during OGTT are ≥140mg / dl.

**2) Glycosylated hemoglobin**

a. Type of variable and level of measurement: Quantitative ratio
b. Indicator: %
c. Conceptual definition: Biochemical test indicating the degree of glycemic control during the last 3 months
d. Operational definition: Glycosylated hemoglobin values measured at the beginning and end of the study by HPLC technique.

**3) Pancreatic beta cell function (Insulin Oral Disposition Index = IDOI)**

a. Type of variable and level of measurement: Dependent, Quantitative of reason
b. Indicator: pmol / mmol
c. Conceptual definition: Pancreatic beta cell's ability to secrete enough insulin and maintain normal glucose levels
d. Operational definition: Value obtained with glucose and insulin values during the OGTT by applying the following formula: (ΔI0-30) / (ΔG0-30), where (ΔI0-30) = difference in the value of insulin measured at 0 and 30 minutes of the OGTT; and (ΔG0-30) = difference in the glucose value measured at 0 and 30 minutes of the OGTT, which will be measured at the beginning and at the end of the intervention in each study group.

**4) Pancreatic beta cell function**

a. Type of variable and level of measurement: Dependent, Quantitative of reason
b. Indicator: pmol / mmol
c. Conceptual definition: Pancreatic beta cell's ability to secrete enough insulin and maintain normal glucose levels
d. Operational definition: This will be obtained using the OGTT glucose and insulin values by applying the following formula: (IncAUCG0-120) / (IncAUCI0-120), where (IncAUCG0-120) = increase in the area under the curve of glucose during the 120 minutes of the OGTT obtained by the trapezoidal rule, and (IncAUCI0-120) = increase of the area under the insulin curve during the 120 minutes of the OGTT obtained by the trapezoidal rule.

**5) Insulin Sensitivity (Matsuda Index)**

a. Type of variable and level of measurement: Dependent, Quantitative of reason
b. Indicator: natural numbers
c. Conceptual definition: The ability of insulin to promote glucose uptake by muscle tissue, to suppress glucose production by the liver and to prevent lipolysis in adipose tissue.
d. Operational definition: It will be evaluated using the Matsuda index with glucose and insulin values during the OGTT using the following formula:

$$\text{Insulin sensitivity} = \frac{10000}{\sqrt{(G0 \times I0) \times (G0\text{-}120 \times I0\text{-}120)}}$$

Where G0 = basal glucose

I0 = basal insulin

G0-120 = average glucose during OGTT

I0-120 = average insulin during OGTT

This measurement will be carried out at the beginning and at the end of the intervention in each of the study groups.

**6) Inflammation markers**

a. Type of variable and level of measurement: Quantitative ratio
b. Indicator: Corresponding measurement unit according to the biochemical marker
c. Conceptual definition: Biochemical markers associated with a pro-inflammatory state
d. Operational definition: Fasting serum levels of TNFa, IL6 and hsPCR
e.

**7) Cardiovascular function**

a. Type of variable and level of measurement: continuous quantitative
b. Indicator: %
c. Conceptual definition: Comprehensive evaluation of the mechanical function of cardiac function, whose objective is to predict the risk of future cardiovascular events.
d. Operational definition: It will be evaluated by the left ventricular ejection fraction, ventricular mass and diastolic and systolic preloads, by standard echocardiography performed by HRAEB Echocardiographic Cardiologist

## GENERAL PROCEDURE

### Identification of patients

The first phase will be carried out in collaboration with SSA First Contact Units, in the external consultation of Endocrinology, Diabetes, Obesity and Nutrition of the HRAEB and in the Metabolism Research Laboratory of the University of Guanajuato, León campus. During this initial phase, patients with obesity and at least 2 risk factors for DM2 will be identified, who will be invited to the study screening, as a first step they will answer a health survey that will include the evaluation of biological factors (physical activity, diet , drug addiction, risk factors for cardiometabolic diseases, etc.), psychological (quality of life, depression, behavior patterns, etc.) and social (family,

work environment, domestic violence, type of family, etc.). For this survey, questionnaires already validated and used in the Cohort study of Health Personnel being carried out at the Autonomous University of the State of Mexico and in the state of Morelos, by the Epidemiological Research Units and Health Services will be used from IMSS [109].

**Patient Recruitment**

Evaluation and metabolic classification of patients: Both patients with a BMI> 30 Kg / m2 and risk factors for DM2 will be invited to the HRAEB outpatient clinic and to the Metabolism Research Laboratory of the University of Guanajuato continue to the scrutiny phase and at this time they will be asked for the signature of informed consent; The next step will be to quote the patients in the Metabolism Research Laboratory of the University of Guanajuato, León Campus for fasting glucose measurement, and only patients who have a glucose level equal to or greater than 100mg / dL will be performed the OGTT to determine the presence of mixed prediabetes.

Oral Glucose Tolerance Test (OGTT): consists of citing the patient to the Metabolism Research Laboratory of the University of Guanajuato with an 8-12 h on fast, first cleaning will be performed from the site with antiseptic solution, the vein will then be channeled to the ulnar with a punzocat and the basal blood samples will be taken, then 75g of glucose will be given to drink and during the next two hours blood samples will be taken every 30 minutes (0, 30 , 60, 90 and 120min), administering after each blood sample 2cc of 0.9% saline, finally at the end of the study the catheter will be removed. During the OGTT, glucose will be determined and then insulin will be measured. As mentioned in the selection criteria, patients who are diagnosed with DM2 during screening (OGTT) will not be included in the study and will be informed of their diagnosis and will be advised to go to their treating physician to carry out their pharmacological treatment. .

According to glucose values at 2 h of the oral load, patients will be classified as:

1) Glucose normotolerant when glucose at 2h during OGTT is <140 mg/dl,

2) Patients with glucose intolerance when the glucose at 2h of the OGTT is between 140 and 199 mg/dl,
3) Patients with newly diagnosed DM2 when the glucose at 2 h of the OGTT is ≥ 200 mg/dl.
Only patients diagnosed with glucose intolerance in the OGTT will be invited to participate in the trial and those who accept will continue with a more thorough metabolic evaluation.

For this purpose, patients will have a complete medical history and the following studies:

1) Fasting baseline blood samples for measurement of: blood count, liver transaminases, lipid profile, inflammation markers (TNF alpha, IL-6, hsPCR, etc.) and insulin for the determination of HOMA-IR and HOMA- B.
2) Weight determination using a digital scale
3) Determination of body composition (% total fat, visceral fat,% total body water, bone mineral density, etc.) by DEXA analysis.

In this phase the screening of patients ends, and if at this time the patient agrees to continue in the intervention phase, the following will be done:
1**)** **Evaluation of cardiovascular function:** This will be done by standard echocardiography that will be performed in the Echocardiography area of the High Specialty Regional Hospital by a specialist in the area.

**2) Evaluation of renal function:** It will be carried out through 24-hour urine collection, in which creatinine clearance and 24-hour albuminuria will be measured.

**3) Randomization:** The patients will be randomized to one of the two interventions. It will be done by using a table of random numbers generated by a computer program. Patients will be randomized to receive, for 12 months, one of the following 2 interventions: 1) Quadruple therapy consisting of: 1 tablet every 24 h of linagliptin 2.5mg / metformin 850mg + 1 tablet every 24 h of empagliflozin 12.5mg / metformin 850mg + lifestyle modifications (personalized diet plan and individualized physical activity

indications), and 2) 1 tablet every 12 hours of Metformin 850mg + lifestyle modifications (personalized diet plan and individualized physical activity indications). The start of the dose in the two study groups will be gradual so that at one month of treatment the patient can be with full doses in each study group. In the quadruple therapy group it will be suggested to take the medication starting with half a tablet of linagliptin 5mg / metformin 850mg every 24 hours with the last meal of the day (dinner) and at 2-4 weeks increase the dose to 1 tablet every 24 hours; and another tablet containing empagliflozin 12.5mg / metformin 850mg starting with half a tablet every 24 hours, it will be indicated to take it with the first meal of the day (breakfast), and at 2-4 weeks complete the dose to 1 tablet every 24 hours; in the metformin therapy group it will be suggested to take the medication starting with half a tablet of metformin 850mg every 12 hours (in the morning with breakfast and half a tablet of metformin 850mg at night with dinner) and at 2 -4 weeks, complete the dose to 1 tablet every 12 hours.

**Intervention and follow-up**

All patients will receive nutritional guidance, meal plan and physical activation recommendations during the study at the Metabolism Research Laboratory of the University of Guanajuato and/or at the Metabolic Unit from the Hospital Regional de Alta Especialidad del Bajío. All patients will have a monthly follow-up with fasting glucose measurement, anthropometry, global clinical evaluation and adherence to treatment (by counting pills consumed monthly by the patient).

Adherence to drug treatment: Information about the disease, its possible complications, the purpose of the treatment to be followed, the exact dose, frequency of administration and duration of therapy, as well as the therapy will be transmitted in an adequate and understandable way to the patient. The consequences of not receiving it. The patient will be suggested to develop a plan as complex as possible and adapted to medications, doses and schedules with daily acts. Sort your medications by time sector (morning and night) and keep them in a visible place.

Nutritional treatment: a feeding plan will be given to each patient, with the aim of achieving a 7% weight loss during treatment. The total energy contribution must be adapted in order to maintain a recommended weight. The energy intake will be between 25 - 30 Kcal / kg/day. The recommended composition of the dietary regimen as follows: total fat 25 -30%, saturated fat <10%, with a predominance of monounsaturated (up to 15%); carbohydrates 50% -60 %; protein 15 – 20 % (1.2 g / body weight (kg) / day). Initially it is recommended to reduce the usual consumption 250 to 500 kcal / day. To determine the adherence to the dietary regimen , the food records of two days of the week and one day of the weekend, and the food consumption frequency questionnaire.

Physical activity: A minimum of 150 minutes per week of cardiovascular exercise and muscular endurance exercises (on non-consecutive days) will be recommended. In the case of sedentary patients, they should be recommended the practice of aerobic exercise, especially the walk, at least for periods of 20 to 30 minutes (periods of 10 continuous minutes are cumulative throughout the day), the application of The above indication should be done gradually, accompanied by the relevant instructions on precautions to avoid injury or other possible problems. In active patients they will be advised to increase time or intensity of physical activity. Physical activity will be assessed through the international physical activity questionnaire (IPAQ).

**Mid-term evaluation**

After 6 months of treatment, the patients of both study groups will be summoned to the Metabolism Research Laboratory of the University of Guanajuato León campus, with fasting from 8-12h for the completion of the OGTT, and the intermediate evaluation on the status of the glucose metabolism During this test blood samples will be taken at 0, 30, 60, 90 and 120 minutes to determine glucose and insulin, and obtain different rates of insulin resistance and insulin secretion. In addition, the evaluation of cardiovascular function and renal function will also be performed, using the methods previously mentioned. The hyperglycemic clamp will also be performed in the patients of both study groups.

**Completion of the study (final measurements)**

At 12 months of treatment, patients will be cited again on an 8-12h fast, on two different occasions:

1) First appointment: OGTT will be performed for the measurement of glucose and insulin, determination of markers of inflammation and endothelial dysfunction will be made. Measurement of body composition will be done using impedance and DEXA.
2) Second appointment: The evaluation of cardiovascular function by echocardiography, and renal function by creatinine and albumin clearance in 24-hour urine will be performed.

**Laboratory procedures and techniques**

For glucose measurement, an Analox GM9 brand glucoanalyzer will be used, which uses the glucose oxidase method. The measurement of TNF alpha, IL-6 and hsCRP will be done by ELISA or chemiluminescence in the Metabolism Research Laboratory of the University of Guanajuato; Insulin measurement will be done by chemiluminescence in the HRAEB laboratory. Lipid measurement, blood count, blood chemistry, liver function tests, will be done using standard techniques. For the determination of body composition, a DEXA equipment for bone densitometry and body composition with a worldwide standard methodology will be used.

Several methods will be used to calculate insulin resistance:

1) HOMA-IR = (Fasting plasma insulin in mU / l / Fasting plasma glucose in mmol / l) /22.5; This only requires fasting glucose and insulin.
2) Matsuda index = 10,000 / √ [(fasting glucose in mg / dl x fasting insulin in mU / l) x (average glucose during OGTT x average insulin during OGTT) [29]
3) QUICKI index = l / (fasting insulin log + fasting glucose log)

The following methods will be used to calculate the beta cell function:

1) HOMA-B = (20 x fasting plasma insulin in mU / l) / (fasting glucose in mmol / l - 3.5), which requires fasting glucose and insulin
2) Calculation of insulin availability index during OGTT

## STATISTIC ANALYSIS

The data will be presented with descriptive statistics, mean and standard or median deviation and interquartile range, as well as percentages and incidence. For comparisons of numerical variables between the study groups, we will use Student's t if the distribution of the data follows a normal pattern, otherwise the Mann-whitney U test will be used; To compare qualitative variables we will use Chi square. Delta analysis will be performed for differences between groups. It will be considered significant when the value of p is <0.05. For the analysis and presentation of the data we will use the SPSS Version 15 and GraphPad Prism 5 programs.

## ETHICAL CONSIDERATIONS

In order to carry out this research, ethical standards, the Regulation of the General Health Law on Health Research and the Declaration of Helsinki of the World Medical Association of the 64th General Assembly, Fortaleza, Brazil in 2013 and International codes and standards in force for good clinical research practices. Special attention will be given to taking care of the privacy and autonomy of the patient. Once the patient has been informed about the study and agrees to participate in it, and since this implies a risk greater than the minimum, the signature of an informed consent letter will be requested (Annex 1).

The protocol was evaluated and approved by the Research Ethics Committee of the Regional High Specialty Hospital of Bajío and the Research Ethics Committee of the University of Guanajuato.

## HUMAN, MATERIAL AND FINANCIAL RESOURCES

This work will be carried out in collaboration with Researchers of the Metabolism Laboratory of the Division of Health Sciences of the University of Guanajuato, Campus León, and therefore will be developed both at the University of Guanajuato and at the High Specialty Regional Hospital del Bajío (HRAEB). Patients will be recruited from the

first contact clinics of the SSA and in the outpatient clinic of the Endocrinology, Internal Medicine and Nutrition services of the HRAEB. The drug will be provided by the HRAEB, as well as the analysis of glycosylated hemoglobin. The oral glucose tolerance curve will be carried out with the inputs of the Metabolism Laboratory of the Division of Health Sciences of the University of Guanajuato, Campus León.

## DECLARATION OF CONFLICT OF INTEREST

There is no conflict of interest since the main commitments and obligations of this project are not influenced by other material or personal interests. The medication will be provided by the Bajío High Specialty Hospital

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## ANNEX 1

## INFORMATION LETTER
## INFORMED CONSENT FORM

**Title:** Effect of quadruple therapy on pancreatic islet function, insulin resistance and cardiovascular function in patients with mixed prediabetes and obesity: Randomized Clinical Trial.

**Place**: University of Guanajuato / Regional Hospital of High Specialty of the Bajío

**Address**: Blvd. Milenio No. 1001, San Carlos la Roncha, León Guanajuato, C.P. 37660

**Researcher:** Dr. Rodolfo Guardado Mendoza.

**Researcher's telephone:** 477 274 4215.

**Introduction:**

Type 2 diabetes mellitus is a very important health problem in Mexico, so different efforts are made to identify it at an early stage (such as prediabetes), to be able to treat it in a timely manner and possibly prevent the disease from progressing. At the University of Guanajuato and the Regional Hospital of High Specialty of Bajío (HRAEB) we are conducting research in people with prediabetes and obesity, whose main objective is to evaluate the usefulness of different treatments to prevent type 2 diabetes mellitus. The document is to inform you about the research we are doing and invite you to participate, since you are at high risk of developing diabetes because you are a carrier of prediabetes, in addition to your participation could serve to identify new diabetes prevention strategies, He would also receive the benefit of some of the interventions to improve his glucose levels and therefore reduce the risk of developing diabetes.

You do not have to decide today if you participate in the research, before deciding, you can talk about the research with someone with whom you feel comfortable, such as a family member or friend.

There may be some words that you don't understand, please stop me as I inform you to give me time to explain. If you have questions later, you can ask me or any member of the team conducting the investigation.

I also inform you that your participation is completely voluntary and that you can withdraw from the study if you wish at any time, without this having any impact on the care you should receive in the health care centers where you go; In addition, all the information obtained in this study will be handled confidentially and will be used only for scientific purposes.

**Purpose of the investigation.**

This study has as main objective to evaluate and compare the effect of two treatments: 1) metformin combined with linagliptin and empagliflozin plus lifestyle modifications (nutrition and physical activity), and 2) metformin alone plus lifestyle modifications (nutrition and physical activity), in the function of the pancreas, insulin resistance and heart function and cardiovascular risk in patients who have prediabetes and obesity.

**Research procedures.**

If you agree to participate in this research work, you will have different types of studies during several appointments:

1) FIRST APPOINTMENT

a. A blood sample will be taken, you must be fasting, to measure: glucose, insulin, cholesterol, triglycerides and inflammation markers.
b. An oral glucose tolerance curve will be performed, which consists of placing a punzocat in one of the veins of the arm so that, after taking a glass with 75g of glucose, blood samples will be taken at 30, 60, 90 and 120 minutes to determine glucose and insulin at each of those times. Venipuncture is a procedure that implies a minimal risk such as bruising and / or slight pain when placing the punzocat inside the vein, sometimes it can occur: feeling dizzy, excessive bleeding and infection.
c. Your weight will be determined by means of a digital scale and your body composition, that is, the percentage of your body made up of visceral fat and water will be calculated; for this measurement you only need to lie on the couch of an apparatus that measures body composition (a densitometer, DEXA. This procedure lasts for 6 min which implies exposure to a very small dose of ionizing radiation to produce images of the interior of the body, which has no risk.
d. Resting echocardiogram: This study consists of measuring different parameters of your heart, lying on a stretcher, it is not painful and will not cause any discomfort, it is performed in approximately 15-20 minutes.
e. Collect 24-hour urine for tests of your kidney function (24-hour creatinine clearance and albuminuria).

2) SECOND APPOINTMENT

After this, you have finished the initial phase of the study and you will receive, at random (like throwing a coin in the air), one of the two treatments for prediabetes, which researchers are using in this research, for 12 months:

1. Lifestyle modification program (includes nutritional guidance and advice on physical activity) plus a tablet of medicine containing linagliptin 2.5mg / metformin 850mg starting with half a tablet every 24 hours (it will be indicated to take it with the last food of the day (dinner) and at 2-4 weeks 1 tablet every 24 hours, and another tablet containing empagliflozin 12.5mg / metformin 850mg starting with half a tablet every 24 hours (it will be indicated to take it with the first meal of the day (breakfast), already 2-4 weeks 1 tablet every 24 hours.
2. Program to change the lifestyle (includes nutritional guidance and advice on the performance of physical activity) plus a medicine called metformin 850mg tablets, starting with half a tablet every 12 hours and at 2-4 weeks 1 tablet every 12 hours.

Metformin is a medication widely used worldwide to treat several diseases (type 2 diabetes mellitus, prediabetes, polycystic ovary in women), its maximum doses are 2550mg / day and the most frequent side effects include: flatulence, diarrhea, malaise intestinal, which are reduced when starting with low doses and usually disappear after 7-14 days. On the other hand, linagliptin is a relatively new medicine that is used worldwide to treat patients with type 2 diabetes mellitus, its side effects are minimal, when they occur they include nausea, mainly. It is important to tell you that you will receive one of the 2 interventions previously described, and each of them covers the basic treatment of a patient with prediabetes. Empagliflozin is a recent medication that is used in patients with diabetes, but because of the beneficial effects it has shown, it favors the elimination of glucose in the urine and reduces the risk of heart disease in some patients, it is believed to be useful. if used early, such as prediabetes; Its side effects are minimal and occasionally genital infections can occur in women, which will be interrogated in each of their visits, and if presented, treatment will be indicated and the medication will be discontinued.

The nutritional orientation you will receive during the 12 months of intervention will be evaluated monthly in which a personalized eating plan will be carried out with the intention of having a weight loss <7% of your current weight and physical activity counseling is included in the performance 150 min of exercise per week at moderate to severe intensity, between cardiovascular exercises and resistance exercises, indicating

techniques and postures to avoid presenting injuries as well as the way to start physical activity in patients with sedentary lifestyle

3) FOLLOW-UP APPOINTMENTS

a. Once the intervention begins, you will go to appointments every month during the 12 months of the study. During these appointments you should always go on a fast, and a blood sample will be taken to determine glucose and insulin. At each appointment you will be given educational information about nutrition and physical activity, as well as any questions you have about the study, monitoring and treatment.

4) APPOINTMENT FOR INTERMEDIATE EVALUATION

a. 6 months after the start of the treatment, you must go to a fasting appointment to perform the Glucose Tolerance Curve, which will allow us to evaluate the progress of your glucose levels; and the exams performed during the FIRST APPOINTMENT will be repeated.

5) POST-INTERVENTION APPOINTMENTS

a. At the end of the intervention (12 months) he will go back to fasting, first to perform another curve of oral glucose tolerance (OGTT) and to measure his body composition using DEXA (body fat, water etc.). At the end of the study, the medication will be suspended with semi-annual follow-up through the OGTT to monitor its evolution.

**Research Risks**

• Any adverse reactions or complications that occur during treatment will be addressed.

• During the first days of treatment, diarrhea or intestinal discomfort is likely, which will disappear in the next 7 to 14 days.

• In some cases, urinary tract infection may occur, which can be identified by the following symptoms: burning when urinating, frequent urination in small quantities,

cloudy urine, strong-smelling urine, pelvic pain in women, especially in women. the center of the pelvis and around the area of the pubic bone.

• The medications used in the project are not associated with hypoglycemia (low blood glucose levels), however, it can be identified by the following symptoms: sweating, chills, confusion, rapid heartbeat, dizziness or dizziness, drowsiness, vision blurred, tingling or numbness of the lips or tongue, headaches, weakness or fatigue, seizures or loss of consciousness.

• If any of these symptoms occur, contact the principal investigator of this project to provide appropriate treatment.

**If I decide so, what do I commit myself to participate in this research?**

If you agree to participate in this research during the duration of this study, you should take the pills with the treatment that you have: (1) metformin with linagliptin and empagliflozin or (2) metformin alone, unless the study doctor considers that You must change treatment. You should go to appointments with the study doctor every month.

**If I accept, will I be paid to participate in this research?**

No, if you decide to participate in this investigation you will not receive any payment for it.

**What are the benefits that I will get by participating in this research?**

The benefit to you of participating in this research study is that the intervention performed will have a beneficial effect on your glucose levels and probably on your body weight, therefore it will be useful to prevent diabetes. In addition to this, you will obtain information and guidance for the duration of the study that will serve to improve your lifestyle with a healthier diet and routine physical activity, in addition to these benefits may be extended to your family members. The procedures performed during the study will have no cost to you and you will not receive any compensation for participating in the study.

**Confidentiality of information**

Remember that your identity will be protected at all times, for this your name and any information that could be used to identify you will be carefully protected. We will assign you a number that we will use to identify your data, and we will use that number instead of your name for the purpose of reporting this investigation. When the results of this study are published or presented at conferences, for example, no information will be given that could reveal your identity.

**Contacts for questions and information about the study and your rights as a participant**

We greatly appreciate your participation in the study “Effect of quadruple therapy on pancreatic islet function, insulin resistance and cardiovascular function in patients with mixed prediabetes and obesity: Randomized Clinical Trial”. If you have questions or want to talk to someone about this research study, you can contact the research staff or the Research Project Coordinator Dr. Rodolfo Guardado Mendoza, by phone: 477 274 4215.

In addition, we provide you with the contact numbers of the Bioethics and Research Committee of the University of Guanajuato, so that you can clarify any questions you have regarding your participation in this research project: (473) 732 00 06, Ext. 5019 or to the email ethica@ugto.mx.

DECLARATION OF INFORMED CONSENT

I _____ have read, agree to participate in the research study entitled: EFFECT OF THE QUADRUPLE THERAPY ON THE FUNCTION OF THE PANCREATIC ISLOT, INSULIN RESISTANCE AND CARDIOVASCULAR FUNCTION IN PATIENTS WITH MIXED PREDIABETES AND OBESITY: Clinical Trial; registered with the Research Ethics Committee and the HRAEB Research Committee, with the number ___ ___, and whose main objective is to evaluate the usefulness of different interventions to prevent type 2 diabetes mellitus. I have been informed that I am a person with a high risk of developing type 2 diabetes mellitus in the future, and that this disease is one of the most frequent chronic health

problems worldwide, so preventive strategies are of vital importance. The principal investigator has informed me that my participation is completely voluntary and that I can withdraw from the study in case I wish at any time during the course of the study, without this having any impact on the care I should receive at my center. medical care; Likewise, I have been informed that the information obtained in said study will be handled confidentially and only used for scientific purposes.

By agreeing to participate in this research work, different types of studies will be carried out during several appointments, and I will be followed up during the 12 months of the research study. Likewise, I will be randomly assigned one of the 2 treatments that are being evaluated in the study, which will be provided to me free of charge by the researchers.

The principal investigator of this project, Dr. Rodolfo Guardado Mendoza, has provided me with all the information regarding the study, has clarified all my doubts regarding the benefits, risks and usefulness of my participation, has provided me with his telephone number (Cel. 044 477 2744215) for in case later I have more doubts, and it has given me a duplicate of this document to keep it.

Participant:
Name and surname: _____
Signature or fingerprint: _____
Date: _____

Legal representative (only if applicable):
Name and surname: _____
Signature or fingerprint: _____
Date: _____

First witness:
Name and surname: _____
Signature: _____

Date: _____

Relationship with the participant: _____

Address: _____

Second witness:

Name and surname: _____

Signature: _____

Date: _____

Relationship with the participant: _____

Address: _____

Researcher of the study and / or responsible for carrying out the informed consent process:

I have explained this research study to the participant. I have answered all the participant's questions.

Name and surname: _____

Signature:_____

Date: _____

Registration or identification or stamp: _____