No. de registro CIIBH:

1. Project title

"Effect on glycemic variability, weight, oxidative stress markers, FGF21 of a three-course low-calorie eating regimen compared to a conventional six-stroke hypocaloric plan in patients with type 2 diabetes mellitus who are overweight or obese."

2. Protocol versión

Versión 2, January 13, 2017

3.Type of investigation

Type of investigation	**Select an option.**
Pharmacologic	
Biological	**X**
Epidemiologic	
Interchangeability	
Other	

4. Investigators

Investigator	**Institutional position.**	**Position in the project**	**Telephone (ext.)**	**e-mail.**
Dr. Miguel Ángel Gómez Sámano	Specialist physician A	Investigador principal	54870900 (2405)	gsamano83@yahoo.com
L.N. Adrián Romero Villaseñor	Nutritionist.	Associate Investigator	54870900 (2405)	adrianromerov@gmail.com
L.N. Aurora Ramos Flores	Nutritionist.	Associate Investigator	54870900 (2405)	nutriologaramos@gmail.com
L.N. Mariana Galindo Guzmán	Nutritionist.	Associate Investigator	54870900 (2405)	
Dr. Daniel Cuevas Ramos	Coordinator of the Neuroendocrinology clinic of the Department of Endocrinology and Metabolism	Associate Investigator	54870900 (2405)	ceptamim@gmail.com
Dr. Adrian Soto Mota		Associate Investigator	54870900 (2407)	
Dr. Francisco J. Gómez	Chief of the	Associate	54870900	gomezperezfco@g

Pérez	Department of Endocrinology and Metabolism	Investigator	(2405)	mail.com
Dr. Alfonso Gulias Herrero	Endocrinólogo, investigador médico y Subdirector médico del INCMNSZ	Associate Investigator	54870900 (2405)	alfonso.tiranicida@gmail.com
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4b. Pertinencia del grupo de investigadores con respecto del proyecto

Investigador	**Belongs to SNI**	**Experience in research studies**
Dr. Miguel Ángel Gómez Sámano	SIN I	Yes
L.N Adrián Romero Villaseñor	No	Yes
L.N. Aurora Ramos Flores	No	Yes
L.N. Mariana Galindo Guzmán	No	Yes
Dr. Adrián Soto Mota	No	Yes
Dr. Daniel Cuevas Ramos	SNI I	Yes
Dr. Francisco Javier Gómez Pérez	SNI III	Yes
Dr. Alfonso Gulias Herrero	SNI I	Yes
Lucía Palacios Baez	No	Yes
Horacio Correa Carranza	No	Yes
Alejandra Dominguez Sanchez	No	Yes

5. Instituciones participantes

Institution.	**Role that will play in the project**	**Did the institution accepted the protocol?**
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán Vasco de Quiroga 15, Colonia Sección XVI, Tlalpan C.P.14000, Mexico City, CDMX	Main Research Center	Yes
Médica Sur. Puente de Piedra 150, Toriello Guerra, 14050 Mexico City, CDMX	Main Research Center	Yes

6. Sponsorship
6a. Sponsoring organizations

None

6b Specify whether researchers receive payment (monetary or in kind) for their specific participation in the investigation

None

7. Summary (400 word limit)

The frequency of meals is a very important aspect of nutrition, with profound effects on human health and, in turn, life expectancy. Excessive energy consumption is totally associated with a significant increase in the incidence of chronic diseases including diabetes (Visscher and Seidell 2001). That is why nutritional therapy is recommended for all people with type 1 and 2 diabetes mellitus as an effective complement to their medical treatment. For type 2 diabetic patients who are overweight or obese, a hypocaloric diet along with healthy eating patterns are recommended for weight loss. Likewise, the modest decrease in body weight can provide clinical benefits in such patients such as the improvement of blood glucose, blood pressure, lipid profile, among others (Evert, Boucher et al. 2013). Data on the role of eating habits and energy density which are important precursors of the obesity and diabetes epidemic are not scarce, but data on feeding frequency, meal schedules and how these relate to body weight if they are (Aljuraiban, Chan et al. 2015).
Typically, hypocaloric plans for patients with type 2 diabetes mellitus are consumed in five or six small meals per day. Eating more frequently is presumed to reduce hunger and thus energy consumption. However, the effects of meal frequency on human health and longevity are unclear (Mattson 2005).

This is why this study will be carried out in which the differences in the impact of nutritional therapy between a conventional 6-course low-calorie meal plan versus a 3-course low-calorie diet plan will be demonstrated.

The procedures will be as follows:

50 patients with DM2 will be recruited from the Institute's Diabetes clinic who are overweight or obese and who are between 40 and 70 years old and who are with oral hypoglycemic agents, with a HbA1c less than 9% and who are less than 10 years old of evolution

Clinical evaluation

A complete medical history and physical examination will be performed in order to confirm the presence of the inclusion criteria and rule out the presence of exclusion criteria. An anthropometric evaluation and body composition (waist and hip circumference, height, weight and percentage of fat) will be made, as well as blood pressure measurement. An interrogation will be conducted to document a history of coronary heart disease, menopausal status and use of hormonal therapy, smoking, alcohol consumption and physical activity (through a validated physical activity questionnaire in the Mexican population).

Nutritional assessment.
Will be performed

Patients will be divided into 2 groups without distinction.

The first group that will be 25 patients will undergo a hypocaloric regimen with caloric restriction of 15% of their usual daily consumption with a distribution of macronutrients of 40% in Carbohydrates, 20% of Protein

and 40% of Lipids in three times of food (TC6) during a period of 12 weeks in which the following variables will be measured (Weight, Glycemic Variability: Monitor, HbA1c, Glycemias, Lipid Profile, Oxidative, FGF-21, Blood Pressure and Hunger-Satiety levels.)
During the first visit, the clinical evaluation will be developed, which consists of a complete physical examination in which vital signs, neck and head examination, chest and abdomen examination, upper and lower limb examination will be measured. As well as a complete nutritional assessment that includes, anthropometric assessment by segments using dry label tape measure, dry brand plyometrometer and dry brand anthropoment; Body composition assessment using impedance OMRON model 512, 72hrs food consumption reminder to collect information on the feeding of each patient. Based on the information collected, the individualized TC6 plan will be prescribed and a 24/7 reminder format will be delivered to be filled out by the patient for a week, in addition to giving the indication to perform a blood glucose measurement at home once a day. in random scheme.

To assess the adherence to the nutritional plan, the patient will be summoned 15 days later to perform daily consumption assessment using the 24/7 reminder format filled out by the patient and a 72hrs reminder made through an interview. Also, Hunger-Satiety levels will be measured with the Joslin institute tables validated for the Mexican population.
If the patient adheres to the diet, blood samples will be taken to measure the variables (HbA1C, Glucose, Lipid Profile, Insulin, FGF-21) and after that place the continuous glucose monitor Medtronic model Ipro2 with in order to monitor the patient's blood glucose for 7 days.
At the end of 7 days, the patient will be summoned to remove the sensor and re-evaluate the diet.
The TC6 plan will be continued for 6 weeks, and at the end of this period the patient will be summoned again to perform blood samples to measure the variables (HbA1C, Glucose, Lipid Profile, Insulin, FGF-21), Weight, Blood pressure and hunger-satiety levels.
The TC6 regime will continue for another 6 weeks. At the end of the period, the patient will be cited to re-perform blood samples to measure the variables (HbA1C, Glucose, Lipid Profile, Insulin, FGF-21), Weight, Blood Pressure and Hunger-Satiety levels.

The second group that will be 25 patients will undergo a hypocaloric regimen with caloric restriction of 15% of their usual daily consumption with a macronutrient distribution of 40% in Carbohydrates, 20% Protein and 40% of Lipids in three times of food (TC3) during a 12-week period in which the following variables will be measured (Weight, Glycemic Variability: Monitor, HbA1c, Glycemia, Lipid Profile, Oxidative, FGF-21, Blood Pressure and Hunger-Satiety levels.)
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8. Background

The frequency of meals is a very important aspect of nutrition, with profound effects on human health and, in turn, life expectancy. Excessive energy consumption is totally associated with a significant increase in the incidence of chronic diseases including diabetes (Visscher and Seidell 2001). That is why nutritional therapy is recommended for all people with type 1 and 2 diabetes mellitus as an effective complement to their medical treatment. For type 2 diabetic patients who are overweight or obese, a hypocaloric diet along with healthy eating patterns are recommended for weight loss. Likewise, the modest decrease in body weight can provide clinical benefits in such patients such as the improvement of blood glucose, blood pressure, lipid profile, among others (Evert, Boucher et al. 2013). Data on the role of eating habits and energy density which are important precursors of the obesity and diabetes epidemic are not scarce, but data on feeding frequency, meal schedules and how these relate to body weight if they are (Aljuraiban, Chan et al. 2015).

Joslin Diabetes Center's Joslin Clinic feeding guidelines indicate that there is strong evidence that weight reduction improves insulin sensitivity and blood glucose control, lipid profile and blood pressure in type 2 diabetes and decreases the risk of developing type 2 diabetes in pre-diabetes and high-risk populations. They also say that a structured life plan that combines diet modification, activity and behavior modification is necessary for weight reduction. A moderate and gradual weight reduction of half to one kilo every one or two weeks should be the optimal goal. The reduction in daily caloric intake should be between 250-500 calories. The total daily caloric intake should not be less than 1000-1200 for women and 1200-1600 for men, or be based on an evaluation of the usual intake. A weight loss of 5-10% can lead to a significant improvement in glycemic control in patients with diabetes and help prevent the onset of diabetes among individuals with pre-diabetes (Giusti and Rizzotto 2006).
Typically, hypocaloric plans for patients with type 2 diabetes mellitus are consumed in five or six small meals per day. Eating more frequently is presumed to reduce hunger and thus energy consumption. However, the effects of meal frequency on human health and longevity are unclear (Mattson 2005). There is only one study that reflects differences between a plan without collations and a conventional one, but the variables to be studied were different, so the effect of T3 vs T6 on glycemic variability, HbA1c, fasting blood glucose, lipid profile is still unknown. , Oxidative stress, FGF21, TA (Kahleova, Belinova et al. 2014).

During the course of human history there has always been a debate about when to eat. In ancient Greece, three to four meals a day were consumed, considering lunch and dinner the most important. (Antonia-Leda Matalas 2001). At the time of the Romans, breakfast was consumed in the early morning,

despite the fact that there was a greater emphasis on eating abundantly later in the day, especially for the upper social classes, unlike the lower social classes that they ate with the labor patterns, therefore their feeding cycle was closer to the Night-Day cycle. (Antonia-Leda Matalas 2001).
In the Islamic world, mealtimes were often dictated by the Light-Dark cycle. It was of utmost importance the consumption of a meal just before dawn, as it was considered a sacred ritual that was believed to prepare the human body for rapid health promotion. This is why the famous doctor Avicenna recommended eating twice a day, one intake before sunrise and a second at night during the dark. (Salas-Salvado, Huetos-Solano et al. 2006).
In ancient Andalusia, doctors also believed in the importance of only consuming two to three meals separated by intervals of between 6-12hrs, taking into account the person and their state of health. (Salas-Salvado, Huetos-Solano et al. 2006).
However, during the Middle Ages in Europe, having breakfast was seen as a sinful act, so doctors at that time warned that having breakfast could be detrimental to health. It was until the 16th century that breakfast was already recognized as one of the most essential meals. (Anderson 2013).
The most recent evidence obtained from trials and studies has documented the consumption of breakfast and its associated health benefits. (Betts, Richardson et al. 2014). Several studies have also investigated the relationship between eating at night and cardiovascular and metabolic disorders, including obesity. (Madzima, Panton et al. 2014).
The importance of the circadian rhythm in the regulation of food intake as human behavior and metabolism has already been described. However, little is known about how energy intake is distributed in the day in existing populations, and its potential association with obesity. (Almoosawi, Vingeliene et al. 2016).

In general, there is no real scientific evidence to support whether a 6-course or one-only three-course meal plan is more beneficial for your health. Therefore, there is no evidence to show the relationship between meal times and glycemic variability in patients with type 2 diabetes mellitus who are overweight or obese.

On the other hand, glycemic variability (VG) is defined as the oscillations in glucose concentration throughout the day, including hypoglycemia and glucose elevation in the postprandial period. It has been proposed that VG is a determinant of the microvascular complications of diabetes mellitus, regardless of hyperglycemia. (Brownlee and Hirsch 2006).
However, recent studies emphasize glycosylated hemoglobin (HbA1c) as a risk determinant for diabetes complications, but no mention is made of how glucose fluctuations influence. (Ceriello and Ihnat 2010).
In a study conducted at Kyoto University Hospital about blood glucose variability if it was associated with quality of life and treatment satisfaction in patients with type 1 diabetes, they mention something very important, neither glucose variability nor hypoglycemia is precisely detected by HbA1c. (Ayano-Takahara, Ikeda et al. 2015).
It has been shown in several studies that the complications associated with diabetes mellitus are largely due to fluctuations in glucose concentration throughout the day. For example in a study that was done with animals where they confirmed that glucose oscillations, increases the risk particularly for all the pathogenesis that is involved in the complications of diabetes. And in studies with humans it was confirmed that glucose fluctuations produces an increase in free radical levels and an increase in endothelial dysfunction. (Cavalot 2013).
In recent years glycemic variability (VG) has become a determining factor in vascular complications of both type 1 and type 2 diabetes mellitus.
In type 1 diabetes, VG data analyzes show conflicting results in both micro and macrovascular

complications and in non-diabetic subjects postprandial glycemia is a stronger predictor of cardiovascular complications than fasting glucose. In type 2 diabetes, both the fasting type coefficients of fasting blood glucose and postprandial blood glucose predict cardiovascular events. In addition, long-term variability of HbA1c has been associated primarily with diabetic nephropathy, less frequently with retinopathy. In an intervention trial that was done to evaluate the effect of postprandial glucose, they have been performed only in prediabetic patients or with type 2 diabetes and the data are inconclusive. In vitro and in vivo they have demonstrated the mechanisms that are at the base of the adverse cardiovascular effects of GBV, mainly associated with oxidative stress; The atherogenic action of postprandial glucose also implies insulin sensitivity, postprandial increase in serum lipids and the glycemic index of foods. Therefore, the correction of the VG emerges as an objective that should be sought in clinical practice in order to reduce the average blood glucose and glycosylated hemoglobin thus safely and by its direct effects on the vascular complications of diabetes. (Cavalot 2013).

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Having described all this, it is essential for science to have studies that reveal the true interaction between meal times and glycemic variability, since this could determine how many feeding times are optimal for patients with DM2 and thus be able to counteract the complications triggered by it.

9. Definition of the problem

The effect on glycemic variability, weight and oxidative stress markers FGF21 of a three-course hypocaloric eating regimen is unknown compared to a conventional six-stroke hypocaloric plan in patients with type 2 diabetes mellitus who are overweight or obese. ”

10. Justification

There is currently a lot of information, bibliography and guides on medical-nutritional treatment for patients with type 2 diabetes mellitus, with full emphasis on the quality of the diet composition, both in the distribution of macronutrients and micronutrients and in the required energy supply, and of course, its relationship with the quality of life of patients; but feeding guidelines for patients with type 2 diabetes

mellitus are unknown in terms of information regarding meal times in hypocaloric diets for patients with type 2 diabetes mellitus and its tangible benefit in the treatment of this condition and its effect on glycemic variability. Conventionally, a hypocaloric diet divided into six meal times is prescribed, but it is presumed that it could have better benefits in patient control if it were only divided into three stages**.**

11. Hypothesis

1. 1. Weight loss in the TC3 group (Three meal times) will be more significant than in the TC6 group (Six meal times).
2. 2. It will significantly improve glycemic variability in patients undergoing the TC3 regimen.

12. Objetives

Primary objetive:

Comparar el impacto en la variabilidad glucémica y pérdida de peso de un régimen alimenticio hipocalórico de tres tiempos (TC3) y un plan convencional de seis tiempos (TC6) en pacientes con DM2 y sobrepeso u obesidad.

Specific objectives.

1. Evaluate whether a TC3 regimen improves HbA1c in patients with DM2 who are obese or overweight compared to a TC6 regimen.
2. Determine if a TC3 regimen improves fasting blood glucose in patients with DM2 who are obese or overweight compared to a TC6 regimen.
3. Assess whether a TC3 regimen improves triglyceride levels in patients with DM2 who are obese or overweight compared to a TC6 regimen.
4. Assess whether a TC3 regimen improves cholesterol levels in patients with DM2 who are obese or overweight compared to a TC6 regimen.
5. Determine if a CT3 timing regimen improves oxidative stress in patients with DM2 who are obese or overweight compared to a TC6 regimen.
6. Assess whether a TC3 regimen better controls FGF21 in patients with DM2 who are obese or overweight compared to a TC6 regimen.
7. Determine if a TC3 regimen improves systolic and diastolic BP in patients with DM2 who are obese or overweight compared to a TC6 regimen.

Measure the level of Hunger-Satiety of a TC3 regimen in patients with DM2 who are obese or overweight compared to a TC6 regimen.

13. Methodology: General design.

a) Study design:

It is a simple blind randomized clinical trial (Information Analyst) in which a group of patients with DM2 and overweight or obesity will be subjected to a 3-course low-calorie diet (TC3), and another to a conventional hypocaloric regimen of 6 meal times (TC6) over a period of 14 weeks with the objective of evaluating the benefits, differences and impact on the health of patients, comparing weight loss, glycemic variability, HbA1c, glycemia, lipid profile , oxidative stress, FGF-21, Blood pressure and Hunger-Satiety level.

d) Treatment allocation mechanism (random / open): Random

e) Treatment groups

It is intended to include two groups of patients with type 2 diabetes mellitus between 40 and 70 years old who meet all the inclusion criteria.

f) Duration of individual follow-up

12 weeks

14. Temporality of the study.

Type of study	Select an option
Retrospective	
Prospective	X

15. Process of assigning the groups under study

Maniobra	Yes (Include the corresponding information)	No	does not apply
Randomization	X		
Open study			
Simple blind study	X		
Double blind study			
Triple blind study			

16.Descripción de las maniobras o las intervenciones

50 patients with DM2 will be recruited from the Institute's Diabetes clinic who are overweight or obese and who are between 40 and 70 years old and who are with oral hypoglycemic agents, with an HbA1c less than 9% and who are less than 10 years old of evolution

Clinical evaluation

A complete medical history and physical examination will be performed in order to confirm the presence of the inclusion criteria and rule out the presence of exclusion criteria. An anthropometric evaluation and body composition (waist and hip circumference, height, weight and percentage of fat) will be made, as well as blood pressure measurement. An interrogation will be conducted to document a history of coronary heart disease, menopausal status and use of hormonal therapy, smoking, alcohol consumption and physical activity (through a validated physical activity questionnaire in the Mexican population).

Nutritional assessment.

Will be performed

Patients will be divided into 2 groups without distinction.

. The first group that will be 25 patients will undergo a hypocaloric regimen with caloric restriction of 15% of their usual daily consumption with a distribution of macronutrients of 40% in Carbohydrates, 20% of Protein and 40% of Lipids in three times of food (TC6) during a period of 12 weeks in which the following variables will be measured (Weight, Glycemic Variability: Monitor, HbA1c, Glycemias, Lipid Profile, Oxidative, FGF-21, Blood Pressure and Hunger-Satiety levels.)

During the first visit, the clinical evaluation will be developed, which consists of a complete physical examination in which vital signs, neck and head examination, chest and abdomen examination, upper and lower limb examination will be measured. As well as a complete nutritional assessment that includes, anthropometric assessment by segments using dry label tape measure, dry brand plyometrometer and dry brand anthropoment; Body composition assessment using impedance OMRON model 512, 72hrs food consumption reminder to collect information on the feeding of each patient. Based on the information collected, the individualized TC6 plan will be prescribed and a 24/7 reminder format will be delivered to be filled out by the patient for a week, in addition to giving the indication to perform a blood glucose measurement at home once a day. in random scheme.

To assess the adherence to the nutritional plan, the patient will be summoned 15 days later to perform daily

consumption assessment using the 24/7 reminder format filled out by the patient and a 72hrs reminder made through an interview. Also, Hunger-Satiety levels will be measured with the Joslin institute tables validated for the Mexican population
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At the end of 7 days, the patient will be summoned to remove the sensor and re-evaluate the diet.
The TC6 plan will be continued for 6 weeks, and at the end of this period the patient will be summoned again to perform blood samples to measure the variables (HbA1C, Glucose, Lipid Profile, Insulin, FGF-21), Weight, Blood pressure and hunger-satiety levels.
The TC6 regime will continue for another 6 weeks. At the end of the period, the patient will be cited to re-perform blood samples to measure the variables (HbA1C, Glucose, Lipid Profile, Insulin, FGF-21), Weight, Blood Pressure and Hunger-Satiety levels

The second group that will be 25 patients will undergo a hypocaloric regimen with caloric restriction of 15% of their usual daily consumption with a macronutrient distribution of 40% in Carbohydrates, 20% Protein and 40% of Lipids in three times of food (TC3) during a 12-week period in which the following variables will be measured (Weight, Glycemic Variability: Monitor, HbA1c, Glycemia, Lipid Profile, Oxidative, FGF-21, Blood Pressure and Hunger-Satiety levels.)
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17. Treatments (if applicable) (include a table for each medication under study)

Medication 1	Include the corresponding information	No	Does

			not apply
Name			X
Does it comply with "Good manufacturing practices"?			X
Codes, labeling, storage, retention and storage of medication samples			X
Pharmaceutical form			X
Dose			X
Administration Interval			X
Learn to pronounce Route of administration			X
Administration speed			X
Learn to pronounce Treatment duration			X

18. Follow-up

	Include the corresponding information	No	Does not apply
Number of phases of the study	1		
Number of visits and their schedule (include schedules)	5 scheduled visits in the endocrinology and metabolism department of the National Institute of Medical Sciences and Nutrition Salvador Zubrian. Visits are scheduled between 7:30 a.m. and 2:00 p.m. from Monday to Friday depending on availability.		
Duration of each phase of the study	12 weeks		
Laboratory and cabinet studies that will be used	HbA1c. Fasting blood glucose Lipid profile FGF21. Continuous Glucose Measurement. Insulin		
Total duration of follow-up	12 weeks		
Sampling methods	Serum		
Treatment options to be offered at the end of the study	SGLT2 inhibitors, DPP4 inhibitors, Insulin, GLP1		

19. Overdose Management

N/A

20. Rescue Therapy

SGLT2 inhibitors, DPP4 inhibitors, Insulin, GLP1

21. Concomitant therapies allowed

Oral hypoglycemic agents (Metformin), Aspirin, Blood pressure medications

22. Concomitant therapies prohibited

Insulin

23. Definition of monitoring variables

HbA1c: The measurement of glycosylated Hb is a laboratory test widely used in diabetes to know if the patient's control over the disease has been good during the last three or four months.
Fasting blood glucose
Lipid Profile: A lipid profile is a series of blood tests used to measure the total cholesterol and triglyceride level of an individual.
FGF21: It is produced in the liver in response to high levels of carbohydrates and then enters the bloodstream, where it sends a signal to the brain to suppress the preference for sweets. This is the first hormone derived from the liver that is known to regulate the consumption of sugar in particular
Hunger-Satiety Levels: There are several tables and measurement scales, for practical purposes we will use the scales of the Joslin Institute validated for the Mexican population.

0	3	5	7	10
Ravenous Hunger	Hungry	Satisfied	Full	Very full

1.- On a scale of 1 to 10, being 1 "voracious" and 10 "very full", classify your hunger just before starting to eat.
2.- Halfway through your meal, classify your hunger again using the same scale from 1 to 10. If you are on a "5", "6" or "7" put down your fork and stop eating.
3.- If you decide to continue eating, finish your meal and classify your hunger. Be honest with yourself, too. If you feel you just ate Christmas dinner, but have a huge plate of ice cream in front of you, chances are that you are eating to help deal with some kind of emotion.

24. Methods to be used for information collection

Initial Interview
Initial assessment by specialist doctor and nutritionist.
Follow-up consultation
Data collection sheets
Sheets for weekly food registration.
Databases.
Hunger-Satiety scales.

25. Monitoring procedure and audits during the development of the study

It is a study initiated by the researcher, so Medtronic is not involved in design, monitoring, monitoring, analysis and publication of results.
The department of endocrinology, where advances, problems in the progression of the protocol, will be submitted for evaluation in the research sessions.

26. Failure and success criteria

Success: Corroborate that the effects of a TC3 diet are more beneficial than a conventional TC6 plan. To achieve this, patients should have better glycemic variability, lose more weight, improve fasting blood glucose, improve HbA1c, optimize their lipid level, improve oxidative stress markers and have better control of Hunger-Satiety.

Failure: That the effects of a TC3 diet be equal or less beneficial to the patient's health than a conventional TC6 plan.

27. Sample size (please include the formula used for the calculation and the source of information on which the assumptions were based)

a) Sample size

The calculation of the sample size was carried out taking as main outcome the body weight and considering the data of a study in which BMI and weight were evaluated. With these data considering a clinically significant difference, the sample size was calculated in 25 patients per group. A difference in weight 2kg, standard deviation of 2.5, alpha of 0.05 and power of 0.8 were used.

with the following formula:

$$n = \frac{2S2\ (Z\alpha + Z\beta)\ 2}{\Delta 2}$$

Allowing to detect this difference with power of 80% (error b = 0.20) and with an alpha error of 5%

28. Description of the techniques, devices and / or instruments that will be used in the measurement (Including: special mechanical, electronic, cybernetic equipment)

An RJL body composition analyzer with stadiometer will be used.
An ergonomic continuous fiberglass tape measure will be used to measure the circumference of the Seca201® brand body
For the continuous measurement of glucose and glycemic variability, the Medtronic Ipro2 Sensor will be used.

The blood samples will be labeled prior to their inclusion in the "Beckman GPKR Centrifuge" equipment to perform their centrifugation and to be able to separate serum samples, to obtain the laboratories and eppendorf containers will be stored, for each time, to be able to consult later before any situation of doubt
Samples will be separated from the tubes to obtain the laboratories.

Beckman-Coulter equipment will be used for the analysis of samples corresponding to blood count.
The Hunger-Satiety scales of the Joslin Institute will be used.

Computing teams of the Department of Endocrinology will be occupied, which have Windows 8.1 pro operating system.
The Easy GV® Software will be used to calculate the glycemic variability indices.
SPSS V21 will be used for data capture and analysis.

29. Description of the evaluation formats, questionnaires, comparison tables, etc., indicating the validity, reproducibility and quality control criteria that are taken of them

-General Data Capture Format
-Formats for capturing capillary glycemia.
-Developed for the application in this protocol, with the objective of documenting adverse events.
- Weekly food registration.
-Format for determining physical activities per day and week.
- Hunger-Satiety scale format.

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

Blood samples will be taken 3 times.
Each sample must be centrifuged, to obtain serum and plasma and stored in eppendorf containers.
A label with the following data will be placed in all containers: patient registration number, date, content (plasma, serum, urine), laboratory to be carried out from the sample.

31. Corresponding information to ensure that the biological samples obtained will not be used for permanent or immortal cell lines or purposes not related to the study

The samples will be stored, due to the possibility that there is a failure in the trial, or the initiative to measure a new hormone, data that will be included and clarified to the patient in the informed consent.

32. Description of the treatment groups

They will be 2 groups each with 25 patients from 30 to 70 years.
One of the groups will have a diet in 3 meal times.
The second group will maintain a diet in 6 meal times.

33.
Mechanisms for treatment allocation

Random: A reference was generated on randomization.com which is attached.
A Randomization Plan
from
http://www.randomization.com
1. a_____
2. b_____
3. b_____
4. b_____
5. a_____
6. a_____
7. b_____
8. a_____
9. a_____
10. b_____
11. a_____
12. a_____
13. a_____
14. a_____
15. b_____
16. b_____
17. b_____
18. a_____
19. b_____
20. a_____
21. b_____
22. a_____
23. a_____
24. a_____
25. b_____
26. a_____
27. a_____

28. a_____
29. a_____
30. a_____
31. b_____
32. b_____
33. a_____
34. b_____
35. b_____
36. b_____
37. b_____
38. b_____
39. b_____
40. b_____
41. b_____
42. a_____
43. a_____
44. a_____
45. b_____
46. a_____
47. b_____
48. b_____
49. a_____
50. b_____

50 subjects randomized into 1 block

To reproduce this plan, use the seed 6312

along with the number of subjects per block/number of blocks

34. If a placebo group is used, include your justification

N/A

35. Criterios para el retiro prematuro del estudio

- Manifest desire of the patient to withdraw from the protocol.
- What is not complying with the meal times that correspond to a patient's personal problem.
- Frequent hypoglycemias, genital infections, urinary tract infections, euglycemic ketoacidosis, anasopharyngitis and orthostatic hypotension that require hospitalization for the assessment and control of the patient's clinical condition.

36. Procedures for the withdrawal of a patient from the study

-Removal of data obtained through patient participation
-Valuation by medical specialists of the department.
- Assessment by department nutritionist.
-A conventional food plan is given.

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

Undesirable side effects

38. Selection criteria

a) Inclusion criteria (It must include the definition of the age groups, sex and the severity of the condition that will be allowed in the study)

a) Individuals with type 2 diabetes
b) Less than 10 years of evolution

c) Age between 40 and 70 years
d) Both genders.
e) With oral treatment scheme (oral hypoglycemic agents).
f) With HbA1c <9%
g) With BMI ≥25 to <40 kg / m2

b) Exclusion criteria

a) Individuals with another type of diabetes.
b) Use of insulin.
c) Evolution time of more than 10 years.
d) Hospitalization in the last 3 months
e) Disease with poor short-term prognosis (for example: cancer)
f) Acute infection or febrile syndrome
g) Chronic disease other than those to be evaluated, for example: HIV, rheumatic diseases, liver cirrosis
h) Pregnancy
i) Who do not wish to participate in the study

c) Elimination criteria

a) Men or women who do not wish to continue in the study
b) Individuals who do not tolerate the placement of the MCGI
c) Individuals who are not attached to the meal plan.
d) Individuals who do not respect the corresponding meal times during the study.

39. Outcome and variables

a) Main variables / outcomes to be measured

BMI (kg / m2)
a) Weight

Glycemic variability, defined with the following indices:
a) MAGE (mean amplitude of glycemic excursions)
b) MODD (mean of daily differences)
c) CONGA-n

b) Secondary outcomes to be measured:
a) Fasting glucose
b) Triglycerides
c) Total Cholesterol
d) HbA1c
e) Oxidative stress
f) FGF21
g) Systolic and diastolic BP
h) Hunger-Satiety Scale

a) Frequency of measurements:

Three measurements will be made in a 12-week period with a 6-week interval between each measurement, however the sensor placed in visit 2 will be left for 7 days during which glucose measurements in the interstitial fluid will be obtained every 5 minutes.

40. Methods that will be used to contact patients

Telephone calls to the contact provided by the patient or by email.

41. Statistical analysis (Description of the processing plan and presentation of the information. Include the justification of the statistical tests that will be used)

The distribution of the variables will be evaluated with the Shapiro-Wilk test. Descriptive statistics will be used for the presentation of the variables, estimating averages and standard deviation for variables with normal and median distribution and interquartile range for variables with non-normal distribution. The prevalence and frequencies will be expressed in terms of percentage. The variables in each group will be compared by Student's T-test or U-Mann Whitney (as appropriate). Categorical variables will be compared by Fisher's exact test or Chi square analysis. A statistically significant difference with a value of $P < 0.05$ will be considered. The statistical program SPSS version 21 will be used for data analysis.

42. Justification of the sample size (include the power of the study and the value of p that will be considered significant)

For the calculation of the sample we consider (study on which we are based):

The calculation of the sample size was carried out taking as main outcome the body weight and considering the data of a study in which BMI and weight were evaluated. With these data considering a clinically significant difference, the sample size was calculated in 25 patients per group. A difference in weight 2kg, standard deviation of 2.5, alpha of 0.05 and power of 0.8 were used.

with the following formula:

$$n = \frac{2S2\,(Z\alpha + Z\beta)\,2}{\Delta 2}$$

Allowing to detect this difference with power of 80% (error b = 0.20) and with an alpha error of 5%

43. Recruitment potential (number of subjects to be recruited)

25 subjects for a diet in 3 meal times.
25 subjects for a diet in 6 meal times.

44. If it is multicentre, include the global number and the local number of the sample

N/A

45. Procedures for reporting deviations from the original statistical plan

Amendments will be sent to the research ethics committee and the institute's research committee if it is intended to report a deviation from the original statistical plan.

46. Possible discomfort resulting from the study

Sensor Placement
Change of eating habits

47. Potential risks

hypoglycemia

48. Early risk detection methods

N/A

49. Security measures for timely diagnosis and risk prevention

N/A

50. Procedures to follow to resolve risks in case they arise

N/A

51. Expected direct benefits

-Weight reduction.
-The study will establish whether the VG is different within a group of individuals with a 3-meal meal plan & one with 6 mealtimes.
-Improvement in glycemic control reflected by fasting glucose and glycosylated hemoglobin.

52. Expected indirect benefits

-Decrease of triglycerides
-Decrease in Cholesterol
- Decrease in systolic blood pressure figures
-Improved recommendations for a meal plan for diabetic patients.
-Better control of Hunger-Satiety.

53. General weighting of risks against benefits of the proposed study

According to the previously published evidence, it is estimated that the benefits outweigh the risks

54. Specify costs (direct / indirect, monetary, in time of participation, visits / transfers) that the research generates for the subjects of the study

The study including consultations, laboratories and medical treatment will have no cost to the patient, the patient will only have to pay the cost of transportation to attend their visits.

55. Specify whether the consultations, laboratory / cabinet exams and medical / surgical treatments generated during the study will or will not be covered by the patient / research subject

The study including consultations, laboratories and medical treatment will have no cost to the patient, the patient will only have to pay the cost of transportation to attend their visits.

56. Report who will cover the costs associated with the investigation

Medtronic glucose sensors

57. If applicable, specify the incentives that will be offered (an incentive is understood as an offer or influence that compels to carry out an action without implying a significant deviation from our general life plan; v. Gr .: give a book for having participated)
Note: A compensation / incentive out of proportion is considered a coercive attitude

N/A

58. Bibliographic citations.

Aljuraiban, G. S., et al. (2015). "The impact of eating frequency and time of intake on nutrient quality and Body Mass Index: the INTERMAP Study, a Population-Based Study." J Acad Nutr Diet **115**(4): 528-536 e521.

Almoosawi, S., et al. (2016). "Chrono-nutrition: a review of current evidence from observational studies on global trends in time-of-day of energy intake and its association with obesity." Proc Nutr Soc: 1-14.

Anderson, H. (2013). Breakfast: AHistory. Plymouth, Rowman and Littlefield.

Antonia-Leda Matalas, A. Z., Vassilis Stavrinos (2001). The Mediterranean Diet: Constituents and Health Promotion. Florida, CRC Press.

Ayano-Takahara, S., et al. (2015). "Glycemic variability is associated with quality of life and treatment satisfaction in patients with type 1 diabetes." Diabetes Care **38**(1): e1-2.

Betts, J. A., et al. (2014). "The causal role of breakfast in energy balance and health: a randomized controlled trial in lean adults." Am J Clin Nutr **100**(2): 539-547.

Brownlee, M. and I. B. Hirsch (2006). "Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications." JAMA **295**(14): 1707-1708.

Cavalot, F. (2013). "Do data in the literature indicate that glycaemic variability is a clinical problem? Glycaemic variability and vascular complications of diabetes." Diabetes Obes Metab **15 Suppl 2**: 3-8.

Ceriello, A. and M. A. Ihnat (2010). "'Glycaemic variability': a new therapeutic challenge in diabetes and the critical care setting." Diabet Med **27**(8): 862-867.

Czerwoniuk, D., et al. (2011). "GlyCulator: a glycemic variability calculation tool for continuous glucose monitoring data." J Diabetes Sci Technol **5**(2): 447-451.

Evert, A. B., et al. (2013). "Nutrition therapy recommendations for the management of adults with diabetes." Diabetes Care **36**(11): 3821-3842.

Giusti, J. and J. A. Rizzotto (2006). "Interpreting the Joslin Diabetes Center and Joslin Clinic Clinical Nutrition Guideline for Overweight and Obese Adults With Type 2 Diabetes." Curr Diab Rep **6**(5): 405-408.

Joubert, M. and Y. Reznik (2012). "Personal continuous glucose monitoring (CGM) in diabetes management: review of the literature and implementation for practical use." Diabetes Res Clin Pract **96**(3): 294-305.

Kahleova, H., et al. (2014). "Eating two larger meals a day (breakfast and lunch) is more effective than six smaller meals in a reduced-energy regimen for patients with type 2 diabetes: a randomised crossover study." Diabetologia **57**(8): 1552-1560.

Madzima, T. A., et al. (2014). "Night-time consumption of protein or carbohydrate results in increased morning resting energy expenditure in active college-aged men." Br J Nutr **111**(1): 71-77.

Mattson, M. P. (2005). "The need for controlled studies of the effects of meal frequency on health." Lancet **365**(9475): 1978-1980.

Rodbard, D. (2011). "Glycemic variability: measurement and utility in clinical medicine and research--one viewpoint." Diabetes Technol Ther **13**(11): 1077-1080.

Salas-Salvado, J., et al. (2006). "Diet and dietetics in al-Andalus." Br J Nutr **96 Suppl 1**: S100-104.

Visscher, T. L. and J. C. Seidell (2001). "The public health impact of obesity." Annu Rev Public Health **22**: 355-375.