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Effectiveness of the Treatment With Dapagliflozin and Metformin Compared to Metformin Monotherapy for Weight Loss on Diabetic and Prediabetic Patients With Obesity Class III

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1. TITLE.

Efficacy of the treatment with dapagliflozin and metformin compared with metformin monotherapy for weight loss and its effects on waist circumference, triacylglycerol concentration, blood pressure and inflammatory cytokines on diabetic and prediabetic patients with obesity class III. Open, randomized clinical trial.

2. ABSTRACT

Introduction: According to the World Health Organization (WHO), obesity is an abnormal or excessive accumulation of fat, which could affect human health. Mexico has one of the highest prevalence of obesity, reported by 32.4% of people over 20 years old, according to National Health and Nutrition Examination Survey (ENSANUT, Encuesta Nacional de Salud y Nutrición 2012). This implies that 22 millions of Mexicans have obesity. Additionally, the prevalence of obesity class III (defined as a body mass index greater than 40 kg/m^2) is 1.8% (IC95% 1.5-2.1%) in males and 4.1% (3.7%-4.5%) in females.

The Mexican Social Security Institute (IMSS, Instituto Mexicano del Seguro Social) (that has one of the widest health coverages in the country) has reported a prevalence of obesity on its affiliates up to 74.4%, but only 50% had received clinical care for weight control (for example, diet or physical activity recommendations). In a recent article published by Barrera-Cruz et al. it is estimated that this disease reduces life expectancy from 6 to 20 years, being responsible for 2.8 million deaths per year in our country. In addition, it is estimated that it generates an annual cost of 3,500 million dollars for direct and indirect costs.

Since 2008, a multidisciplinary clinic focused on obesity treatment and related comorbidities has been organized at the Hospital de Especialidades of Centro Médico Nacional Siglo XXI. Currently, the "Obesity Clinic" attends a 400-patient population, of which 150 patients have undergone bariatric surgery (gastric bypass or gastric sleeve) and 250 patients are on waiting list and preparation for this procedure. According to previous data (not yet published) the mean age of these patients is 41 ± 9 years, 46% have pre-diabetes or type 2 diabetes mellitus, 66% has hypertension and 33% has dyslipidemia (hypertriglyceridemia, hypoalphalipoproteinemia or both). The management of glucose alterations on this unit is done with personalized diets supervised by the Nutrition Service of the Obesity Clinic and with the use of metformin at doses greater than 2 g/day and/or basal insulin at doses of 0.5 to 1 U/kg of patient's weight.

Recently, a group of drugs known as inhibitors of sodium-glucose transporter protein 2 (iSGLT2) has been approved for T2D treatment. These low-affinity and high-capacity transporter proteins are found at proximal convoluted tubules and are responsible for glucose re-absorption; therefore, its inhibition induces glycosuria and subsequently a decrease of plasmatic glucose. Dapagliflozin is a selective iSGLT2 with a sustained effect on the reduction of glycated hemoglobin (HbA1c) at 0.4 to 0.8%. Additionally, due to the induction of glycosuria up to 20-85 g/day, it has been calculated that its use induces a caloric deficit at 80 to 340 kcal/day. This has been observed in patients with T2D in which induces a weight loss of 2 to 3 kg and in combination with metformin even a weight loss up to 5.07 kg (-6.21 to 3.93 kg) without regain (at least for 2 years) as

previously demonstrated. Furthermore, it decreases systolic blood pressure (4-5 mmHg), increases concentration of HDL cholesterol (1.8 to 4.4%) and decreases triglycerides concentration (2.4 to 6.2%). Due its mechanism of action is independent of insulin production; there exists a possibility of using dapagliflozin in patients with prediabetes or even on other types of diabetes. Regarding safety considerations, main adverse effects related to its use are genitourinary infections and it has been demonstrated that it does not induces kidney injury.

A previous study by Bolinder et al. that included 182 patients with T2D inadequately controlled with metformin, assessed the effect of dapagliflozin 10 mg in weight loss after 24 weeks of treatment and compared it to placebo. They found a decrease of 2.08 kg (2.8 to 1.31 kg, $p<0.001$), decrease in waist circumference of 1.52 cm (2.74 to 0.31, $p=0.014$), decrease in total fat mass assessed with densitometry (DEXA) of 1.48 kg (2.22 to 0.74, $p=0.001$), decrease of visceral fat mass of 258.4 cm^3 (448.1 to 68.6, $p=0.008$) and decrease of subcutaneous fat of 184.9 cm^3 (359.7 to 10.1, $p=0.03$). However, those studies on weight are performed in patients with obesity class I or II and there is a lack of information on inflammatory markers after dapagliflozin treatment.

Primary objective: To determine if combined treatment with dapagliflozin and metformin is more effective than monotherapy with metformin for weight loss and assess its effects on waist circumference, triglycerides, blood pressure and inflammatory cytokines (resistin, adiponectin, interleukin-6 and interleukin-10) in prediabetic and diabetic patients with obesity class III. **Secondary objective**: To determine the effect of combined treatment with dapagliflozin and metformin in comparison with monotherapy with metformin on serum concentrations of glucagon, ghrelin and insulin.

Patients, material and methods: The protocol includes patients with previous or recently diagnosed diabetes or prediabetes from the Obesity Clinic of CMN SXXI from July 2018 to August 2023 age 18 to 60 years old, both male or female and not treated with insulin. Patients requiring initial treatment with insulin, with obesity secondary to other comorbidities and patients who do not wish to enter the study or not provide their informed consent will be excluded. Patient's data that during the study are advised to undergo bariatric surgery will be used until the moment of surgery. Other treatments will be adequately recorded. Patients will be randomized by computer software and assigned in a group treated with metformin (>2 g/day, group 1) or with metformin (>2 g/day) and dapagliflozin 10 mg (group 2). All patients will receive dietary treatment and follow-up by the Nutrition service during the study. Anthropometric and biochemical variables will be recorded at the beginning and at months 3, 6 and 12. Adherence to treatment will be assessed, defined by consumption of at least 90% of provided pills. The adverse events will be recorded since the beginning of study and will be ranked according to the CTCAE lists (Common terminology criteria for adverse events version 4.03). An intention-to-treat analysis will be performed in case of patients that require treatment with insulin.

3. RATIONALE FOR THE STUDY

Mexico has one of the highest prevalence of obesity worldwide. According to data from ENSANUT 2012, 32.4% of people older than 20 years have obesity, which means that 22 millions of Mexicans have this pathology. This survey described that 1.8% of male and 4.1% of female population have obesity class III. This disease is characterized by an increased risk of comorbidities as T2D, atherogenic dyslipidemia, HTN, obstructive sleep apnea hypopnea syndrome, as well as an increase in proinflammatory cytokines from adipose tissue, which results in a decrease in life expectancy in the economically active population, increased mortality and high costs for the healthy system derived from direct or indirect attention.

Since 2008 in the Hospital de Especialidades Centro Médico Nacional Siglo XXI, the Endocrinology Department organized an Obesity Clinic with the purpose of generating learning strategies of changes in lifestyle, control of comorbidities related with obesity and achieve the weight decrease of patients with obesity class III needed to undergo for bariatric surgery. However, many patients fail to meet weight reduction despite reporting good adherence to diet and exercise strategies. Despite pharmacological treatment is required in these patients, the deleterious cardiovascular effects of current treatments contraindicate its use in this population.

At this point, new pharmacological strategies focused on people with obesity class III are required. Those strategies need to help in weight decrease and should induce benefits on other cardiometabolic parameters such as HTN, increased glucose concentration, hypertriglyceridemia, decreased HDL-c and abdominal obesity.

4. JUSTIFICATION FOR THE STUDY

Recently, it has been approved for the treatment of T2D, a group of drugs known as inhibitors of sodium-glucose co-transporter type 2 (iSGLT2) whose main mechanism of action is the induction of glycosuria. Dapagliflozin was the first marketed drug in this class and has a large number of studies that have demonstrated its effect on glucose lowering with good safety profile and tolerability Furthermore, it has been observed that induces weight loss and also decrease in blood pressure, triglycerides, waist circumference by loss of fat mass and increased HDL-c and adiponectin concentrations. These effects have been evaluated in patients with T2D with normal BMI, overweight and obesity grade I and II but have not been evaluated in patients with class III obesity or higher. Furthermore, there is a lack of information about its effects on inflammatory cytokine concentrations (resistin, IL-6, IL-10 and TNF-α) or in the glucagon concentration.

Based on previous studies (not yet published), up to 46% of patients at Obesity Clinic have prediabetes or diabetes. The use of dapagliflozin in this population could increase weight loss and improve other cardiovascular factors. This could be translated into decreased time needed to undergo surgical treatment and better control of associated comorbidities.

RESEARCH QUESTION

What is the efficacy of dapagliflozin and metformin combination in comparison with monotherapy with metformin for decrease on weight, waist circumference, triacylglycerol, blood pressure and inflammatory cytokines in patients with prediabetes or diabetes and obesity class III?

5. HYPOTHESIS

Dapagliflozin in combination with metformin induce 10% of weight lost and 5% of decrease in waist circumference, triacylglycerol concentration, blood pressure and inflammatory cytokines in patients with prediabetes or diabetes with class III obesity, in comparison with the 5% of weight lost and lack of effect in the rest of parameters in patients treated with metformin monotherapy.

6. OBJECTIVES

a. **Primary objective:** To determine if combined treatment with dapagliflozin and metformin is more effective than monotherapy with metformin for weight loss and assess its effects on waist circumference, triglycerides, blood pressure and inflammatory cytokines in patients with prediabetes and diabetes with obesity class III.

b. **Secondary objectives:**

i. To determine efficacy of metformin monotherapy for weight loss and decrease on waist circumference, triglycerides and blood pressure in patients with prediabetes and diabetes with obesity class III.

ii. To compare efficacy of metformin monotherapy and combined treatment with metformin and dapagliflozin.

iii. To determine the percentage of patients that with combined treatment achieves a decrease in total weight of 5%, 10% and 15% and the percentage of patients that achieve a decrease in 10% of excess weight.

iv. To evaluate if combined treatment allows a decrease in number of drugs needed for comorbidities' control.

v. To evaluate prevalence of adverse effects commonly associated with dapagliflozin use (urinary and genital tract infections, hydroelectrolytic alterations) in patients with obesity class III.

vi. To determine serum concentration of adiponectin, resistin, IL-6, IL-10 and TNF-α before and after treatments, and compare it between groups.

viii. To determine serum concentration of insulin, glucagon and ghrelin before and after treatments, and compare it between groups.

7. BACKGROUND

Definition and classification of obesity

According to the World Health Organization (WHO), obesity is defined as an abnormal or excessive fat accumulation that impairs health. Recently, clinical guidelines developed by the Mexican Social Security Institute (IMSS, Instituto Mexicano del Seguro Social) defined it as a multi-causal, chronic and systemic disease that involves individuals from different ethnic groups, independently of age or social class [1].

Obesity is classified according to body mass index (BMI), defined as a person's weight in kilograms (kg) divided by the square of its height in meters (m). Current WHO classification defines overweight as a BMI of 25 to 29.9 kg/m^2, obesity class I with a BMI of 30 to 34.9 kg/m^2, obesity class II with a BMI of 35 to 39.9 kg/m^2 and extreme obesity or class III with a BMI above 40 kg/m^2 [2]. However, there are some patients with BMI greater than those ranges, with different clinical characteristics and worst cardiovascular outcomes. In this way the term "super-obesity" was coined to define patients with BMI greater than 50 kg/m^2 [3]and later the term "super-super obese" emerged to define those patients with a BMI greater than 60 kg/m^2 [4].

In Mexico obesity is defined using WHO criteria, however, according Mexican Official Standard NOM-008-SSA-2010 published by Department of Health, overweight must be defined as a BMI between 23 to 25 kg/m^2 and obesity with a BMI higher than 25 kg/m^2 in male with a height lesser than 1.60 m or females with a height lesser than 1.50 m [5].

Epidemiology

Mexico has one of the higher prevalence of overweight and obesity worldwide. According to the National Health and Nutrition Survey 2012 (ENSANUT 2012), 38.8% of Mexicans over 20 years were overweight and 32.4% had obesity, with a prevalence of 26.9% in male and 37.5% in female population. This implies that almost 22 millions of Mexicans had some grade of obesity around the country. This survey also showed that higher prevalence of obesity is reported in population from 40 to 49 years old (40.5%), higher prevalence of obesity class III is found among people from 50 to 59 years old (14.3%) and this prevalence increases in locations with a higher socio - economic level (28.5%), urban areas (28.5%) and in the north side of the country (29.4%). Additionally, it was reported a prevalence of obesity class III in male of 1.8% (CI95% 1.5-2.1%) and in females of 4.1% (CI95% 3.7-4.5%). However, this survey did not identify individuals with a BMI higher than 40 kg/m^2 [6].

Obesity is related with 2.8 million deaths per year around the world. It is estimated that obese persons died 8 to 10 years earlier that those with normal weight and that every 15 kg above ideal weight increases the risk of early death by 30% [7]. Furthermore, it has been found that mortality due to cardiovascular diseases increases up to 50% in patients with obesity and up to 90% in patients with extreme obesity, in comparison with people with normal weight [8]. In fact, in United States it is estimated that obesity is responsible of 300,000 deaths per year [9]. Mexico lacks

statistics about number of deaths per year attributed to obesity; however in 2012 the National Institute of Geography and Informatics (INEGI) reported 109,309 deaths caused by cardiovascular diseases of which 74,057 deaths were due to myocardial infarction; 85,055 deaths were related to diabetes and 31,905 deaths were caused by stroke. All of those diseases are related to obesity [10].

Obesity Clinic of the Centro Medico Nacional Siglo XXI, IMSS

The Mexican Social Security Institute (IMSS) has the broader coverage of health of the country. According to ENSANUT 2012, 74.4% of its beneficiaries are obese and it is estimated that only 50% receive medical attention focused on weight control (e.g. diet or physical activity recommendations). Considering the current increase in the prevalence of obesity and an imperious need of reducing the prevalence of co-morbidities and high mortality rate associated with this disease, since 2008 the Hospital de Especialidades from the Centro Medico Nacional Siglo XXI organized a multidisciplinary team composed by members from departments of Endocrinology, Clinical Nutrition, Bariatric Surgery, Psychiatry and Internal Medicine, under the name of "Obesity Clinic".

The requirements for admission are a BMI greater than 35 kg/m^2 with a major comorbidity (diabetes, hypertension, dyslipidemia or sleep apnea syndrome) or obesity class III. Patients are initially evaluated by nutritionists authorized to assess anthropometric measures, who also adjust diet based on weight and initial consumption of calories and evaluate patients monthly recording weight, waist and hip circumference, percentage of adherence to diet and amount of physical exercise per week (measured in minutes per week). At the same time, the endocrinologists perform clinical assessment and laboratory tests for detection and control of comorbidities such as altered metabolism of glucose (Type 2 diabetes [T2D] or pre-diabetes), hypertension (HTN) and dyslipidemia and request assessment and treatment of other specialists if necessary. Meanwhile, psychiatrists perform tests for detection and treatment of anxiety disorders or depression, eating disorders, personality disorders and other psychiatric comorbidities that could influence negatively on the management of patients and finally, once the patients lost 10% of excess of weight, they become candidates for bariatric surgery (laparoscopic Roux-Y bypass or gastric sleeve, depending on the evaluation of surgeons).

Several studies support that weight reduction before surgery decreases surgical risk and improves time to recovery. It has been observed that patients with super-obesity have a higher incidence of complications during bariatric surgery associated with increased technical difficulties (for example an increased liver volume due to steatosis), increased anesthetic risk and risk of treatment failure.

Santo et al. in 2014 conducted a prospective study for evaluating possible benefits related to pre-surgical weight decrease in patients with super-obesity. In this study, 20 patients had in-hospital strict dietary control with diets between 600 and 1500 kcal plus aerobic physical activity (in those allowed to do it). Patients obtained a reduction of 19.7% of initial weight, with decrease of BMI

from 67 kg/m^2 to 55 kg/m^2 over a 24-weeks period. In this group, they observed a lower incidence of complications and decreased time of hospitalization [11].

Prevalence of comorbidities in the Obesity Clinic

The Obesity Clinic evaluates a population of approximately 400 patients, 150 patients have undergone bariatric surgery, 250 patients are pending of approval for such proceedings and up to 10 new patients per month are assessed for determine its possible admission to the clinic.

Sanchez-Ruiz et al. previously performed a retrospective study with a sample of 109 patients from the Obesity Clinic who underwent bariatric surgery between January 2009 and December 2011 (in press). In this study, the mean age was 45.3 ± 10.1 years, 70% were female, 85% reported attachment to diet (up to 80%), 31% performed physical activity (defined as at least 30 minutes of daily walking), 21% were smokers, 74% had a family history of obesity and 67% reported overweight or obesity since childhood. In this study, we observed that 46% of patients had prediabetes (defined by the American Diabetes Association, [ADA] as a plasmatic fasting level greater than 100 mg/dl and less than 126 mg/dl or a 2-h plasma glucose level measured after a 75-g oral glucose load, greater than 140 mg/dl and less than 200 mg/dl) or T2D (defined as fasting glucose greater than 126 mg/dl or a glucose level post-load greater than 200 mg/dl) [12], 66% had HTN and 33% had dyslipidemia (hypertriglyceridemia, hypoalphalipoproteinemia, or both). At this clinic, patients with prediabetes or T2D are usually treated with metformin 2 g/day or Neutral Protamine Hagedorn insulin (NPH) with calculated doses of 0.5 to 1 U/kg of weight when necessary and are evaluated every three months for treatment adjustment depending on their glycated hemoglobin as stipulated by ADA guidelines [12].

Pharmacological treatment of obesity

According to 2015 clinical practice guidelines of the Endocrine Society co-sponsored by the Obesity Society and the European Society of Endocrinology, pharmacologic treatment is justified for weight loss in those individuals with BMI > 30 kg/m^2 or BMI> 27 kg/m^2 with comorbidities (TD2, hypertension, dyslipidemia), in addition to diet and exercise, with doses according to patient characteristics and tolerance for the drug. They also recommend suspension and change of treatment if patients do not decrease at least 5% of initial weight after 12 weeks [13]. Current weight loss drugs authorized by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) are:

a) Orlistat. Approved since 1999, this drug inhibits pancreatic lipase, enzyme responsible for the degradation of triacylglycerols in mono and diacylglycerols for their absorption in the intestine. It induces fat excretion through feces (30% of triacylglycerols ingested) but had a modest effect on weight: a 60 mg dose is associated with a decrease of -2.5 kg (CI95% -1.5 to -3.5 kg) and 120 mg is associated with reduction of -3.4 kg (IC95% -3.2 to -3.6 kg). Furthermore, due its mechanism of action, it reduces cyclosporine and levothyroxine concentrations, has been associated with decrease on fat-soluble vitamins and is contraindicated in patients with gastrointestinal diseases, chronic renal failure (CRF) and those in risk of cholelithiasis. Finally, it has a poor rate of adherence

due to its adverse effects (steatorrhea and urgency to defecation), being 40% approximately [14].

b) Phentermine/topiramate. Phentermine is a sympathomimetic amine with anorexigenic effect at central level (acting directly on the secretion of pro-opiomelanocortin [POMC], hormone responsible of satiety from paraventricular nucleus of hypothalamus). Topiramate is a monosaccharide that acts like an agonist of gamma aminobutyric acid (GABA) receptor and antagonist of calcium channels, with anorexigenic action [15]. The combination of phentermine 7.5 mg with extended-release topiramate 46 mg and 15/92 mg was assessed in the CONQUER study, a randomized, double-blind, placebo-compared study with follow-up of 56 weeks performed at 93 centers in the United States [16]. This study found that the combination of diet, exercise and a dose of 7.5/46 mg (n = 488 patients) achieved a decrease of 5% of initial weight in 62% of the population and decrease of 10% of initial weight in 37% of the population meanwhile a dose of 15/92 mg (n = 981) achieved a decrease of 5% of initial weight in 70% of the population and decrease of 10% of initial weight in 48% of the population in comparison to placebo (21% decreased 5% and 7% decreased 10% of initial weight, n = 979), with $p < 0.001$ for all determinations. They also found a significant decrease in waist circumference, systolic and diastolic blood pressure, total cholesterol, LDL-cholesterol, triacylglycerols and glycated hemoglobin in patients that used both combinations with greater differences at higher doses. The effects of this drug were confirmed by the SEQUEL study, which consisted in monitoring patients from the CONQUER study for 108 weeks [17]. Adverse effects observed in both studies were dry mouth, paresthesia, constipation, dysgeusia, insomnia, dizziness, anxiety, irritability and impaired attention. It is noteworthy that this study only contemplates patients with BMI of 27-45 kg/m^2 [16, 17].

c) Naltrexone/bupropion. Naltrexone is a non-selective opioid antagonist originally used to treat opiate addiction and for the treatment of alcohol withdrawal syndrome. Their main mechanism of action for weight reduction lies in inhibiting the μ receptor from hypothalamic neurons that secrete POMC, thereby decreasing the self-inhibitor effect generated by co-secretion of β-endorphins generated by POMC cleavage. Bupropion is an anorexigenic amine that directly induces POMC secretion [14]. The combination of naltrexone 32 mg with bupropion 360 mg was tested in four randomized, double-blind, compared with placebo, phase III clinical trials for 56 weeks: COR-I which included 1742 patients, COR-II that included 1496 patients from the previous study, COR-BMOD that evaluated 793 patients with intensive lifestyle modification and COR-DM that included patients with T2D [18]. Those studies found that treatment with naltrexone/bupropion allowed that 53% to 80% of patients achieved a decrease of 5% of initial weight and 26% to 55% of patients achieved a decrease of 10% ($p < 0.001$). However, due to common adverse effects of nausea, constipation, vomiting, diarrhea, headache, dizziness, insomnia and upper airways symptoms [19], only 50 to 54% of patients completed proposed treatment [18].

d) Lorcaserin. It is a 5-hydroxytryptamine 2c receptor agonist that stimulates hypothalamic POMC secretion. In a previous clinical trial, randomized and compared with placebo, 1538 patients were assigned to lorcaserin 10 mg for 104 days. After this

time, 47.5% of patients achieved a reduction of 5% of initial weight and 22.6% a decrease of 10% compared to 20.3% and 7.7% achieved with placebo, p <0.001. Additionally, a significant reduction in waist circumference, systolic and diastolic blood pressure, cholesterol, triglycerides and HbA1c was observed [20]. Main adverse effects reported were headache, upper airways infections, nasopharyngitis, dizziness, nausea and sinusitis [20].

e) Glucagon-like peptide type 1 receptor agonists (GLP-1). Initially they were used for the treatment of T2D due to its mechanism of action, based on the incretin effect that induce insulin secretion and decrease glucagon secretion [21]. Additionally, it was observed that they slow gastric emptying and intestinal motility thereby increasing the feeling of gastric fullness; had a direct anorexigenic effect through GLP1 receptors in hypothalamus; and increase the activity of the sympathetic nervous system, thereby generating increased lipolysis and insulin secretion [22]. A recent clinical trial, controlled, randomized, double-blinded, compared with placebo (n = 1225) assessed subcutaneous administration of liraglutide 3.0 mg in 2437 patients without T2D. It was observed that after 56 weeks of treatment, 63.2% of patients decreased 5% of the initial weight, 33.1% decreased 10% of initial weight and 14.4% decreased 15% of the initial weight (compared to placebo which decreased 27.1%, 10.6% and 3.5%, respectively; p <0.001) [23]. Furthermore, it was observed a decrease in waist circumference, HbA1c, triacylglycerols and systolic and diastolic blood pressure [23]. Main adverse effects included nausea, diarrhea or constipation, vomiting, dyspepsia and nasopharyngitis. This clinical trial (SCALE) also included Mexican patients; minor effects were observed in patients with BMI greater than 40 kg/m^2.

Mexico's COFEPRIS has approved only orlistat and, more recently, liraglutide for obesity treatment.

Sodium-glucose co-transporter 2 inhibitors (iSGLT2)

In 1835 a phenolic glycoside called phlorizin was isolated from the bark of the apple tree. Initially, it was used for research on electrolytes and glucose transport at the kidney. This compound was initially tested in animals, where it induced glycosuria and weight loss independently of glycaemia, and later it was also observed in humans [24]. However, until 50’s it was discovered that this compound blocks the facilitated glucose transport in erythrocytes and inhibits glucose transport in the kidneys and small intestine, and at early 90's it was characterized that such transportation was mediated by sodium-glucose co-transporter type 2 (SGLT-2) at the kidney.

Families of glucose transporters

There are three families of glucose transporters in the human genome: the SLC2 family of facilitated transporters, also known as GLUTs; the SLC5 family of "active glucose transporters" characterized by transport against the concentration gradient, and the SLC50 family of uniporters (SWEET) [25].

Nowadays there are 13 members of GLUT family identified [26], being the most common: GLUT1, an insulin-independent transporter expressed in erythrocytes, endothelial cells and brain; GLUT2, transporter at pancreatic **β** cell with a fundamental role for insulin secretion; GLUT3, transporter at neuronal tissue, responsible for glucose transference to cerebrospinal fluid; GLUT4, widely spread in muscle and adipose tissue and GLUT5, a fructose transporter located in enterocytes at small intestine [27].

Meanwhile, there are 12 human genes identified in family SCL5, which are expressed in different tissues as small intestine, kidneys, brain, muscle and thyroid gland. One of the most important is SLC5A1, expressed in 22q12.3, encodes SGLT1, a transporter located in the small intestine, trachea, kidneys, heart, brain, testis and prostate. SGLT1 is a high affinity but low-capacity transporter with little renal effect and their main substrates are glucose and galactose. SLC5A2 gene is expressed in 16p11.2 and encodes SGLT-2, a low affinity but high-capacity transporter located in kidneys, heart, liver, heart muscle and salivary glands [24, 25]. Other transporters are SGLT-3 encoded by SLC5A4 in 22q12.3, located in the small intestine, skeletal muscle, kidney, uterus and testis; SGLT4 encoded by SLC5A9 in 1p33, located in kidney, intestine, brain, heart, lung and uterus and SGLT5 encoded by SLC5A10 in 17p11.2, located only in renal cortex [25].

At kidney, SGLT2 is responsible of 90% of glucose reabsorption and it's located at apical domain of epithelial cells in the proximal tubule (S1 segment) meanwhile remaining 10% is reabsorbed by SGLT1 at the end of the proximal tubule (S3 segment) [24].

SGLT2 inhibitors (iSGLT2): Dapagliflozin

Once discovered, there was an attempt to use phlorizin in humans; however, this compound has poor oral bioavailability and is rapidly inactivated in the intestine to its inactive metabolite aglycone phloretin. Pharmacological tests further led to the synthesis of compounds such as T-1095, a methylated compound that is converted to its active metabolite in the liver and has high affinity and selectivity for human SGLT2. At initial evaluation, it was observed that this compound improved HbA1c levels, glucose and triglyceride levels and improved insulin sensitivity, with beneficial effects on weight gain [28, 29]. Subsequently, other glycosides derived from phlorizin were synthesized: 29, 45 and 46, sergliflozine and finally dapagliflozin [24].

First studies using dapagliflozin in rats, observed an induced glycosuria without hypoglycemia [30]; subsequently Komoroski et al. evaluated the dose of 5 mg, 25 mg and 100 mg in 47 patient's natives to treatment or with current treatment with metformin (monotherapy) for 14 days (with baseline HbA1c of 6 to 10%). In these patients they found that dapagliflozin 5, 25 and 100 mg induced glycosuria of 40, 73 and 82 g/day, respectively. At the end of the study, they observed a rate of urinary glucose excretion of 35, 68 and 66 g/day and improvement in plasma glucose concentrations and glucose tolerance [31]. At the same time, two randomized, double-blinded clinical trials assessed the use of sergliflozine in non-diabetic overweight or obese patients. In this group they also found elevation in GLP-1 concentration and a decrease of weight of 1.5 kg compared to placebo. This was the first study that proposed a possible effect on weight [32].

Dapagliflozin in T2D

Clinical guidelines for treatment of diabetes recommend metformin as first-choice drug for patients with HbA1c below 9% [12]. However, in patients with drug intolerance, an iSGLT2 could be used as monotherapy. A previous study with dapagliflozin 10 mg for 12 weeks, observed a decrease in HbA1c of -0.12% (CI95% -0.41 to 0.17%), like that produced by metformin [33]. In studies using dapagliflozin as second-line treatment (patients treated with metformin), there has been a decrease in HbA1c of -0.30% (CI95% -0.51 to -0.09%) compared to glipizide (sulfonylurea) [34]. Similarly, in patients previously treated with pioglitazone (a thiazolidinedione), addition of dapagliflozin induced a decrease in HbA1c of -0.67% (95% CI -0.88 to -0.46% compared to placebo) [35]. Furthermore, in patients previously treated with sulfonylureas as first-line treatment, addition of dapagliflozin had a similar glycemic effect to GLP-1 (difference in HbA1c of 0.11% with 95%CI -0.18 to 0.4%) [36]. Finally, it has been used as third-line treatment in patients with maximum doses of metformin and sitagliptin. In this group, addition of dapagliflozin reduced HbA1c by 0.6% (95%CI -0.8 to -0.4%) [37].

Because of their mechanism of action independent of the functionality of the β cell it has been proposed that this drug can be used in patients with type 1 diabetes or prediabetes [38].

Dapagliflozin and α and β cell function

It has been observed that use of dapagliflozin improves β cell function, as assessed using homeostatic valuation models of β cell (HOMA-β). It is believed that this protective effect is due to decrease in glucose, thereby decreasing the glucotoxicity phenomenon [38]. However, Bonner et al. recently found that alpha cells express SGLT1 and SGLT2 and proposed that this inhibition induces glucagon secretion [39]. This theory is supported by observation that some patients with T2D increase hepatic glucose production and serum levels of glucagon [40]. This effect may attenuate improvement on plasma glucose concentrations relative to glycosuria induced by dapagliflozin without counteracting its effect [40].

Dapagliflozin and blood pressure

It has been observed that use of dapagliflozin induces a decrease in blood pressure from 12 weeks of use, comparable to hydrochlorothiazide [41]. A recent meta-analysis reported that the use of dapagliflozin decreased systolic blood pressure by -3.78 mmHg (95%CI -4.49 to -3.07 mmHg) and diastolic blood pressure by -1.41 mmHg (95% CI -1.8 to -0.96 mmHg) [42]. The effects on blood pressure have been associated with the induction of osmotic diuresis in association with a decrease in weight; however, it has also been suggested that increased release of sodium at juxtaglomerular level and therefore affects renin-angiotensin-aldosterone system [38].

Dapagliflozin and dyslipidemia

Some clinical trials have reported moderate effects on total cholesterol and HDL cholesterol. In a phase III study conducted in patients with metformin at doses higher than 1500 mg/day with inadequate glycemic control, dapagliflozin increased HDL-c in 1.8% to 4.4% and decreased triacylglycerols in -2.4 -6.2%, compared to placebo. Another study at 52 weeks in patients using metformin and sulfonylureas showed that canagliflozin (other iSGLT-2) increased levels of HDL-C by 7.6% (95% CI 4.6 to 9.3%); however, this study also observed an increase in LDL-C concentrations of 11.7% (95% CI 1.7 to 11.2%), with no changes on atherogenic index [43].

Weight loss associated with metformin

Metformin is the main member of the biguanide drug class and is widely used in T2D treatment, estimated that 150 million people worldwide use it. However, although it has been marketed since 1957, its precise mechanism of action is not fully understood. Its proposed mechanism is that is internalized in the hepatocyte through the organic anion transporter (OCT), it accumulates in the mitochondria and inhibits complex I, which decreases ATP and raises AMP, generating activation of cAMP kinase (AMPk) [44]. This enzyme inhibits transcription of genes involved in gluconeogenesis and inhibits lipogenesis via phosphorylation of acetyl-CoA carboxylase, inhibition of the transcriptional activity of sterol regulatory element-binding protein (SREBP-1) and carbohydrate-responsive element binding protein (ChREBP). However, there are AMPk independent mechanisms as well as antineoplastic, aging-related and intestinal microbiota regulatory mechanisms [45].

Different studies have reported a modest decrease in weight or stability compared with other hypoglycemic medications [46]. Hermann et al. were the first to describe the body weight effects of metformin [47]. In 1991 they studied 25 patients with a mean age of 59 years (range 36-69 years) with an average weight of 76.4 kg (63-97 kg) for women and 77.4 kg (64.5-92 kg) for men previously treated with glyburide. In this group, they observed a decrease of -2.64 kg of total weight (95% CI -4.23 to -1.05 kg) after one year of treatment. Subsequently Stumvoll et al. conducted a study with 10 patients with T2D (6 men and 4 women), aged 58 ± 9 years and average BMI of 32.1 ± 3.2 kg/m^2 to those treated with initial doses of metformin 850 mg increments every two weeks to 2550 mg (850 mg three times a day) [48]. After 16 weeks observed a total weight loss of -2.7 ± 1.3 kg (95.1 ± 14.9 vs. initial 92.4 ± 14.5 kg end, $p < 0.001$) and -2.4 ± 2.2 kg of fat mass, determined by bioimpedance (initial 31.2 ± 11.4 vs. 28.9 ± 10.1 kg end, $p = 0.02$) (48). That same year, DeFronzo et al. conducted a study to compare the metabolic effects of metformin, glyburide, and the combination of both. In that study the group with metformin (n=210 patients, average age 55 ± 1 years, mean BMI 29.4 ± 0.3 kg/m^2) reported a loss of -3.8 ± 0.2 kg, compared with less weight loss observed in the group with glyburide or the weight gain observed in the group with combination therapy for 29 weeks [49].

Moreover, Mogul et al. conducted a retrospective analysis of 30 non-diabetic women who previously tried to lose weight with diet and exercise and grouped them into two categories of BMI: 25 to 32.9 kg/m^2 (group 1) and 33 to 41.7 kg/m^2 (group 2). In addition to a hypocaloric diet, they received a dose of 1500 mg and 2000 mg metformin, respectively. In group 1, a loss of 3.47 kg (SE, standard error 0.68), 6.41 kg (0.72) and 8.06 kg (0.96) at 3, 6 and 12 months of treatment

was observed and in group 2 a weight loss of 4.4 kg (0.8), 9.7 kg (2.3) 15.1 (3.3) kg was observed at those times. In this study, 96% of patients achieved a loss of more than 5% of body weight at 6 months and 81% lost more than 10% of weight at 12 months [50].

Weight loss associated with dapagliflozin

Obesity is generated by an increase in caloric intake associated with deficit of caloric expenditure. Dapagliflozin induces glycosuria of 20 to 85 g/day in humans and 0.5 to 1.9 g/day in rats (weighing 200 g), which means that would generate a caloric deficit of 80-340 kcal/day in humans and 2 to 7.6 kcal/day in rats. Assuming a consumption of 6.16 kcal/g of fat in rodents and 3,500 kcal/lb of fat in humans, the use of dapagliflozin theoretically induces a weigh loss of 0.32 to 1.2 g/day in rats and 0.022-0.097 lb/day (9.97 43.9 g/day) in humans [51].

As monotherapy, dapagliflozin has shown to be as effective as metformin in terms of weight decrease (-1%, 95%CI -2.04 to 0.04%) [33]. In association with metformin, dapagliflozin induced a loss of -5.07 kg (95%CI -6.21 to -3.93 kg) compared to glipizide [34]. When used in combination with pioglitazone for 48 weeks, a loss of -2.3 kg (95%CI -3.37 to -1.23 kg) was observed in comparison to placebo [35] and when used in patients previously treated with sulfonylurea induced a loss of -1.54 kg (95%CI -2.16 to -0.92 kg) compared with the group with GLP-1 (loss -0.65 kg, 95%CI -1.37 to 0.07 kg) and DPP4 inhibitor (gain of 0.57 kg, 95%CI 0.09 to 1.06 kg) [36]. Finally, even in the metformin and sitagliptin group, the addition of dapagliflozin induced a loss of -2.1 kg (95%CI -3.2 to -1 kg) compared to placebo [37].

According to this data, Zhang et al. proposed that urinary glucose excretion of 71.2 g/24 h should decrease 3.05 kg. However, only 2.5 kg was achieved (20% less than expected) [52]. This led to Devenny et al. to evaluate possible compensatory mechanisms that attenuate the effect of dapagliflozin. In a group of rats with obesity induced by diet (DIO), dapagliflozin was orally administered in doses of 0.5 mg/kg, 1 mg/kg and 5 mg/kg, inducing a weight and fat mass loss in a dose-dependent (greater in the 5 mg/kg). In their study, the effect on weight and fat mass was greater in those animals with restricted food consumption (4 times more weight loss for 10 days of treatment). However, in the group of animals with diet *ad libitum*, they observed an increase in appetite since day 7 in animals with 0.5 mg/kg and since day 10 in rats with 0.1 mg/kg. This increase in appetite was maintained throughout the study. Other changes measured were the increase in water consumption, increased rate of respiratory turnover (especially in the group with dietary restrictions), increased concentration of beta-hydroxybutyrate (ketone body) and decreased insulin and non-esterified fatty acids (NEFA) concentration. This study proposed: 1) the effect of dapagliflozin on weight can be altered by an increase in appetite, 2) due to the increase in the rate of respiratory exchange ratio, associated with a decrease on NEFA and increase of ketone bodies, the weight decrease could be associated with increased lipolysis as a metabolic attempt to maintain energy balance and 3) decreasing insulin levels may be associated with a decrease on insulin resistance [51]. The effect on appetite has not been evaluated in humans. Furthermore, the quantification of appetite is complicated, so it is proposed to determine ghrelin concentration, a hormone synthesized by the stomach that modulate brain activity in the

areas that control appetite [53]. However, there are currently no studies on the effect of dapagliflozin on the ghrelin concentration.

In a previous study, Bolinder et al. included 182 patients with type 2 diabetes inadequately controlled with metformin (mean HbA1c 7.17%) and evaluated the effect of dapagliflozin 10 mg on total weight after 24 weeks in comparison to placebo. This group found decrease on weight by 2.08 kg (2.8 to 1.31 kg, p <0.001), waist circumference in 1.52 cm (2.74 to 0.31 cm, p = 0.014), total fat mass assessed by densitometry (DEXA) in 1.48 kg (2.22 to 0.74, p = 0.001), visceral fat mass in 258.4 cm^3 (448.1 to 68.6, p = 0.008) and subcutaneous fat mass in 184.9 cm^3 (359.7 to 10.1 , p = 0.03) [54].

It is noteworthy that all studies have been conducted in patients with BMI under 40 kg/m^2, so its effect on weight and other metabolic factors has not been studied in populations with extreme obesity.

Dapagliflozin and inflammatory markers

The term meta-inflammation is used to describe chronic, low-grade inflammation caused by obesity and it is associated with an increased cardiovascular risk [55]. Adipose tissue is an endocrine organ formed by adipocytes and stromal tissue containing precursors of adipocytes, nerves, vessels and cells of the immune system and together can produce adipokines (cytokines), growth factors and proteins [56-60]. It was observed that adipose tissue of patients with obesity have increased the production of leptin, visfatin, vaspin, apelin, retinol binding protein 4 (RPB-4), resistin and lipocalin 2 (cytokines and proinflammatory proteins), and decrease the production of adiponectin, fatty acid-binding protein type 4 (FABP-4) and perilipin (cytokines and anti-inflammatory proteins) [56, 59-64]. Furthermore, it has been observed an increased number of macrophages in this tissue, that also form corona structures and polarize from type 2 to type 1, with a subsequent increased secretion of cytokines and proinflammatory proteins as interleukin-6 (IL-6), interleukin 1B (IL-1B), tumor necrosis factor alpha (TNF-α), hepatocyte growth factor (HGF), inhibitor of plasminogen activator-1 (PAI-1), C-reactive protein (CRP), interleukin-18 (IL-18), monocyte chemoattractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF-1) and decreased secretion of anti-inflammatory cytokine: interleukin 10 (IL-10) [59-68]. It has been observed that weight decrease and specifically the decrease in abdominal fat, decreases meta-inflammation defined by the presence of high concentrations of CRP, IL-6, TNF-α, resistin and diminished levels of IL-10 and adiponectin, especially when patients decrease more than 10% of their body weight [59-68].

There is only one article where the effect of dapagliflozin on the concentration of inflammatory cytokines was evaluated. Okamoto et al. observed the effect of 5 mg of dapagliflozin administered for 12 weeks, in highly sensitive CRP (hs-CRP), adiponectin and PAI-1. This study included 27 Japanese patients, 63% male (n = 17), aged 49.7 ± 8.8 years and BMI of 32.7 ± 6.5 kg/m^2 and a significant decrease in the concentrations of hs-CRP (2410 ± 2814 ng/ml at baseline vs. 1607 ± 1960 ng/ml at the end of 12 weeks, p <0.01), and increased adiponectin levels (5.1 ± 2.3 mg/ml

vs. 6.7 ± 4.2 mg/ml, p <0.01) was observed. No significant changes were observed in the concentrations of PAI-1 (30 ± 16.8 mg/ml to 26.8 mg/ml, p = 0.07). Furthermore, they found an inverse correlation between changes in HbA1c/body weight changes and adiponectin (ρ = -0.787 and -0.696, respectively, p <0.05) and positive correlations of such parameters with changes in PAI-1 (ρ = 0.382 and 0.435, respectively, p <0.05) [69]. Finally, the authors evaluated the effect of dapagliflozin (comparison between before and after administration) on concentrations of glucagon and insulin in 11 patients before and after a test meal tolerance, finding a significant increase in glucagon before test (p <0.01), after an hour (p = 0.02) and after two hours (p <0.01) post-test and a significant decrease in the post-test insulin after an hour (p = 0.04) [69].

According to the effect of decreased weight and waist circumference previously reported in patients with obesity grade I and II who are given dapagliflozin, we expected a decrease of concentrations like resistin, IL-6 and TNF-α and an increase of concentrations of IL-10 and adiponectin.

Adiponectin

It is also known as adipoQ, Acrp30 (Adipocyte Complement Related Protein) or apM1. It's a protein exclusively secreted by adipose tissue, with a serum concentration of 5 to 10 mg/ml (10 to 30 nM). It has been observed that its concentration is reduced in obese and diabetic mice and humans. Subsequently it was found that hypoadiponectinemia is present before insulin resistance and the return to adequate concentrations decreased this resistance in animal models. In addition to its role as insulin sensitizer, it has anti-inflammatory and anti-apoptotic properties [70-72].

Resistin

It's a protein discovered by Steppan et al., which is encoded on chromosome 19 and leads the family of resistin-like molecules (RELMs). There has been observed an increase in concentration of patients with type 2 diabetes and patients with obesity. Although their functions are not fully understood, it has been observed to cause insulin resistance in the liver and has a possible role as regulator of the immune system [70, 72].

Interleukin 6 (IL-6)

IL-6 was characterized as a pro-inflammatory cytokine secreted by adipose tissue. It has been linked with the downregulation of IRS-1 (insulin receptor substrate type 1), impaired phosphorylation of tyrosine kinase, and decreased expression of GLUT4 (glucose transporter 4). Additionally, it increases resistance and decreases adiponectin levels [73-75].

Interleukin 10 (IL-10)

It is also known as Cytokine-synthesis Inhibiting Factor (CSIF). IL-10 is a cytokine with anti-inflammatory properties due to their ability to inhibit the synthesis of pro-inflammatory cytokines from T-lymphocytes and macrophages. Its presence has been proven in human atherosclerotic plaques and it has been that its low concentration favors the development of larger and unstable atherosclerotic lesions [76].

Tumor necrosis factor alpha (TNF-α)

Initially, TNF-α was described as a pro-inflammatory cytokine released by macrophages and monocytes as part of the immune response. Soon after, its expression and release were discovered in adipocytes and was quickly linked to insulin resistance in rodents and humans with obesity. It is known that TNF-α is involved in several processes related with development of insulin resistance, such as [77-79]:

1) Reduces expression of GLUT4, consequently decreasing glucose caption in the cell.
2) Induces serine phosphorylation at insulin receptor, affecting signaling cascades.
3) Induces down-regulation of IRS-1.
4) Increases expression of IL-6 and leptin and decreases adiponectin expression.
5) It has been associated with adipocytes apoptosis through increased concentration of intra-cellular free fatty acids.

Security of dapagliflozin

Based on combined analysis of three clinical trials, it was observed that dapagliflozin did not cause hypoglycemia compared to other oral hypoglycemic drugs (OR 0.49, 95%CI 0.18 to 1.39). In fact, hypoglycemia rate was 10 times lower compared with sulfonylureas.

The most common side effect observed in patients treated with dapagliflozin is genital tract infection (OR 3.48, 95%CI 2.33 to 5.20): vulvar-vaginal mycotic infections in women and balanitis in men, none of whom was reported as serious or requiring treatment discontinuation. It was also observed an increase in the incidence of urinary tract infections (OR 1.43, 95%CI 1.05 to 1.94), which were reported as mild to moderate intensity, were more frequent in females and caused a low rate of discontinuation (0.3% vs. 0.1% with placebo) [80].

There have been some cases of breast cancer in patients treated with dapagliflozin; however, diagnosis was made since day 6 to 334 of use (9 of 5501 patients), so it is unlikely that dapagliflozin could be involved due to the short exposure time. In this way, it has been reported to have an incidence of 0.17% of bladder cancer (10 of 6045 patients). This could be associated with a lack of scrutiny prior to inclusion in the study [41].

Additionally, its use is not recommended for patients with chronic kidney disease (CKD) or in those with glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 due to loss of effectiveness and a possible increased risk of fractures in this population. It's also not recommended in people at risk of inadvertent volume depletion (like patients over 65 years with renal impairment or using loop diuretics) [41].

Diabetic ketoacidosis in patients using iSGLT2

Recently the FDA issued a warning statement of a possible association between the use of iSGLT2 with a type of diabetic ketoacidosis, characterized by minimal to moderate glycemic elevation, named as "euglycemic diabetic ketoacidosis" [81]. This warning was based on 20 cases reported between March 2013 and June 2014, most patients had type 1 diabetes (T1D), other comorbidities, had decreased food and liquid intake or a history of alcohol consumption [82].

Subsequently, EMA announced that until May 2015, 101 cases of diabetic ketoacidosis in patients with T2D have been reported. However, until September 2015, information came from case reports and any clinical trials that clearly explain the frequency of this association had been performed [81].

Erondu et al. from Janssen Company informed that in CANVAS study (CANAGLIFLOZIN Cardiovascular Assessment Study) until May 2015, there have been 15 cases of diabetic ketoacidosis associated with canagliflozin from 17,596 participants (incidence rate of 0.5, 0.8 and 0.2 per 1,000 patients/year for canagliflozin at 100 mg, 300 mg and comparator, respectively) [83]. Meanwhile, AstraZeneca Company has reported a frequency of 0.1% of possible events related to dapagliflozin from DECLARE's study (Dapagliflozin Effect on Cardiovascular Events) and Boehringer has reported the same incidence in the study EMPA-REG that includes 7,000 patients in treatment with empagliflozine [81]. From these data, canagliflozin is the drug most associated with ketoacidosis, however up to 50% of the reported cases are patients with T1D, in whom the use of iSGLT2 is not authorized.

Regarding euglycemic ketoacidosis' pathophysiological mechanism, it has been observed that iSGLT2 induced glycosuria up to 85 g/day by 24 hours and therefore in a 60 year old patient with overweight (BMI 28 kg/m^2) and in a diet with 50% of carbohydrates, this glycosuria should cause a loss of 17 to 34% of carbohydrates consumed in men and 22 to 44 % in women [84]. These drugs induce insulin release and decrease of glucagon routinely, but in those patients with a strict glucose control, the persistent action of iSGLT2 could decrease insulin release due to a decrease in glucose (main effector). This hormonal change releases the inhibition of gluconeogenesis in the liver causing moderate fasting and postprandial hyperglycemia (glucose remain being eliminated by the kidneys), and subsequently stimulates lipolysis with an increase on serum free fatty acids (up to 40%) and lipid oxidation (up to 20%) [85]. Free fatty acids released are transported to the liver where β-oxidation increases with formation of ketone bodies. Finally, iSGLT2 also induces volume depletion, which aggravates that condition. Furthermore, it seems that reported cases were associated with special conditions where patients decreased insulin doses due to iSGLT2 effect on serum glucose, but there were increased requirements (such as stress, infections or use of alcohol).

Further studies are needed to determine conclusively if ketoacidosis depends on type of drug used (which in turn depends on variability of the inhibition's degree of SGLT2 between drugs) and if their existence restricts the widespread use of these drugs.

8. PATIENTS, MATERIAL AND METHODS

Type of study: Phase II/III, controlled, randomized, open clinical trial.
By study groups: two parallel groups' design.
By maneuvering: experimental
By measurements in the population: longitudinal
By temporality: prospective
By the nature of the study: clinical
By purpose: Therapeutic

Study Population

- Patients with obesity class III from the "Obesity Clinic" in preparation for bariatric surgery.
- Study period: December 2016 to October 2018
- Place of study: Mexico City, Mexico
- Time of clinical assessment of each patient: one year

Workplan

1) A convenience sampling will be done for patients diagnosed with diabetes or prediabetes according to ADA criteria [12] (Appendix 1), who come to Obesity Clinic in the indicated period and that meet the selection criteria. Since the first assessment, patients are informed that they would become candidate for bariatric surgery (bypass or gastric sleeve, depending on surgeons' criteria) once they achieve a weight reduction of 10%.
2) Due patients achieve weight loss needed for the surgical procedure at different times of follow-up (3, 6 or 12 months), clinical, biochemical, and initial and final determinations of inflammatory cytokines and hormones, will be collected until the previous valuation before surgery. Data will be collected even if the patients require treatment with sulfonylureas or insulin (adverse event grade 3).
3) Prevalence of comorbidities at baseline and the type and dose of drugs used for treatment will be recorded (Appendix 2. Data Collection Sheet). Treating physicians based on the clinical response of patients, will decide the dose and number of drugs and will record changes in the corresponding data sheet.
4) Patients will be randomized by a random numbers system generated with a computational software and will be assigned to a group: 1) metformin (2 g/day) or 2) metformin (2 g/day) and dapagliflozin 10 mg. All patients will receive dietary treatment and follow-up during the study by Nutrition service. Dietary treatment is individualized, as it is based on the initial weight of the patient and their eating habits at the time of valuation. It is expected that dietary treatment does not influence the results because all patients will undergo diet and those with little attachment (defined as failure to comply with at least 80% of the prescribed diet) are discharged of the surgical protocol. The Nutrition service calculates the percentage of adherence to diet.
5) Once assigned to the corresponding group, patients will receive an identification code that will be retained throughout the study. One of the researchers not directly involved in patient care, will assign tablets needed for daily intake for a month and then the number of tablets required for 3 months in a sealed envelope.
6) A run-in period will be used to assess tolerance to treatments. This period will be for a month. At this time patients will be advised of a possible increase on uresis and will be instructed in the relevance of increasing fluid intake.
7) The anthropometrical and biochemical variables will be recorded at baseline and at 1, 3, 6 and 12 months (Appendix 2. Data Collection Sheet). A clinical nutritionist accredited as Level 1 Anthropometrist by the International Society for the Advancement of Kinanthropometry (ISAK) will assess the weight on a scale with a

maximum capacity of 300 kg and precision of 100 g; height using an stadimeter, and waist circumference using a fiberglass tape, according to the manual of procedures for nutrition projects of the Centro de Investigación en Nutrición y Salud de Instituto Nacional de Salud Pública [86], and also in accordance with the procedures manual for the assessment of clinical and anthropometrical measurements in the adult and elderly of the Ministry of Health [87] (Appendix 3. Clinical and anthropometric measurements).

8) The determination of blood pressure will be performed by a nurse from the Obesity Clinic, using an aneroid sphygmomanometer, recording the average of two measurements taken with a 5-minutes difference, according to the procedure's manual for the assessment of clinical and anthropometrical measurements in the adult and elderly of the Ministry of Health (Appendix 3. takes clinical and anthropometric measurements).
9) The determination of insulin, glucagon, ghrelin, adiponectin, resistin, IL-6, IL-10 and TNF-α will be held at baseline and before surgery (depending on the response of each patient). For the determination of these cytokines and peptides, an ELISA kit (enzyme linked immunosorbent assay) will be used (Appendix 4. ELISA technique).
10) Adherence to treatment will be evaluated and will consist in consumption of 90% of pills granted. The registration of adverse events will take place from the start of treatment and throughout the study. Each event will be evaluated by researchers and classified according to CTCAE list (Common terminology criteria for adverse events version 4.03). CTCAE considers that an adverse event grade 4 or 5 indicates discontinuation of treatment; grade 3 must be submitted to review by the medical team, and grades 1 or 2 require registration and intervention but not discontinuation of treatment (Appendix 5. CTCAE list). Adverse events will be recorded in the report sheet (Appendix 6. Sheet report for drug side effects).
11) The patients that do not achieve weight loss at one-year follow-up will be discarded to their primary care hospital as pre-specified by the Obesity Clinic internal regulation.

Selection criteria

Inclusion criteria

1. Age from 18 to 60 years
2. Any gender
3. BMI greater than or equal to 40 kg/m^2
4. Diagnosis of diabetes or prediabetes according to the criteria of the ADA. Patients must have glycated hemoglobin less than 9%.
5. Patients who sign informed consent letter (Appendix 7. Charter informed consent)
6. Candidates to bariatric surgery

Exclusion criteria

1. Current use of insulin or sulfonylureas
2. Chronic kidney disease with glomerular filtration rate <60 ml/min/1.73 m^2
3. Use of loop diuretics with no possibility to suspend.

4. Concomitant illnesses that predispose to volume depletion, weight loss (including oncological diseases), or metabolic acidosis (including drug users)
5. History of recurrent genital or urinary tract infections
6. Use of drugs for weight control.
7. Untreated or uncontrolled hypothyroidism (defined through TSH higher than 4.2 μU/ml)
8. Pregnant or lactating mother.
9. Tobacco use (in the last month)
10. Current use of steroids or nonsteroidal anti-inflammatory drugs

Withdrawal criteria

1. Becoming pregnant during the study
2. Tobacco use
3. Use of steroids during the study

Sample size

Weight loss after dapagliflozin treatment has not been previously used as primary outcome in randomized controlled trials (RCT) of patients with prediabetes or diabetes and grade III obesity. Zhang et al. performed a meta-analysis that included seven RCTs evaluating the effect of metformin combined with SGLT2-inhibitors (dapagliflozin 10 mg/day, canagliflozin 300 mg/day, empagliflozin 25 mg/day, or ipragliflozin 300 mg/day) vs. metformin (at doses of 1.5 to 3 g/day) with placebo on HbA1c; fasting plasma glucose; and body weight over 24 weeks, 1 year, and 2 years. The total sample was 2847 patients with a mean BMI of 31.7 ± 4.9 kg/m2. After a year of treatment, the placebo-metformin control group showed a reduction in body weight of 1.1 ± 3.39 kg, whereas the SGLT-2 inhibitor-metformin group showed a 3.6 ± 4.22 kg reduction with a mean difference of− 2.6kg (− 3.17 to− 2.03 kg), $p < 0.0000$. Using these data, we calculated a sample size using a mean difference formula. With a power of 80% (alpha level of 0.05) in a two-sided T-test, the sample size required is 90 patients: 45 patients in the M group and 45 patients in the D/M group. The final sample size required is 108 patients: 54 patients in metformin and 54 patients in the dapagliflozin group, allowing for a 20% dropout rate.

Variable definition

Variable name	Variable Type	Scale	Conceptual definition	Operational definition	Units
INDEPENDENT VARIABLES					

Type of treatment	Qualitative, dichotomic	Nominal	Metformin is a biguanide with effect in cyclic AMP kinase that diminishes insulin resistance. Diabetes treatment guidelines consider it as the first line therapy. Dapagliflozin is an SGLT2 inhibitor that induces glycosuria and has been approved for monotherapy in patients with metformin intolerance and also as a second or third line therapy for diabetes.	Use of metformin 2 grams per day or use of metformin 2 grams per day in combination with 10 mg of dapagliflozin.	**0 = metformin, 1 = metformin/dapagliflozin**
DEMOGRAPHIC VARIABLES					
Age	Quantitative, continuous	Ratio	Time elapsed from birth to	Time elapsed from birth to the baseline registry of the patient.	**years**

			the current date.		
Gender	Qualitative, dichotomic	Nominal	Taxon that gathers members of the same species.	Gender assigned in the registry documents.	**0 = female** **1 = male**
Height	Quantitative, continuous	Ratio	Length of a human body when measured in a standing position from the top of head to the floor.	Height registered in the baseline documents using the same stadiometer in all patients during the first evaluation.	**meters (m)**
Body mass index (BMI)	Quantitative continuous	Ratio	Measurement that associates height and size in the same individual and classifies them as underweight, normal, overweight or obese.	Ratio between weight in kilograms (kg) divided by the square of the height in meters. Calculation is performed in every clinical evaluation during the protocol.	**kg/m^2**
DEPENDENT VARIABLES					
Weight	Quantitative continuous	Ratio	The force that gravitation exerts upon a body.	Total amount of kilograms registered in the same person using the same calibrated scale at baseline and months 3, 6 and 12.	**kilograms (kg)**

Waist circumference (WC)	Quantitative continuous	Ratio	Measurement of the body circumference measured at the minimum circumference between the iliac crest and the rib cage.	Perimeter registered in the documents using the same flexible tape at baseline and months 3, 6 and 12.	**centimeters (cm)**
Fasting Glucose	Quantitative continuous	Ratio	Glucose concentration in the blood serum after an 8 hour fast.	Blood glucose reported at baseline, months 3,6 and 12.	**mg/dl**
Fasting Insulin	Quantitative continuous	Ratio	Insulin concentration in the patients serum determined by radioimmunoassay after an 8 hour fast.	Blood insulin reported at baseline and months 3,6 and 12.	**mU/L**
HOMA-IR	Quantitative continuous	Ratio	The homeostatic model assessment (HOMA) is a method used to quantify insulin resistance and beta-	HOMA = [glucose (mg/dl)/18 *insulin (mU/L)]/22.5 at baseline and months 3,6 and 12.	**Dimensionless**

			cell function.		
Glycated hemoglobin (HbA1c)	Quantitative continuous	Ratio	Is the most abundant of the minor components in hemoglobin, formed by the condensation of glucose in the N-terminal portion of the beta chain of the hemoglobin molecule, representing an average of blood glucose concentration for the last three months.	Percentage of glycated hemoglobin in the blood of patients at baseline and months 3,6 and 12, using immunoanalysis with turbidimetric inhibition.	**%**
Cholesterol	Quantitative continuous	Ratio	Total cholesterol in the serum of the patient after an 8-12 hour fast.	Magnitude obtained in lipid profile of patients at baseline and months 3,6 and 12 after an 8-12 hour fast.	**mg/dl**

Triglycerides	Quantitative continuous	Ratio	Triglyceride concentration in the serum of the patient.	Magnitude obtained in lipid profile of patients at baseline and months 3,6 and 12 after an 8-12 hour fast.	**mg/dl**
c-HDL	Quantitative continuous	Ratio	High-density cholesterol concentration in the serum of the patient.	Magnitude obtained in lipid profile of patients at baseline and months 3,6 and 12 after an 8-12 hour fast.	**mg/dl**
c-LDL	Quantitative continuous	Ratio	Low-density cholesterol concentration in the serum of the patient calculated using the Friedwald formula.	c-LDL = total cholesterol– (c-HDL + triglycerides/5) at baseline and months 3,6 and 12 after an 8-12 hour fast.	**mg/dl**
Systolic and diastolic blood pressure	Qualitative nominal	Nominal Dichotomic	Measurement of the pressure of the blood against the inner walls of the blood vessels.	Blood pressure determined by the same investigator in two different measurements after 30 minutes of resting in a sitting position, performed 5 minutes apart from each other.	**mmHg**
Adiponectin	Quantitative continuous	Ratio	Protein secreted exclusively by the adipose tissue and directly related to	Concentration determined using the method ELISA.	**pg/ml**

			insulin resistance.		
Resistin	Quantitative continuous	Ratio	Protein, member of the RELM family, related to insulin resistance in the liver tissue.	Concentration determined by using the method ELISA.	**ng/ml**
Interleukine 6 (IL-6)	Quantitative continuous	Ratio	Pro-inflammatory cytokine, secreted by adipose tissue and related to insulin resistance mediated by the insulin receptor.	Concentration determined using the method ELISA.	**pg/ml**
Interleukine 10 (IL-10)	Quantitative continuous	Ratio	Anti-inflammatory cytokine related to the development of atherosclerosis	Concentration determined using the method ELISA.	**pg/ml**
Ghrelin	Quantitative continuous	Ratio	Hormone secreted in the gastric fundus that induces appetite.	Concentration determined using the method ELISA.	**pg/ml**

Glucagon	Quantitative continuous	Ratio	Hormone produced by pancreatic alpha cells that counteracts the effects of insulin.	Concentration determined using the method ELISA.	**ng/ml**

Statistical analysis: Quantitative variables were described using measures of central tendency and dispersion according to the data distributions. Qualitative variables were described using frequencies or percentages. The Shapiro-Wilk test was used to establish normality in the distribution of quantitative variables. Because information on the different quantitative variables was available up to different time periods, a mixed model analysis was performed using the restricted maximum likelihood (REML) method, as well as a mixed model considering the fixed effects of assigned treatment, sex, and prediabetes or diabetes status, and the random effects of variability in weight loss over time. Comparisons between groups were analyzed using Sidak's post-hoc test. Pearson's chi-square test or Fisher's exact test were used to evaluate the association between qualitative variables. Statistical significance was defined as a $p < 0.05$. Data analysis was performed using SPSS version 17.0 and STATA version 11.0.

4. ETHICAL ISSUES

For the realization of this protocol, we will apply for approval by the Instituto Mexicano del Seguro Social (IMSS) National Ethics Committee. The proposed procedures are compliant with ethical regulations, the General Health Law regarding Health Research, the institutional regulations for investigation, the declaration of Helsinki in 1975 and its amendments. It also is compliant with the currently approved international codes and norms regarding Good Clinical Practice in Clinical Investigation.

Risk: according to the General Health Law regarding Health Research, this study represents a "higher than minimum risk" for the participants since it is a clinical trial (Article 17).

Contributions and benefits of the study for the participants and society: patients will not be directly benefited from this study. This statement will be stipulated into informed consent form. Regarding utility of the study, in case that we can determine higher effectiveness of the combination of metformin with dapagliflozin in terms of weight control, glucose concentrations, blood pressure and triglycerides, we will be able to recommend its inclusion in the treatment for the metabolic control of patients with extreme obesity.

Confidentiality: Patient will be guaranteed that personal data will be kept confidential according to the Article 21 Fraction VIII of the General Health Law regarding Investigation Research.

Conditions under which the informed consent will be requested: The informed consent form will be requested before the inclusion of the patient in the study**.** The patients will be invited personally when they attend to their regular visits to the outpatient clinic. (See appendix 7. Informed Consent Form). The principal investigator and co-investigators will sign the consent.

The patient will be explained about the freedom to withdraw consent at any time during the investigation according to Article 21 Fraction I-VII of the General Health Law regarding Investigation Research.

Participant selection: all patients meeting the inclusion criteria and no exclusion criteria that sign informed consent will be included in the study.

11. RESOURCES AND ENVIRONMENT

Human resources. All the investigators participating in this study have expertise in clinical trials, they are trained in the management of the patients attending to the clinic and they are authorized to perform and handle blood samples and patient data.

The investigator group includes one attending physician registered in the obesity clinic in the Endocrinology department, which will also be in charge of this protocol as part of the work to achieve his Doctors degree in Science (D.Sc.); other members of the group are two attending general endocrinologists with experience in clinical investigation in obesity and two other endocrinologists from the Experimental Endocrinology Unit which constitutes the laboratory area of the Endocrinology department. These endocrinologists can perform the laboratory techniques required for this protocol and one of them has been previously involved in protocols with pharmaceutical companies.

Material resources. The investigation unit and the endocrinology department have the necessary equipment required for the procedures. We will apply for financing for the purchase of the ELISA kits as well as for some of the supplies required for the protocol.

Feasibility. According to the large number of patients existing in the Obesity Clinic that are waiting for bariatric surgery and the frequency of metabolic disturbances found in the extremely obese, we consider that the number of patients required for the study is feasible.

The clinical and laboratory investigators involved in this protocol have the necessary expertise for the management of patients with extreme obesity, taking blood samples and information gathering. The Investigation Unit has ample experience in the management of biological samples and analysis with ELISA. They have also performed studies in the field of immunoendocrinology.

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11. APPENDIX

Appendix 1. Criteria for diagnosis of prediabetes and diabetes according American Diabetes Association (ADA) guidelines.

	Prediabetes	Diabetes
Glycated hemoglobin	5.7 - 6.4%	≥ 6.5%
Fasting plasma glucose	100 - 125 mg/dl	≥ 126 mg/dl
Oral glucose tolerance test	140 - 199 mg/dl	≥ 200 mg/dl
Random plasma glucose		≥ 200 mg/dl

Considerations

1. The fasting glucose test must be performed after caloric deprivation for at least 8 hours.
2. Oral glucose tolerance test must be performed as follows:
    a. The patient must have at least an 8-hour fasting.
    b. Basal venous plasma glucose will be determined.
    c. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water and a new serum glucose determination will be performed after two hours of the glucose administration.
    d. The test will be considered invalid if the patient is not able to take the complete glucose load or if vomiting is present during the test
    e. The test will not be performed in patients with a basal glucose higher tan 200 mg/dl.
    f. Glucose tolerance test is required for patients classified as prediabetic.
    g. A random glucose higher than 200 mg/dl is a valid test only for patients with evident symptoms of hyperglycemia or hyperglycemic crisis.
    h. The ADA recommends that all patients classified as obese by BMI should be tested for diabetes or prediabetes.

Appendix 2. Collecting data sheet
INSTITUTO MEXICANO DEL SEGURO SOCIAL

Name:_____
Social security ID number: _____ Treatment code_____
Birthdate: _____Inclusion date: _____
Direction:
_____
Phone number: _____ Gender: _____ Height: _____ Age: _____

Relevant history (Cross out the appropriate option)
Smoking (Yes) (No) Age from beginning: _____ Number of years_____
Cigarettes/day: _____
Alcoholism (Yes) (No) Age from beginning: _____ Number of years _____
Drinks/week: _____
Cancer history in family (father, mother, aunt, uncle, grandfather/mother) (Yes) (No)
Type:

Comorbidities	**Yes**	**No**	**Age from diagnosis**	**Drugs (Frequency/doses)**
Hypothyroidism				
Prediabetes				
Diabetes Mellitus				
Hypertension				
Hypertriglyceridemia				
Myocardial infarction/angina				
OSAH syndrome				

Osteoarthritis				

Labs	**Initial**	**Final**	**Labs**	**Initial**	**Final**
Glucagon (ng/ml)			**Creatinine clearance (ml/min)**		
Ghrelin (pg/ml)					
Adiponectin (pg/ml)			**iPTH (ng/ml)**		
Resistin (ng/ml)			**Calcium (mg/dl)**		
IL-6 (pg/ml)			**Phosphorus (mg/dl)**		
IL-10 (pg/ml)			**Magnesium (mg/dl)**		

Name:_____NSS:_____

Parameter	1 month	3 months	6 months	12 months
Weight (kg)				
BMI (kg/m^2)				
Waist circumference (cm)				
Systolic blood pressure (mmHg)				
Diastolic blood pressure (mmHg)				
Glucose (mg/dl)				
HbA1c (%)				
TAG (mg/dl)				
TC (mg/dl)				
HDL-c (mg/dl)				
LDL -c(mg/dl)				
Urinary infection (y/n)				
Ketonuria (y/n)				
Proteinuria (y/n)				
Leukocyturia (y/n)				
Nitrite (y/n)				
Sodium (mEq/L)				
Potassium (mEq/L)				

Calcium (mg/dl)				
Phosphorus (mg/dl)				
Magnesium (mg/dl)				
Drugs (hypoglycemic agents, antihypertensives, treatment for dyslipidemia)				

Appendix 3. Procedures for clinical and anthropometrical measurements

Weight assessment

Required material: adult scale, with a maximum capacity of 300 kg and precision of 100 g, not mobile. This scale is calibrated with known tare weight (10 kg) each month and is in one of the offices of the Obesity Clinic.

Process:

- The measurement is carried out with the least possible clothing and shoes.
- The subject must be step on the scale by placing its feet parallel in the center of the scale, facing the examiner. Subjects must be upright, staring straight ahead, unmoving, with their arms falling naturally at their sides.
- The lecture is taken when the central needle is in the middle of the 2 margins and unmoving.

Height assessment

Required material: a stadimeter fixed to the wall, which consists of a measuring tape with 2 m long and a mobile bracket with a 90 ° angle.

Process:

- Before starting the measurement, subjects will be asked to take off their shoes and any object over the head, as pins, bows, high hairstyles, braids, etc
- The height is measured standing, with the back and head fully supported on the wall.
- The midline of the body must coincide with the midline of the tape stadimeter.
- The anthropometrist must be placed on the left side of the subject. With his left hand, he takes subjects' chin to control his head and direct it towards the plane of Frankffort; with his right hand slide the movable piece vertically to the tape, touching the coronal part of the head at an angle of 90 °.
- The data observed is registered.

Assessment of waist circumference.

Required material: fiberglass tape with a capacity of 200 cm and accuracy of 1 mm.

Process:

- Waist circumference is taken with the subject at standing position.
- The tape should be parallel to the floor.
- Another person must ensure that in the back of the body the tape is horizontally and not creating pressure on the skin.
- In very obese people waist is difficult to evaluate, so the tape should measure the minimum circumference of the abdomen in the area between the costal margin and the iliac crests. The measurement is read in cm.

Blood pressure measurement.

Required material: aneroid sphygmomanometer with special bracelet for obese adults

Process:

- The patient should refrain from smoking, drinking coffee or cola products at least 30 minutes before measurement.
- The patient must be sitting with good back support and his arm flexed at heart level.
- The measurement is carried out after at least 5 minutes of rest.
- A bracelet of suitable size will be used to ensure an accurate measurement. The air chamber must cover at least 3/4 of the length of the arm and at least 80% of the arm circumference.
- Both systolic and diastolic pressures must be registered; the appearance of the first sound defines the appearance of diastolic pressure and the last noise is used to define diastolic pressure.
- The blood pressure value is the average of two measurements, separated by two minutes or more. If the two pressures differ by more than 5 mm Hg, two additional measurements are made and the average will.

Appendix 4. ELISA technique for cytokine determination.

Required material:

- Capture antibody (360 µg/ml of anti-human mouse cytokine reconstituted with 1 mL of PBS).
- Detection antibody (9 mg/ml of anti-human goat cytokine biotinylated reconstituted with 1 mL of reaction diluent).
- Standard (120 ng/ml recombinant human cytokine reconstituted with distilled or deionized water).
- Streptavidin-HRP (1 ml of streptavidin conjugated with horseradish peroxidase).
- PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO4, 1.5 mM KH_2PO4, pH 7.2-7.4, filter 0.2 µm.
- Washing Buffer: 0.05% Tween 20 in PBS, pH 7.2-7.4.
- Diluent reaction: 1% BSA in PBS, pH 7.2-7.4.
- Substrate Solution: 1:1 mixture of Reagent A (H_2O2) with Reagent B (tetramethylbenzidine)
- Stop solution ($2NH_2SO_4$).
- Microplate reader.

Plate's preparation:

1) Dilute capture antibody at a concentration that will work on PBS without carrier protein. Immediately cover the 96-well microplate with 100 L of diluted capture antibody. Seal the plate and incubate overnight at room temperature.
2) Vacuum and wash each well with washing buffer, repeating the process twice in a total of three washes. Wash by filling each well with 400 µL of washing buffer. Complete removal of liquid at each step is essential for good performance. After the last wash remove any remaining of washing buffer by aspiration or by inverting the plate on a paper towel.
3) Block plates by adding 300 µL of the dilution reaction to each well. Incubate at room temperature for at least one hour.
4) Repeat washing / extraction process (Step 2). The plates are ready to add the sample.

For samples:

1. Add 100 µL of sample or controls in the reaction diluent to each well. Cover it with tape and incubate 2 h at room temperature.
2. Repeat aspirate / wash process. (Step 2)
3. Add 100 uL of detection antibody (diluted in the reaction diluent) to each well. Cover with a new adhesive tape and incubate 2 h at room temperature. Repeat aspirate / wash process. (Step 2)
4. Add 100 µL of dilution of HRP-streptavirine to each well. Cover the plate and incubate for 20 min at room temperature, without exposing the plate.
5. Repeat wash / aspirate process (Step 2).
6. Add 100 µL of substrate solution to each well. Incubate for 20 min at room temperature.
7. Add 50 µL of stop solution to each well. Cover the plate and mix thoroughly.
8. Determine the optical density of each well immediately, using a microplate reader at 450 nm. If available, adjust wavelength at 540 nm or 570 nm. If is not available, subtract readings of 540 or 570 to 450 nm. This subtraction corrects the optical imperfections on each plate. The readings at 450 nm without correction may be high or low accuracy.

Results

1) Make a standard curve using the appropriate cytokine at a concentration of 1, 10, 100 and 1000 pg/ml, obtaining and plotting the obtained absorbances.
2) Extrapolate the results of the sample and calculate the concentration according to the absorbance.
3) If sample dilution was performed, the concentration should be corrected by multiplying by the dilution factor.

Appendix 5. List of adverse events CTCAE (Common Terminology Criteria for Adverse Events v4.0)

Grade	Description
Grade 1	Minimal, asymptomatic or mild symptoms. No intervention required, only clinical follow-up.
Grade 2	Moderated. Non-invasive intervention, minimal or local intervention, it may impair activities of daily living (ADL) for their age.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.
Grade 4	Life-threatening, urgent intervention indicated.
Grade 5	Death related to adverse event.

Consideration: impaired daily activities refers to activities required for health and quality of life, such as preparing meals, buying garments, using the telephone, handling Money, etc. Self-care activities include bathing, getting dressed or undressed, feeding, toilet use, taking medicine and being bedridden.

Example of the use of CTCAE: Infections and infestations

Adverse event	1	2	3	4	5
Urinary tract infection Definition: A disorder caused by the infectious process involving the urinary tract, usually the bladder and urethra.	-	Localized; local intervention (antibiotic, antifungal or antiviral as needed)	IV antibiotic, antifungal, or antiviral intervention indicated; radiologic or operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Vaginal Infection Definition: A disorder characterized by an infectious process involving the vagina	-	Localized; local intervention indicated (e.g., topical antibiotic, antifungal, or antiviral)	IV antibiotic, antifungal, or antiviral intervention indicated; radiologic or operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death

Vulvar infection. Definition: A disorder characterized by an infectious process involving the vulva	Localized, local intervention indicated	Oral intervention indicated (e.g., antibiotic, antifungal, antiviral)	IV antibiotic, antifungal, or antiviral intervention indicated; radiologic or operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death

Appendix 6. Adverse event registry form
Adverse events (effects/reactions) presented by patients included in investigation protocols.

México City, _____, 20___
I, Aldo Ferreira Hermosillo, M. Sc. as the Principal Investigator of the protocol titled "Effectiveness of the treatment with dapagliflozin and metformin compared to metformin monotherapy for weight loss and its effects on waist circumference, triacylglycerol concentration, blood pressure and inflammatory cytokines on diabetic and prediabetic patients with obesity class III. Open, randomized clinical trial."
Registered number: _____
Date of authorization: _____
Site: **Hospital de Especialidades CMN SXXI**

Inform that I have reviewed each and every adverse event (effect/reaction) presented by the patients included in this investigation protocol. These events are described as follows:

Date	Case number	Reaction description	Outcome	Suspected relationship to the protocol intervention	Initial or follow up report

Please mark with an X one of the two options. In the cases where the second line is marked, please explain the motives:
( ) After careful analysis, I declare that none of the adverse events described previously demands for suspension or cancelling of the investigation protocol.
( ) Protocol has been suspended due to the following reasons:
_____
_____