EFFECTS OF EARLY USE OF DUAL THERAPY OF DAPAGLIFLOZIN WITH METFORMIN ON GLYCEMIC VARIABILITY IN MEXICAN PATIENTS WITH TYPE 2 DIABETES MELLITUS. AN OPEN-LABEL RANDOMIZED CLINICAL TRIAL.

4th Version, May 6, 2021

NCT NUMBER NCT04090580

Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán

Research Ethics Committee/Research Committee

Application for evaluation of research protocols

Project Title

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Type of Research

Type of research	**Select an option**
Pharmacological	x
Biomedical	
Epidemiological	
Interchangeability	
Other	

Investigators

Investigator	**Institutional Position**	**Project Position**	**Phone ext.**
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Valerie Paola Vargas Abonce	UNAM Master's in Medical Sciences Student	Associate Investigator	2405
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Relevance of the research group to the project

Investigator	Research study experience
Miguel Ángel Gómez Sámano	Extensive
Valerie Paola Vargas Abonce	In training
Anna Paula Guerrero Castillo	In training
Francisco J Gómez Pérez	Extensive
Gerardo Gamba Ayala	Extensive
Daniel Cuevas Ramos	Extensive
Sigfrido Benítez Rentería	Extensive
Alejandra Silva Giordano	Extensive
Lucía Palacios Báez	Extensive
María Guadalupe López Carrasco	Extensive
Sandra Rodríguez Carranza	Extensive
Jessica Bahena López	Extensive
Lorena Rojas Vega	Extensive
Griselda Xóchitl Brito Córdoba	Extensive
Iliana Manjarrez	Extensive

Participating Institutions

Institution	(Company name and address)	Role in the project	Granted project approval?
"Instituto Nacional de Ciencias de Médicas y Nutrición, Salvador Zubirán"		Site	Yes

Sponsorship

Sponsoring organizations

The study was designed by the principal investigator, without interference from the pharmaceutical industry.

The project will receive financial support from AstraZeneca, which will provide the research product and financial support for its execution (supplies, laboratory costs, and associated publication costs).

Specify if investigators receive payment (monetary or in-kind) for their specific participation in the research.

No payment of any kind is received.

Summary (Limit 400 words)

Type 2 Diabetes is one of the most prevalent chronic diseases and constitutes the eighth leading cause of death and disability due to its micro and macrovascular complications.

Chronic hyperglycemia induces a proinflammatory cascade and the formation of free radicals, such as superoxide and hydrogen peroxide, which generate a state of oxidative and mitochondrial stress, causing irreversible damage to cell membranes and interfering with various cellular signaling pathways, being the main pathophysiological mechanism for the development of microvascular complications.

Therefore, the treatment of Diabetes focuses on reducing exposure to hyperglycemia, and intensive glycemic control has been shown to decrease the risk of microvascular complications, especially if implemented from diagnosis.

However, most pivotal studies on intensive glycemic control were developed before modern medications such as SGLT2 inhibitors and GLP-1 agonists.

Glycated hemoglobin (HbA1c) is the standardized marker to assess glycemic control, since it reflects the average glycemia of the last 3 months. However, it does not detect short-term glucose fluctuations or hypoglycemic episodes.

Glycemic variability (GV), which refers to daily glucose fluctuations, including preprandial, postprandial glycemia, and hypoglycemic episodes, could be associated with an increase in oxidative stress and the development of microvascular complications in Type 2 Diabetes (T2D).

Although SGLT2 inhibitors have been shown to reduce GV, there is no evidence regarding their effect in newly diagnosed T2D patients when used at the initiation of pharmacological therapy, nor regarding their impact on oxidative stress.

Main objective: To evaluate the effect of dual therapy with Dapagliflozin + Metformin (DAPA/MET) versus Metformin monotherapy (MET) over 12 weeks on GV in patients with T2D.

Secondary objectives: To evaluate the effects on HbA1c, insulin, plasma antioxidant capacity, body composition, and cardiometabolic risk factors.

Hypothesis: The reduction in glycemic variability (measured using the mean amplitude of glycemic excursions, MAGE) will be greater in patients receiving dual therapy with Dapagliflozin + Metformin compared to those receiving Metformin monotherapy.

Methods: 88 patients with T2D without catabolic symptoms or complications, with HbA1c >7.5% and <12% and BMI >25 kg/m2 will be included. Patients will be randomized 1:1 to receive Dapagliflozin 10 mg/day + Metformin 2000 mg/day (n=44) or Metformin 2000 mg/day (n=44). GV will be measured with the iPro™ CGM system (Medtronic) at baseline and the end of the 12 weeks of treatment.

Statistical analysis: The primary dependent variable will be MAGE as a marker of GV. Other variables such as HbA1c, serum insulin, plasma antioxidant capacity, VCAM-1, and adiponectin will be analyzed. Change deltas will be calculated for each group and compared using ANCOVA. A p-value ≤0.05 will be considered significant. All analyses will be performed with SPSS 20.0.

Background

There is growing evidence regarding the beneficial effects of sustained intensive glycemic control on the incidence and progression of complications in T1D and T2D [1]. HbA1c has been the main marker used to define glycemic control because it provides an average of glycemia in the 2 or 3 months before its measurement, with the American Diabetes Association recommending that HbA1c levels be maintained between 6.5% and <8%, depending on the individual characteristics of each patient [2]. Although HbA1c is a standardized, validated, and evidence-based marker, it is not useful for evaluating glycemic fluctuations over short periods [1].

Glycemic variability (GV) is defined as fluctuations in blood glucose throughout the day, including preprandial, postprandial glycemia, and hypoglycemic episodes [2]. It is important to evaluate the effect of oral hypoglycemic treatments on GV because evidence suggests it could be significantly involved in the generation of oxidative stress and consequent endothelial dysfunction [3], and thus has been proposed as a determinant in the development of microvascular complications of T2D [6-8].

In a cross-sectional study of 26 patients with T1D, GV was found to be associated with endothelial and cardiovascular damage, even when the average HbA1c was reported as 6.7% [4]. In a retrospective cohort of 748 patients with T2D and heart failure, the GV index showed an independent association with mortality, also independent of the presence of hypoglycemic events [5]. In a sub-analysis of the ADVANCE study involving 4399 T2D patients, visit-to-visit GV in HbA1c was independently associated with the risk of vascular events ($p=0.01$) and mortality ($p<0.001$). Furthermore, variability in HbA1c was positively associated with the risk of macrovascular events, and variability in fasting glucose was associated with micro and macrovascular complications [6]. In another study of 68 T2D patients followed for 4.8 years, visit-to-visit HbA1c variability was associated with cognitive decline [7]. GV estimated by MAGE was the most important risk factor associated with diabetic peripheral neuropathy in T2D patients with controlled glycemia (HbA1c <7.0%) [8]. In another study of 110 T2D patients, the coefficient of variation and HbA1c variability were independently associated with the presence of cardiac autonomic neuropathy [9]. In a sub-analysis of the DEVOTE study, increased day-to-day GV was associated with a higher presence of major cardiovascular events (HR 1.36, 95% CI 1.12, 1.65) and higher all-cause mortality (HR 1.58, 95% CI 1.23, 2.03) [10]. Finally, a systematic review and meta-analysis found that HbA1c variability was associated with kidney disease (RR 1.56), cardiovascular events (RR 1.98), and retinopathy (RR 2.11) in T1D patients, and with kidney disease (RR 1.34), macrovascular events (RR 1.21), presence of ulcers and gangrene (RR 1.5), cardiovascular disease (RR 1.27), and mortality (RR 1.34) in T2D patients [11].

It is known that "total glycemic exposure" (mean HbA1c values and duration of diabetes) accounts for 11% to 23% of microvascular complications [17,18]. This implies that more than 75% of these complications are based on etiopathogenic and pathophysiological mechanisms different from sustained hyperglycemia. Exposure to glycemic disorders can be described based on 2 components: the duration and magnitude of chronic sustained hyperglycemia (HbA1c), which depends on interprandial and postprandial hyperglycemia, modulating the percentage of each contributor by the degree of control [19], and acute glucose fluctuations during a daily period [20-24].

Hyperglycemic states trigger harmful metabolic events through a single process: the overproduction of superoxide by the mitochondrial electron transport chain. This theory seems to indicate that the activation of oxidative stress by hyperglycemia plays an important role in the pathogenesis of diabetic complications. Because all these metabolic alterations occur more particularly in endothelial cells, it has been postulated that they can cause endothelial dysfunction and contribute to vascular damage [25-26]. The results of Monnier et al. [5] in patients with T2D suggest a very significant relationship between GV, determined by continuous glucose monitoring and calculated using MAGE, and the urinary excretion of 15(S)-8-iso-PGF2α, a marker of oxidative stress. In patients with T1D, Ceriello et al. [27] report that acute hyperglycemia seems to worsen endothelial function and increase the inflammatory response and oxidative stress, regardless of the duration of the disease and previous glycemic control.

Some epidemiological studies have reported that obesity, atherosclerosis, insulin resistance, and diabetes mellitus are associated with a chronic inflammatory state, less intense than the acute phase reaction secondary to the secretion of different adipocytokines (IL-6, TNF-α, leptin, adiponectin), which influence the regulation of the atherogenic process and insulin resistance [28]. Low plasma levels of adiponectin have been detected in diabetes mellitus [29], and Stefan et al. [30] have found a negative correlation between plasma adiponectin and endogenous glucose production, supporting a role for this hormone in glucose metabolism. Different studies have shown that adiponectin has anti-atherogenic and anti-inflammatory properties, including suppression of macrophage phagocytosis and inhibition of foam cell formation, reduction of TNF-α secretion, migration of smooth muscle cells to the arterial wall, and blockade of excessive platelet adhesion [31].

There is no conclusive information in the literature regarding the possible contribution of GV to the development of chronic complications in diabetic patients. However, in vitro experiments, animal models, and some clinical trials conducted in humans show that acute fluctuations in glucose concentrations increase oxidative stress, inflammatory response, and endothelial damage [5, 27, 32]. Currently, GV measures such as having a coefficient of variation < 36% are recommended as treatment goals for T2D [12], and the use of drugs such as exenatide [13], acarbose [14], saxagliptin [15], dapagliflozin [16], liraglutide [17], and dulaglutide [18] has been shown to improve GV.

Sodium-glucose cotransporter 2 inhibitors [19] (SGLT2i) are a new therapeutic option for glycemic control in T2D patients. As their name suggests, they act by inhibiting the sodium-glucose cotransporters responsible for renal glucose reabsorption in the proximal convoluted tubule (SGLT1 and SGLT2). Various formulations of these inhibitors have been developed, among which

Canagliflozin, Dapagliflozin, and Empagliflozin are the most studied and approved. Their clinical use results in the urinary excretion of 70 to 90 g of glucose [20], mostly reducing HbA1c by 0.6 to 1% [59]. They modestly reduce blood pressure and affect weight loss. The risk of hypoglycemia is low because their effect decreases when the filtered glucose level is lower than the reabsorption capacity of SGLT1 [21]. Their main associated complications are an increased risk of fungal and bacterial genitourinary infection, dizziness, and postural hypotension. The ADA recommends their use in combination therapy with Metformin as a second line in patients not meeting targets with monotherapy and those who have a high risk of cardiovascular disease, chronic kidney disease, and/or heart failure [22].

Dapagliflozin is a highly selective, reversible inhibitor of the sodium-glucose cotransporter type 2 (SGLT2i) that improves glycemic control independently of insulin secretion or action by reducing the reabsorption of filtered glucose and increasing urinary glucose excretion. By inhibiting SGLT2 with dapagliflozin, the renal threshold for glucose reabsorption (90%) is reduced, causing increased glucose excretion in the urine, thereby lowering fasting and postprandial hyperglycemia. The use of dapagliflozin is associated with a reduction in body weight, secondary to increased glucose elimination and related caloric loss, and also with a reduction in blood pressure, apparently related, at least in part, to its mild diuretic effect and the associated reduction in body weight.

The guidelines of the American Association of Clinical Endocrinologists recommend the use of combination therapy with MET + another glucose lowering medication when HbA1c at diagnosis is >7.5 and ≤ 9%, and in patients with HbA1c >9% without classic symptoms of diabetes; dual or triple therapy can also be initiated [23]. In a study of 16 T2D patients, dapagliflozin 5 mg showed statistically significant improvement in body composition (fat mass) from week 8 [49]. In another study of 40 hypertensive patients treated with dapagliflozin vs hydrochlorothiazide, an impact on blood vessel vasodilation was found after 4 weeks of dapagliflozin treatment [50]. The effect of administration of dapagliflozin decreased glycemic excursions, reduced GV, and prevented atherosclerosis damage in preclinical studies [45]. In a case report of a patient with MODY, administration of gliclazide with dapagliflozin improved GV [46].

Henry et al. [37], using CGM, determined GV in patients with uncontrolled T2D (HbA1c 7.5% to 10.5%) who were previously treated with Metformin >1500 mg/day (50%) or insulin > 30 units/day (50%), to whom dapagliflozin or placebo was additionally administered. A decrease of 18.2 mg/dL in average glucose with CGM and a decrease of 15.3 mg/dL in MAGE were reported. Based on this study, the sample size calculation was performed.

9. Problem Definition

HbA1c reflects the mean glucose concentration over the last 8 to 12 weeks. It is well documented that elevated HbA1c levels are associated with an increased risk of diabetes complications. However, evidence has shown that glycemic variability, particularly MAGE, is associated with greater vascular endothelial dysfunction and oxidative stress, consequently increasing the risk of developing retinopathy, nephropathy, and coronary artery disease. A frequently used measure of GV is MAGE, which reflects the mean of the differences between consecutive peaks and nadirs of blood glucose concentrations and is therefore independent of the mean glucose level [47]. Correcting GV is currently considered a clinical objective to reduce average glycemia and determine its direct effects on vascular complications in diabetes. Sodium-glucose cotransporter type 2 inhibitors have been shown to be effective in improving glycemic control, in terms of fasting glycemia, glycated hemoglobin, and glycemic variability determined by MAGE. However, the evidence primarily considers their use in Type 2 Diabetes patients diagnosed more than 5 years prior and previously treated with other pharmacological regimens, including insulin. Therefore, the effect of SGLT2i on glycemic variability and oxidative stress when used in patients with a recent diagnosis of Type 2 Diabetes is unknown.

10. Justification

Type 2 Diabetes is one of the most prevalent chronic diseases worldwide and constitutes the eighth leading cause of death and disability due to its micro and macrovascular complications. Chronic hyperglycemia, through the production of advanced glycation end products, induces a proinflammatory cascade involving mainly Interleukin 6, Tumor Necrosis Factor-alpha, and the formation of free radicals, such as superoxide and hydrogen peroxide. This generates a state of oxidative and mitochondrial stress, causing irreversible damage to cell membranes and interfering with various cellular signaling pathways, representing the main pathophysiological mechanism for the development of microvascular complications. For this reason, the current treatment of Diabetes focuses primarily on reducing exposure to hyperglycemia, and intensive glycemic control has been shown to decrease the risk of microvascular complications, especially if implemented early after diagnosis.

However, most pivotal studies on intensive glycemic control predate the existence of medications such as SGLT2 inhibitors and GLP-1 agonists. Glycated hemoglobin (HbA1c) is the most widely used standardized marker for evaluating glycemic control, as it reflects the average glycemia over the last 3 months. However, it does not detect short-term glucose fluctuations or hypoglycemic episodes. Glycemic variability (GV), which refers to daily glucose fluctuations including preprandial, postprandial glycemia, and hypoglycemic episodes, could be associated with increased oxidative stress and the development of microvascular complications in Type 2

Diabetes (T2D). There is increasing information about the reduction in the risk of microvascular complications when decreasing glycemic variability is considered a treatment goal in both Type 1 and Type 2 Diabetes. SGLT2 inhibitors have demonstrated reduced GV and their beneficial cardiovascular and renal effects are now well known. Although the American Diabetes Association recommends their use in concomitant therapy with Metformin as a second line in patients not meeting targets with monotherapy and those with high risk of cardiovascular disease, chronic kidney disease, and/or heart failure, clinical endocrinologists are increasingly inclined to initiate T2D treatment with dual therapy including Metformin and an SGLT2i or a GLP-1 agonist when HbA1c at diagnosis is >7.5% and ≤ 9%, and in patients with HbA1c >9% without classic diabetes symptoms. Understanding the effect of SGLT2i on glycemic variability and markers of oxidative stress when used early in the diagnosis of Diabetes would allow establishing a line of research on their effect in reducing the risk of microvascular complications, thereby having a positive impact on the global burden of the disease.

Hypothesis

The reduction in glycemic variability (measured using the mean amplitude of glycemic excursions, MAGE) will be greater in patients receiving dual therapy with Dapagliflozin + Metformin compared to patients receiving Metformin monotherapy.

Objectives

Main Objective

To evaluate the effect of dual therapy of Dapagliflozin + Metformin (DAPA/MET) versus Metformin monotherapy (MET) on GV in patients with T2D after 12 weeks of treatment.

Specific Objectives

- To compare the effect of dual therapy of Dapagliflozin + Metformin versus Metformin monotherapy on HbA1c levels (%).
- To compare the effect of dual therapy versus monotherapy on plasma antioxidant capacity.
- To compare the effect of dual therapy versus monotherapy on inflammation biomarkers (VCAM-1 and adiponectin).
- To compare the effect of dual therapy of Dapagliflozin + Metformin versus Metformin monotherapy on cardio-metabolic risk factors (weight (kg), BMI (kg/m2), total cholesterol (mg/dL), HDL (mg/dL), LDLc (mg/dL), albuminuria (mg/24 hr), and blood pressure (mmHg)) in patients with T2D, after 12 weeks of treatment.

Methods

GENERAL DESIGN

STUDY DESIGN. Randomized, open-label, prospective, longitudinal phase IV clinical trial.

POPULATION. Adult men and women aged 18 to 77 years who meet the following Inclusion Criteria: Diagnosis of Type 2 Diabetes, with HbA1c 7.5% <12%, with BMI ≥ 25 kg/m2.

Exclusion Criteria: HbA1c >12%, T2D-related complications, HbA1c >9% with catabolic symptoms, creatinine clearance by CKD-EPI <60 ml/min/1.73m2, Type 1 Diabetes or LADA, Concomitant clinically significant disease such as: liver, hematological, oncological, psychiatric or rheumatic disease, symptoms of severe glycemic dyscontrol such as marked polyuria or polydipsia + 10% weight loss within the last 3 months before enrollment, Known hypersensitivity to dapagliflozin or metformin or any of the drug excipients, Patients with bariatric surgery, Any major CV event / vascular disease within 3 months before signing consent at enrollment, pregnancy or lactation period, being hospitalized or having less than one month since hospital discharge for any cause.

SAMPLE SIZE. Using the difference of means formula and based on Henry et al., a reduction in glucose variability from 4.85 to 2.2 was estimated with an expected standard deviation of 2.8 in the MAGE index, with a statistical power of 80% and a confidence level of 95%. A total of 88 participants were obtained, 44 individuals in each treatment group. The CDC Epi InfoTM software was used. Patients will be randomized 1:1 to receive Dapagliflozin 10 mg/day + Metformin 2000 mg/day (n=44) or Metformin 2000 mg/day (n=44). GV will be measured with the iPro™ CGM system (Medtronic) at baseline and at the end of the 12 weeks of treatment.

VARIABLES. The primary dependent variable will be MAGE as a marker of GV. Other variables such as HbA1c, serum insulin, plasma antioxidant capacity, VCAM-1, and adiponectin will be analyzed. The independent variable will be the treatment group.

STATISTICAL ANALYSIS. Quantitative variables with normal distribution according to the Kolmogorov-Smirnov test will be expressed as Means ± Standard Deviation. Quantitative variables with non-normal distribution will be expressed as Medians (Interquartile Range 25-75). Categorical variables will be expressed as proportions (percentages). Change deltas will be calculated for each group and compared using ANCOVA, considering age, sex, BMI, baseline HbA1c, and time since Diabetes diagnosis as covariates. A p-value ≤0.05 will be considered significant. All analyses will be performed with SPSS 22.0.

Study Timeline

Study Type	Select an option
Retrospective	
Prospective	X

Process of assigning study groups

Maneuver	Yes (Include corresponding information)	No	Not applicable
Randomization	X		
Open-label study	X		
Single-blind study		X	
Double-blind study		X	
Triple-blind study		X	

Description of maneuvers or interventions

- **Clinical History:** Will be conducted via telemedicine (Phone call/Videocall, Whatsapp, Teams, Zoom, Google Forms) at visit 1 DP of the treatment period.
- **Diet Plan Prescription:** Will be conducted via telemedicine (Phone call/Videocall, Whatsapp, Teams, Zoom, Google Forms) at visit 2 DP of the treatment period.
- **Anthropometric Measurements and Physical Exam:** Will be performed at visits 1 DP, 3 DP, 4 DP, and 6 DP of the treatment period.
- **GV Monitoring:** Both groups will be monitored before treatment and at the end. The iPro ™ CGM system (Medtronic, Northridge, CA) will be used. Baseline continuous glucose monitoring will begin in week 1 (Visit 1DP) and end seven days later at Visit 2DP (day 1, Week 2). End-of-treatment continuous glucose monitoring will begin at Visit 5 DP (week 12) and end 7 days later (Visit 6 DP).
- **Measurement of HbA1c and Insulin Levels:** Will be performed at visits 1DP and 6DP of

the treatment period.

- **Spot Urine Sample:** Will be collected at Visits 1DP, 3DP, 4DP, and 6DP.
- **Measurement of Oxidative Stress and Inflammation Biomarkers:** Will be performed at visits 1DP, 3DP, 4DP, and 6DP of the treatment period.
- **WHO Physical Activity Questionnaire:** Will be evaluated via telemedicine (Phone call, Whatsapp, Teams, Zoom, Google Forms) at visits 1DP, 3DP, 4DP, and 6DP of the treatment period.
- **Food Record:** Will be performed at visits 1 FP, 2 FP, 1 DP, 2 DP, 3 DP, 4 DP, 5 DP, and 6 DP of the treatment period. The patient will keep a daily record, and data will be collected via Phone call/Videocall, or e-mail.

Table of Times and Events

Procedure	**Visit 1 FP**	**Visit 2 FP**	**Visit 1 DP**	**Visit 2 DP**	**Visit 3 DP**	**Visit 4 DP**	**Visit 5 DP**	**Visit 6 DP**
	Week -2	Week -1	Week 0	Week 1	Week 5	Week 9	Week 12	Week 13
Informed Consent			x					
Weight and Height			x		x	x		x
Pre-randomization Inclusion Criteria*	x							
Clinical History			x					
MET Tolerance Evaluation		x						
Inclusion and	x							

Exclusion Criteria								
Anthropometric Measurements (weight, waist circ., blood pressure)			x		x	x		x
Physical Exam			x		x	x		x
Concomitant Diseases	x							
Concomitant Medications	x							
Glycemic Assessment Monitoring			x				x	
CGM Removal				x				x
WHO Physical Activity Questionnaire	x		x		x	x		x
Food Record (Consumed Diet Assessment)	x		x	x	x	x	x	x
HbA1c Levels			x		x			x
Fructosamine					x	x		
Insulin Levels			x		x	x		x

Oxidative Stress			x		x	x		x
Inflammation Biomarkers			x		x	x		x
Body Fat Quantification			x		x	x		x
Nutritional Counseling and Food Plan				x				
FP: Off-protocol, DP: On-protocol, MCG: Continuous Glucose Monitoring * Determination of HbA1c levels and BMI ** Lipids (total cholesterol, HDL, LDLc, triglycerides), glucose, ALT, AST, creatinine, creatinine clearance, uric acid, albuminuria)								

Treatments (if applicable) (include a table for each study medication)

Medication 1

Item	**Include corresponding information**	**No**	**Not applicable**
Name	Dapagliflozin		
Does it comply with "Good Manufacturing Practices"?	Yes		
Codes, labeling, storage, retention, and safeguarding of drug samples	Yes		
Pharmaceutical form	Tablets of 10 mg		
Dose	1 tablet		

Administration interval	Every 24 hours		
Route of administration	Oral		
Administration speed	Immediate, with a glass of water		
Duration of treatment	12 weeks		

Medication 2

Item	**Include corresponding information**	**No**	**Not applicable**
Name	Metformin		
Does it comply with "Good Manufacturing Practices"?	Yes		
Codes, labeling, storage, retention, and safeguarding of drug samples	Yes		
Pharmaceutical form	Tablet 1000 mg Tablet 500 mg		
Dose	1 tablet of 1000 mg or 2 tablets of 500 mg		
Administration interval	Every 12 hours		
Route of administration	Oral		
Administration speed	Immediate, with a glass of water		
Duration of treatment	12 weeks		

Follow-up

Item	Include corresponding information	No	Not applicable
Number of study phases	2		
Number of visits and scheduling (include times)	8 Patient visits will be scheduled in the morning, from 8 am to 12 pm, ensuring that appointments during the study occur at the same time for each patient.		
Duration of each study phase	Phase 1, pre-randomization: 2 weeks. Phase 2, treatment: 12 weeks		
Laboratory and imaging studies	HbA1c, insulin, fructosamine, lipid profile, uric acid, serum creatinine, urinary creatinine, serum albumin, urinary microalbumin, inflammation markers, and transaminases.		
Total duration of follow-up	13 weeks		
Sampling methods	Randomisation		
Treatment options offered at the end of the study	Standard glucose lowering medication		

Overdose Management

- **Metformin:** In cases of overdose, gastric lavage and supportive measures according to symptoms will be performed, with close monitoring every 4 hours until clinical resolution.
- **Dapagliflozin:** Due to its mechanism of action, dapagliflozin very rarely causes hypoglycemia even at doses up to 50 times that used in this study. However, in case of

overdose, appropriate supportive treatment will be provided as indicated by the patient's clinical status, with close monitoring every 4 hours until clinical resolution.

Rescue Therapy

According to the ADA/EASD 2019 guidelines [49], at 12 weeks, it will be assessed whether the patient reaches HbA1c targets. If the individualized target is not met, or if the patient has fasting glucose > 140 mg/dL at any visit, rescue therapy will be administered:

- **Group 1:** Dapagliflozin 10 mg/day + Metformin 2000 mg/day + glimepiride or glibenclamide or gliclazide or glipizide.
- **Group 2:** Metformin 2000 mg/day + glimepiride or glibenclamide or gliclazide or glipizide.

If the patient has postprandial glucose > 180 mg/dL on capillary blood glucose monitoring at home, rescue therapy will be added:

- **Group 1:** Dapagliflozin 10 mg/day + Metformin 2000 mg/day + glimepiride or glibenclamide or gliclazide or glipizide.
- **Group 2:** Metformin 2000 mg/day + glimepiride or glibenclamide or gliclazide or glipizide.

Permitted Concomitant Therapies

Medications for concomitant medical conditions will be permitted. However, caution should be taken when using the following drug groups, given their interaction with Metformin:

- Drugs that may adversely affect renal function, which may increase the risk of lactic acidosis, e.g., NSAIDs, including selective cyclooxygenase (COX) II inhibitors, ACE inhibitors, angiotensin II receptor antagonists, and diuretics, especially loop diuretics. Standardized medications and dose ranges will be established to homogenize the sample as much as possible.
- In case of elevated blood pressure, Losartan will be administered, titrating the dose based on response, always within ranges of 25-100 mg/day.
- In patients with dyslipidemia, Rosuvastatin 2.5-40 mg/day will be preferred.
- In case of elevated triglycerides, the preferred therapy will be Bezafibrate 50-600 mg/day.
- Elevated uric acid levels will be managed with Allopurinol at doses of 100-300 mg/day, dose-response.
- In case of newly diagnosed hypothyroidism, the Levothyroxine dose will be calculated at 1.6 µg/kg/day.

- For patients already under treatment for any previously mentioned conditions, the same management will continue and will be noted in the analysis section.
- For patients experiencing pain, fever, or needing non-steroidal anti-inflammatory drugs, Paracetamol 2-3 g/day will be the administered.

Condition	**Medication and Dose**
Hypertension	Losartan 25-100 mg/day
Hypercholesterolemia	Rosuvastatin 2.5-40 mg/day
Hypertriglyceridemia	Bezafibrate 50-600 mg/day
Elevated uric acid	Allopurinol 100-300mg/day
Hypothyroidism	Levothyroxine at 1.6 µg/kg/day
Pain, fever	Paracetamol 2-3g/day

*The exact dose will be defined using the dose-response method and adjusted to each patient's requirements.

Prohibited Concomitant Therapies

- Medications with intrinsic hyperglycemic activity (e.g., glucocorticoids (systemic and local) and sympathomimetics).
- Organic Cation Transporters (OCT). Metformin is a substrate of OCT1 and OCT2 transporters.
    - Co-administration of Metformin with:
        - OCT1 inhibitors (like verapamil) may reduce the efficacy of Metformin.
        - OCT1 inducers (like rifampicin) may increase gastrointestinal absorption and efficacy of Metformin.
        - OCT2 inhibitors (like cimetidine, dolutegravir, ranolazine, trimethoprim, vandetanib, isavuconazole) may decrease Metformin elimination, potentially increasing plasma Metformin concentration.
        - Inhibitors of both OCT1 and OCT2 (like crizotinib, olaparib) may alter efficacy and renal elimination of Metformin.

- Relevant considerations will be taken according to the approved prescribing information for the product.

Definition of Follow-up Variables

- **Primary Variable:** Change from baseline in GV (MAGE) in mg/dL after 12 weeks of treatment between the DAPA+ MET group versus the MET monotherapy group.
- **Secondary Variables:**
    - Change from baseline (absolute difference) in HbA1c levels (%) after 12 weeks of treatment between the DAPA + MET group versus the MET monotherapy group.
    - Change from baseline (total difference) in insulin levels (µU/mL) after 12 weeks of treatment between the DAPA + MET group versus the MET monotherapy group.
    - Change from baseline (absolute difference) in oxidative stress measurement (total antioxidant capacity) after 12 weeks of treatment between the DAPA + MET group versus the MET monotherapy group.
    - Change from baseline (total difference) in the measurement of inflammation biomarkers (VCAM-1 and adiponectin) after 12 weeks of treatment between the DAPA + MET group versus the MET monotherapy group.
    - Change from baseline (absolute difference) in body fat quantification after 12 weeks of treatment between the DAPA + MET group versus the MET monotherapy group.
    - Change from baseline (total difference) in the parameters of cardio-metabolic risk factors (weight (kg), total cholesterol (mg/dL), HDL (mg/dL), LDLc (mg/dL), albuminuria (mg/mg creatinine), and blood pressure (mmHg)) after 12 weeks of treatment between the DAPA + MET group versus the MET monotherapy group.

Methods for Data Collection

Electronic Record containing the clinical history collected via telemedicine (Phone call/Videocall, Whatsapp, Teams, Zoom or Google Forms), information gathered at each visit, notes containing patient physical examinations, physical activity questionnaires answered via telemedicine (Phone call/Videocall, Whatsapp, Teams, Zoom or Google Forms), Nutrition notes collected via telemedicine (Phone call/Videocall, Whatsapp, Teams or Zoom), and laboratory results. Patients will only be identified by their initials, date of birth, and the internal protocol folio number.

Monitoring and Auditing Procedures During Study Development

Not applicable

Criteria for Failure and Success

- **Failure:** No decrease in GV in the DAPA + MET group compared to the MET monotherapy group.
- **Success:** A decrease in GV in the DAPA+ MET group compared to the MET monotherapy group.

Sample Size

The sample size was determined using the difference of means formula:

$n = (Z\alpha / 2 + Z\beta)\ 2 * 2 * \sigma 2 / d2,$

A reduction in glucose variability from 4.85 to 2.2 was estimated with an expected standard deviation of 2.8 in the MAGE index (values based on the study by R. R. Henry et al., "Effects of Dapagliflozin on 24-Hour Glycemic Control in Patients with Type 2 Diabetes: A Randomized Controlled Trial," Diabetes Technol. Ther., vol. 20, no. 11, pp. 715–724, Nov. 2018.), with an alpha value of 0.05 and a power of 0.80, resulting in a sample size of 88 subjects, with 44 subjects in each treatment group. No adjustments were included in the sample size calculation to estimate the subjects required for the estimated differences in secondary variables.

Description of techniques, devices, and/or instruments to be used (Including special mechanical, electronic, cybernetic equipment)

Continuous glucose monitoring will be performed using the iPro™2 Continuous glucose monitor, from Medtronic®, to determine the MAGE value as follows:

- **Calculation of Mean Amplitude of Glucose Excursion (MAGE):**
  - In the first step, all local maximum/minimum values are determined.
  - The next step is an evaluation of maximum/minimum pairs against the standard deviation (SD).
  - If the difference from minimum to maximum is greater than the SD, this variation from the mean measurement is retained.
  - If the local maximum/minimum is less than 1 SD, it is excluded from further calculations.
  - These channels are retained and summed to achieve the MAGE
- Glycated hemoglobin (HbA1c) will be measured using HPLC (Variant II Turbo, Biorad).
- Insulin levels will be measured with Access-2 equipment (Beckman Coulter, Inc.).
- Oxidative stress will be measured with the BioAssay Systems antioxidant assay kit (DTAC-

100) (QuantiChrom Antioxidant Assay Kit) (BioAssay Systems).

- VCAM-1 will be measured using ELISA kits (Merck Millipore, USA).
- Adiponectin measured using ELISA kits (Merck Millipore, USA).
- Body fat quantification by bioelectrical impedance with the Jawon scale, model[5] IOI 353 - JMW160.
- Anthropometric variables will be measured using standardized techniques; these include height, weight, waist, and hip circumferences.
- BMI will be calculated using the equation: weight / height² (kg/m2).
- Blood pressure will be measured considering the mean of two determinations after sitting for at least 5 minutes.
- Mean arterial pressure (MAP) will be calculated with the formula: (systolic blood pressure * 0.33) + (diastolic blood pressure * 0.66).
- After at least eight hours of fasting, glucose, lipid profile (total cholesterol (mg/dL), HDL (mg/dL), LDL (mg/dL), triglycerides (mg/dL)), creatinine (mg/dL), uric acid (mg/dL), alanine aminotransferase (ALT) (U/L), aspartate aminotransferase (AST) (U/L) will be measured using the Unicell Dxc600 analyzer (Beckman Coulter, Inc.).
- Albuminuria will be measured using the Unicell Dxc600 analyzer (Beckman Coulter, Inc.).
- Exosome quantification: a 30ml urine sample is collected. Protease inhibitors are immediately added to this urine, and it is stored at -80°C. For exosome extraction, the urine is thawed on ice and vigorously shaken to re-homogenize it. A total of 8 ml is taken and centrifuged at 17,500 g for 15 minutes at 4°C to remove sediments. From this procedure, the supernatant (1) will be isolated into a clean tube, and the pellet (1) will be processed with 200mg/ml of dithiothreitol (DTT) dissolved in a volume of 100µl of isolation solution (10mM triethanolamine and 250mM sucrose at pH 7.6), incubated for 5 minutes at 37°C, and subsequently centrifuged at 17,500 g for 10 minutes at 37°C. From this procedure, supernatant 2 will be obtained and mixed with supernatant 1, while pellet (2) will be discarded. The supernatant mixture is centrifuged at 200,000 g for 2 hours at 4°C, the supernatant (3) is discarded by decantation, and the pellet (3) is dissolved in 50µl of lysis buffer with phosphatase inhibitors and will be analyzed via Western Blot.
- LDL Cholesterol will be estimated using the Friedewald equation (LDL = total cholesterol - HDL - triglycerides / 5) [23].
- Creatinine clearance will be calculated using the CKD-EPI equation.
- A WHO physical activity questionnaire will be administered.
- A food record sheet will be completed at each visit.

- All samples will be measured in the Laboratory of the Department of Endocrinology and Metabolism, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (http://www.innsz.mx/opencms/contenido/departamentos/labcentral/). In the case of analytes whose processing is not immediate or within the first few days, the sample will be preserved by ultra-freezing (-70°C) until processing. 100% of the samples are certified by the College of American Pathologists (71893-07-01).

Description of evaluation formats, questionnaires, checklists, etc., indicating validity, reproducibility criteria, and quality controls

Not applicable

Does the protocol involve the handling and labeling of biological samples? If applicable, mention the procedures that will be used

Yes, with the patient's initials, date of birth, internal laboratory patient number, and randomization number.

Information to ensure that obtained biological samples will not be used for permanent or immortal cell lines or purposes unrelated to the study

Qualified personnel will take samples for this activity and will be processed within the institution. Samples will not be sent to any other institution/company, so there is no risk of being used for purposes other than those stipulated in the protocol.

Description of treatment groups
Included subjects will be randomized 1:1 to receive:

- **Group 1:** Dapagliflozin 10 mg/day + Metformin 2000 mg/day and, if necessary, as mentioned previously, rescue treatment.
- **Group 2:** Metformin 2000 mg/day and, if necessary, as mentioned previously, rescue treatment. Treatments will be administered for 12 weeks. Patients who do not tolerate Metformin at a dose of 2000 mg will have their dose adjusted to 1500 mg daily. If patients do not tolerate 1500 mg daily, the dose will be titrated to 1000 mg daily. If they do not tolerate this dose, they will be withdrawn from the study.

Mechanisms for treatment assignment

The https://www.google.com/search?q=randomization.com tool will be used (The Table is attached as Appendix 1 of the protocol).

If a placebo group is used, include its justification

Not applicable

Criteria for premature withdrawal from the study

- Allergic reaction to any of the study medications or their excipients.
- Patients who do not tolerate Metformin 1000 mg/day.
- Severe life-threatening illness.
- Pregnancy during the study.
- Presence of acute renal failure (decrease in > 40% of glomerular filtration rate).
- Lactic acidosis or ketoacidosis.

Procedures for withdrawing a patient from the study

The patient will be notified of withdrawal from the protocol and will receive standard treatment, according to AACE 2019 guidelines (MET, GLP1 agonist, SGLT2 inhibitor, DPP4i, acarbose, thiazolidinediones, insulin, or sulfonylurea) and will be referred for follow-up at the secondary care level.

Criteria for premature (partial or complete) suspension of the study

NA

Selection Criteria

a) Inclusion criteria

- Subjects > 18-77 years old.
- Both men and women.
- HbA1c ≥ 7.7% <12% (HbA1c from 9 to <12%: without catabolic symptoms).
- BMI > 25 kg/m2.
- Diagnosis of T2D: if a patient has taken any drug and/or insulin, they will be prescribed only Metformin as a washout period in the pre-randomization phase for 2 weeks.

b) Exclusion criteria

- Complications related to T2D and catabolic symptoms.
- HbA1c >9% with catabolic symptoms.

- CKD-EPI creatinine clearance: <60 ml/min.
- LADA or T1D.
- Concomitant clinically significant disease such as: liver, hematological, oncological, psychiatric, or rheumatic disease.
- Symptoms of markedly uncontrolled diabetes: (marked polyuria or polydipsia + 10% weight loss within the last 3 months before enrollment).
- Known hypersensitivity to dapagliflozin, metformin, or any of the drug excipients.
- Unstable or rapidly progressing kidney disease.
- Patients with severe liver failure (Child-Pugh class C).
- Patients with bariatric surgery.
- Any major CV event / vascular disease within 3 months before signing consent at enrollment, as assessed by the investigator.
- For women only: currently pregnant (confirmed with a positive pregnancy test) or lactating.
- Being hospitalized or having less than one month since hospital discharge.

c) Elimination criteria

- Allergy to dapagliflozin, severe life-threatening illness, or pregnancy during the study.
- Patients presenting with acute renal failure (decrease in > 40% of GFR)
- For women only: pregnancy confirmed with serum chorionic gonadotropin. In case of pregnancy during the study, the subject must be eliminated from the trial, offered standard treatment indicated in point 36, and the Pregnancy Outcome Report (Annex) must be completed to record essential details of the mother. Part I should be completed after pregnancy identification and Part II is to record the pregnancy outcome.

39. Outcomes and Variables

- **Primary Variable:** Change from baseline in GV (MAGE) in mg/dL after 12 weeks of treatment between the DAPA+ MET group versus the MET monotherapy group.
- **Secondary Variables:**
    - Change from baseline (absolute difference) in HbA1c levels (%) after 12 weeks of treatment between the DAPA + MET group versus the MET monotherapy group.
    - Change from baseline (total difference) in insulin levels (µU/mL) after 12 weeks of treatment between the DAPA + MET group versus the MET monotherapy group.
    - Change from baseline (absolute difference) in oxidative stress measurement (total antioxidant capacity) after 12 weeks of treatment between the DAPA + MET group versus the MET monotherapy group.

- Change from baseline (total difference) in the measurement of inflammation biomarkers (VCAM-1 and adiponectin) after 12 weeks of treatment between the DAPA + MET group versus the MET monotherapy group.
- Change from baseline (absolute difference) in body fat quantification after 12 weeks of treatment between the DAPA + MET group versus the MET monotherapy group.
- Change from baseline (total difference) in the parameters of cardio-metabolic risk factors (weight (kg), total cholesterol (mg/dL), HDL (mg/dL), LDLc (mg/dL), albuminuria (mg/mg creatinine), and blood pressure (mmHg)) after 12 weeks of treatment between the DAPA + MET group versus the MET monotherapy group.

Methods to be used to contact patients

Personal contact data such as landline and mobile phone numbers, email address will be obtained via Phone call/Videocall, Whatsapp, Teams, Zoom, or Google Forms. Due to the SARS-CoV-2 pandemic, investigators will conduct home visits and/or collect data and hold medical consultations via Phone call/Videocall, Whatsapp, Teams, Zoom, or Google Forms.

Statistical Analysis (Description of the information processing and presentation plan. Include justification for the statistical tests to be used)

Stratified randomization in both groups to balance patient types and control potential bias. The normality of variables will be analyzed using the Kolmogorov-Smirnov test. According to the distribution of variables, they will be expressed as mean and standard deviation (SD) or median and interquartile range (IQR) for normally or skewed distributed variables, respectively. GV will be determined using the MAGE index. Comparison between the two groups will be evaluated using ANCOVA. A p-value based on two-tailed tests ≤0.05 will be considered significant. All analyses will be performed with SPSS 20.0 (Chicago, IL). Patients experiencing an acute stress event during the study will be excluded from the analysis due to the impact on GV.

Justification of sample size (include study power and the p-value considered significant)

The sample size was determined using the difference of means formula:

$n = (Z\alpha / 2 + Z\beta)\ 2 * 2 * \sigma 2 / d2,$

A reduction in glucose variability from 4.85 to 2.2 was estimated with an expected standard deviation of 2.8 in the MAGE index, with an alpha value of 0.05 and a power of 0.80, resulting in a sample size of 88 subjects, with 44 subjects in each treatment group. No adjustments were included in the sample size calculation to estimate the number of subjects required for the

estimated differences in secondary variables.

Recruitment potential (number of subjects intended to recruit)
It is estimated that approximately 15% of the subjects who start the project and enter the pre-randomization period will not meet the criteria for the treatment period. Therefore, it is estimated that approximately 102 subjects will be assessed.

If multicenter, include the global and local sample number
Not applicable

Procedures for reporting deviations from the original statistical plan
A letter will be sent to the research committee for approval.

Possible discomforts resulting from the study
Frequency of follow-up visits, blood sample collection.

Potential risks
Those inherent to blood sampling.

Methods for detecting anticipated risks
Patients will be asked to report any side effects 24 hours a day. Patients will be able to contact the participating medical staff at any time.

Safety measures for timely diagnosis and prevention of risks
Patients reporting a side effect will be scheduled for evaluation.

Procedures to follow to resolve risks if they occur
Depending on the case, treatment for the complication or side effect will be provided, either with an outpatient prescription or the patient will be referred to the emergency department for assessment and treatment. Medical expense insurance will be available to cover treatment costs for patients associated with adverse effects secondary to the use of study medications.

Expected direct benefits

Patients who agree to participate in this research study will be comprehensively evaluated and more frequently compared to the standard treatment (see point 36) available at the time, giving the patient an advantage in better managing their disease. The additional tests performed could improve glucose control and also evaluate the function of other organs and systems. The medications administered during the study will lower blood glucose, thus, it is expected that microvascular complications will be prevented. Improvement in GV and control of plasma glucose levels will reduce the short-term risks associated with hypoglycemic episodes and the long-term risk of microvascular complications.

Expected indirect benefits

The study results may contribute to evaluating the impact of oral glucose-lowering medication on GV in Mexican patients with T2D who have significant glycemic dyscontrol.

General risk-benefit assessment of the proposed study

The oral glucose-lowering agents used in the study have potential risks such as hypoglycemia, gastrointestinal disorders, and increased frequency of urinary tract infections, which occur in only a small percentage of users, versus the benefit of controlling elevated glucose levels associated with micro and macrovascular complications.

Specify costs (direct/indirect, monetary, time participation, visits/travel) generated by the research for the study subjects

Patients must attend 8 visits, transportation costs will be covered by the study group.

Specify whether consultations, laboratory/imaging tests, and medical/surgical treatments generated due to the study will be covered by the patient/research subject or not

Expenses for emergencies or complications associated with the use of study medications will be covered by medical expense insurance.

Report who will cover the costs associated with the research

The study was designed by the principal investigator, without interference from the pharmaceutical industry. The project will receive financial support from AstraZeneca, which will provide dapagliflozin, consumables, and analysis costs, as well as publication fees. None of the research team members will receive financial compensation for their participation.

If applicable, specify the incentives offered (incentive is understood as an offer or influence that compels an action without implying a significant deviation from our general life plan; e.g., giving a book for participating)

Note: Compensation/incentive out of proportion is considered coercive.

There will be no additional incentives for patients.

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