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Comparative study of the response to weight loss and metabolic conditions using two Nonpharmacological nutritional programs.
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1. INTRODUCTION

Obesity is a chronic disease characterized by an excessive body fat accumulation, which has a negative impact on health and affects the physical, social and emotional wellbeing. A double-blind study will be carried out using a weight loss program based on a low-fat, normoprotein, controlled ketogenic diet, followed by a metabolic adaptation process. During the weight loss process, psycho-emotional support and personalized physical activity supervision will be provided in order to modify lifestyle habits along with nutrition. In this study, supervision during 24 months of the weight lost is proposed to ensure the stability of the results obtained. In the initial phase where the diet process is fully controlled, the control group will receive the weight reduction program proposed by the WHO task force.

The weight loss program is developed according to the characteristics of each patient and includes medical control, dietary-nutritional supervision, introduction of a physical activity program and psycho-emotional support.

The primary objective of this study is to test the effectiveness, safety and security of this intervention method for weight loss and compare it with a conventional method for weight loss.

This document includes all the background information for the use of the so-called very low-calorie ketogenic diet, the hypotheses to be tested in this study, the methodology of how the objectives will be demonstrated and the statistical analyses used, the table of variables to be studied, the bibliography consulted and finally the materials used for data collection and management.

2. BACKGROUND.

Obesity is a chronic disease characterized by an excessive accumulation of body fat, which impacts health negatively and affects the physical, social and emotional wellbeing. It is a growing health problem with a high prevalence worldwide and a high impact in Mexico (1). According to a study conducted by the Food and Agriculture Organization of the United Nations (FAO), Mexico has become the country with the highest obesity rate, with 32.8% obese people, above the United States with 31.8% (2), such data definitely underestimates the problem in Mexico, since according to the National Health and Nutrition Survey (ENSANUT) 2018, 42.5% of men over 20 years old are overweight and 30.5%, are obese; while in women these figures correspond to 36.6 and 40.2%, respectively (3).

Higher total energy intake and sedentary lifestyles are most immediate contributors for the increase in the number of people with obesity. This is mainly attributed to an increase in the consumption of refined sugars, and to the consumption of a greater variety of foods with high energy content, without leaving aside the increase in commercial portions consumed (4).

The increase in the prevalence of obesity represents a growing public health risk, since obesity is not only an aesthetic problem or a body image appreciation problem, but also has a direct impact on health. Obesity is the underlying pathology of many chronic diseases, especially those related to lipid metabolism such as dyslipidemia, metabolic syndrome, or coronary and vascular diseases. In addition, obese patients also face other pathologies derived from weight gain, such as sleep apnea or osteoarticular disorders (5).

To effectively treat obesity, three goals must be met: to lose weight, to maintain the new weight, and to not regain the lost weight. The achievement of these goals is fundamentally based on three pillars: patient motivation, individualization of treatment, and realistic goals. Motivation is important because it has been proven that unmotivated patients show low adherence to the diet. (6) Treatment must be individualized, according to the patient's lifestyle and food preferences. For that purpose, it is necessary not only to regulate the diet, but also to encourage physical exercise and, if necessary, use medication. Setting realistic goals, agreed with the patient, allows the patient to become involved with the diet and facilitates adherence to it. (5).

Nowadays, there are a large number of diets available to treat and control obesity. One of the most commonly used by nutritionists is the hypocaloric diet. This type of diet consists of a daily caloric intake around 10% lower than the patient's basal metabolism, which normally represents an intake of between 800 and 1,500 kcal per day. To achieve this, in hypocaloric diets the consumption of the basic nutrients required by the body is reduced in a balanced way, maintaining their proportions: between 25% and 35% of fats, between 50% and 65% of carbohydrates, and between 10% and 20% of proteins. This type of diet has proven to be effective in short-term weight loss, but not in maintaining the weight loss (7). The possible causes of the low efficiency of hypocaloric diets in the long term are the lack of adherence to this type of diet, and the reduction of basal energy expenditure (4).

Due to the ineffectiveness of hypocaloric diets in the long term, a large number of diets have been developed that are based on the intake of different percentages of macronutrients that seek to optimize weight loss, especially in the long term. Among them is the protein diet, developed by Blackburn in 1976. This diet results in weight loss through a protein-rich diet and reduced intake of lipids and carbohydrates. In addition, as a consequence of the nutritional imbalance, the patient should receive supplementation with vitamins and minerals to ensure the supply of the micronutrients required by the body (8). The basis of the protein diet is the reduction of carbohydrate intake to a minimum. This ensures that in a 48-72 hour period, the body resorts to fats as an energy source, and ketone bodies are formed so that stored lipids are used as a source of energy. Studies on the efficacy of the ketogenic diet with very-low carbohydrate, high-protein and reduced lipid content show that it produces, not only a very rapid decrease in the weight of patients in the short term, but is also effective in the long term (9, 10).

So far, different variants of Blackburn's protein diet have been developed. Among them is the multidisciplinary Zélé program which, like the rest of the high-protein diets, is based on maintaining protein intake according to the calculated requirement, while reducing lipids and carbohydrate intake. However, this method does not only consist of weight loss by means of controlled ketosis, but also by patient re-education to promote changes in eating habits. . This program must be carried out under the supervision of a multidisciplinary group that includes a physician to control the possible side effects of ketosis, a nutritionist to supervise the nutritional habits and conditions, a

psychologist to strengthen and help maintain the patient's motivation for weight loss, and a physical trainer to adapt a personalized program for the preservation and strengthening of muscle mass.
The Zélé method consists of five stages: frank ketosis stage, mixed ketosis stage, transition stage, integral stage and maintenance. The ketogenic stage, in which a high-protein diet with high biological value (HBV) proteins is followed, supplemented with vitamins and minerals. In this stage, the expected weight is reduced by 70 to 80%, divided into frank ketosis and mixed ketosis, with a variable duration depending on the patient's initial weight and the kilograms to be lost, although for the purposes of the study a duration of between two and three months is proposed. In the transition stage, carbohydrates are incorporated with the purpose of leaving the metabolic state of ketosis. Later, in the integral stage, carbohydrates, natural proteins and fats are progressively incorporated, instilling in the patient the habits to achieve a balanced diet. In this stage, a loss of between 20% and 30% of the remaining excess weight is sought. Throughout the maintenance stage, with a supervised duration of two years, the patient is followed up and monitored to maintain the weight loss, with the advice of a team of dietitians and nutritionists.

The studies carried out to date with diets that are very-low in carbohydrates, high-protein and low in fat have demonstrated their efficacy in reducing weight both in the short and long term (9,10). There is increasing evidence that rapid weight loss, such as that achieved with this type of nutritional intervention, is associated with better long-term results, and initial weight loss has even begun to be considered as a predictor of long-term weight maintenance.(11,12,13,14) Furthermore, in 2012, George Blackburn himself acknowledged in an article that the tendency to use commercial rapid weight loss programs is appropriate, since they achieve better results.(15,16,17.)

The anecdotal experience obtained in daily practice with the Zélé method and products has shown great effectiveness in weight reduction, however, so far there is no scientific evidence of its efficacy or its medium and long term effects. By conducting a double blind study, our objective is to evaluate the efficacy and safety of this nutritional intervention for the reduction and maintenance of weight loss, due to the fact that it is possible, according to Zélé's food engineering system, to build a reduced carbohydrate diet under the same concept of Zélé's products for a controlled ketogenic diet, as well as to perform a 12 and 24 months follow-up of the cohort to assess its efficacy not only in the initial stages, but also during the 24-month follow-up. The aim of this study is also to evaluate patient adherence to treatment, which in most studies reports a dropout rate of over 40% (4). In addition,

the characteristics of weight loss and the preservation of muscle mass and strength and finally patient satisfaction with their treatment will be evaluated.

3. PROBLEM STATEMENT

Obesity is one of the biggest pandemics in human history. According to the WHO, in 2016, more than 1.9 billion adults aged 18 or older were overweight, of which, more than 650 million were obese. Overweight and obesity are defined as an abnormal or excessive accumulation of fat that can be detrimental to health.

Until the end of the 20th century, the health problems of the population in developing countries were related to their nutritional status, especially stunting in children and anemia in women of reproductive age. But all that has changed in just a few decades; the world has gone from a nutritional profile in which the prevalence of undernutrition more than doubles that of obesity, to the current situation in which there are more obese people in the world than underweight people. Obesity and overweight, once considered characteristic of affluent societies, are now on the rise in low- and middle-income countries, especially in urban areas, where the increase is particularly marked.

This evolution toward obesity affecting the entire population is occurring at a frightening speed. In Mexico City, for example, obesity in the urban adult population has risen from 16% in 2000 to 35.6% in 2018. By then, the urban child population between 5 and 11 years old with obesity or overweight had already reached 35%. As for the country as a whole, it is estimated that currently seven out of ten Mexicans are overweight and that one third of those affected can be considered medically obese (ENSANUT 2018). Obesity increases the risk of diabetes mellitus, cardiovascular diseases, obstructive sleep apnea-hypopnea, and some types of cancer are within the group of conditions that have shown a direct association. Currently, the condition in which obesity, as an independent risk factor, has the greatest impact is type II diabetes mellitus with its costly complications, such as blindness, amputation of limbs and the need for dialysis, so finding a method accessible to the general population that achieves the reduction or control of obesity rates could have an impact on the reduction of cases of obesity that progress to DM-II and thus even have an impact on the national health budget.

Therefore, we ask ourselves if a structured multidisciplinary program based on a normoprotein ketogenic diet low in fat and carbohydrates is effective, safe for short-term weight loss and allows to maintain weight loss in the long term.

4. JUSTIFICATION

Overweight and obesity have been associated with an increased risk of multiple diseases including cardiovascular conditions and DM-II. If a person already has type 2 diabetes and gains weight, it will be even more difficult for him or her to control his or her blood sugar level.

In Mexico we do not know the current status of cardiovascular diseases associated with obesity, but in relation to DM-II presents high incidence in the population, in this regard the National Health and Nutrition Survey (ENSANUT) in 2018 revealed that approximately 8.6 million Mexicans have diabetes mellitus, which we can associate with the increase in the rates of overweight and obesity, which in children aged 5 to 11 years is 35.6%, from 12 to 19 reaches 39.7% and in the adult population over 18 years 76.8% in women and 73% in men, which represents an increase of at least 3% between 2012 to 2018. As commented, obesity and overweight is one of the most important risk factors for triggering Diabetes Mellitus. Good nutrition is an indispensable tool for the good control of all chronic degenerative diseases such as diabetes. Nutritional management is one of the determining factors to improve the control of many of these chronic degenerative diseases, mainly DM-II and hyperlipidemia. It is imperative to mention that diabetes is one of the main causes of death and the tendency is that the figures continue to increase due to the high rates of obesity and sedentary lifestyle in the world. The implementation of diets that help to reduce weight will favor the control and improve the quality of life of these patients, since it will reduce the risk of complications, and will also help to improve their self-esteem and avoid depression. The results obtained in this study are expected to be disseminated in research forums and scientific journals, as well as a technical report for the company that finances the project.

The direct benefits provided to the study population will be close monitoring of their clinical and metabolic evolution, awareness of their nutrition and how it impacts their health. There is no evidence of risk compared to the benefit that the rapid and safe reduction will provide to the study

population and to all those subjects who will be managed in the future with the proposed nutritional intervention.

5. OBJECTIVES

5.1 *General Objective*

To evaluate differences in the response of weight, body composition and metabolic biochemical parameters among obese patients following a normoprotein low-fat controlled ketogenic diet compared to a balanced BIEM).

5.2 *Specific Objectives*

- Intra- and intergroup comparison of patient weight and BMI reduction between baseline and subsequent follow-up monitoring visits.
- To determine the percentage of patients with weight loss equal to or greater than 10% of initial weight and the time it took for patients to reach this figure.
- Evaluate the evolution of comorbidities associated with obesity (HT, hyperglycemia or diabetes, hypercholesterolemia, hypertriglyceridemia, apneas), control of clinical and analytical parameters and changes in medication during the weight loss process and during follow-up.
- To know the tolerability of the diet: Incidence of side effects during the development of the study,
- Safety of the intervention diet: appearance of analytical alterations, especially of the ionic profile, renal function and hepatic function, incidence of serious adverse events.
- Assess diet adherence and dropout rate and cause.
- To know patient satisfaction with the assigned nutritional program.

6. HYPOTHESIS

Assumption 1: **Weight Loss** .

H1: The use of the "Zélé method" (**Tx1**) allows a greater weight loss (*pp*) than a hypocaloric diet (**Tx2**) in the same time (*t*).

Assumption 2.

H2: Tx1 is safe and with low or no adverse effects.

Assumption 3. **Metabolic conditions**

H3: *pp* with Tx1 allows the regulation of metabolic syndrome and its components.

H4: The state of controlled ketosis that occurs with Tx1 does not change pH or raise blood lactate levels.

Assumption 4 . **Body Composition**.

H5. Tx1 will produce a selective loss of fat and preservation of muscle mass.

7. METHODOLOGY

7.1. Study setting.

The study will be conducted in Mexico City under the sponsorship of Zélé® and in collaboration with the VIME Weight Loss and Wellness Center of Mexico. During the year 2021 and with follow-up for 24 months. An open call will be made through different media including social networks for the recruitment of patients who meet the inclusion criteria and must present themselves for a clinical evaluation in which pathological history, clinical status, heart rate, blood pressure, oxygenation and temperature will be recorded. Nutritional assessment in which anthropometry and multifrequency electrical bioimpedance analysis (MFBIA) will be performed to determine body composition. Patients will have to bring a Complete Blood Count, a biochemical profile that includes the following determinations: Fasting blood glucose, glycosylated hemoglobin, urea, creatinine and uric acid, cholesterol, triglycerides, LDL, HDL and VLDL, direct and indirect bilirubins, alanine and aspartate aminotransferases, gamma-glutamyl transferase and albumin, determination of serum electrolytes sodium, chloride, potassium, magnesium, phosphorus and calcium, Fasting Insulin , HOMA index calculation. In addition to a thyroid function panel and arterial blood gases. They will be instructed during recruitment for the studies to be performed in a clinical laboratory, under the current standardization and with standardized and perfectly calibrated automated equipment, and a request will be made to keep the vials of the patients' plasmas. Patients who meet all the inclusion criteria will be selected and will have an interview with the principal investigator in which they will sign their letter of commitment and informed consent, then they will be subjected to the randomization process with a 2:1 allocation for treatment and controls, respectively. Subsequently, they will be submitted to the two nutritional treatments and will be clinically evaluated every week and will receive nutritional, psycho emotional and physical activity counseling. At each visit, adverse effects, changes in clinical status will be recorded and the presence of ketone bodies in capillary blood will be determined with the Freestyle Abbott optium H β-Ketone (β-hydroxy-Butyric Acid) device; during nutritional counseling, MFBIA will be performed using the In-Body 570 body composition analyzer. When ketone body levels above 0.8 mmol/liter are detected, a new arterial blood gas analysis will be taken; for patients who do not have elevated ketone bodies, an arterial blood gas will be taken between the first and second week of treatment. A new biochemical profile determination will be performed when patients have lost 40% of their overweight or between 10 and 12 kg, whichever

occurs first. And new biochemical evaluations when the decrease of Ketone Bodies below 0.4 mmol/l is detected or when patients have reached a loss of 60 to 70% of their overweight and one more when they reach their weight loss goal. Subsequently, only clinical and nutritional follow-up will be given along with psycho-emotional support and physical activity advice every 3 months up to 12 months and every 6 months up to 24 months.

7.2 Study design.

Longitudinal, Prospective, Controlled Randomized Clinical Trial.

7.3 Study population.

Patients of either sex, between 18 and 60 years of age, with a body mass index (BMI) between 30 and 35 kg/m2.

7.4 Sample.

The sample will be obtained by open call through social media and all patients who come with their clinical analysis and meet the inclusion criteria will be evaluated until the sample number is reached. In order to obtain 95% power in this Controlled Clinical Trial and with a 95% CI, a 3:1 case-control ratio is established, considering a 35% attrition rate, 56 cases and 32 controls will be included and subsequently crossed to become cases. (Calculated with EPIinfo STATCALC for sample size)
Two blinded arms are proposed for the physician and the patient.

Obese patients assigned to the low-fat normoprotein controlled ketogenic diet group.

Obese patients assigned to a balanced hypocaloric diet. And who will subsequently be subjected to a controlled normoproteic low-fat ketogenic diet.

*7.5 Inclusion, exclusion and elimination criteria**.***

7.5.1 *Inclusion*:

- Men and Women
- 18 to 60 years of age
- With BMI between 30 and 35 Kg/m2
- Who has not taken weight loss treatment in the last 6 months

7.5.2 Exclusion

- Pregnant or breastfeeding patients.
- Patients with severe eating disorders, alcoholism, or drug addiction.
- Patients with severe psychological disorders (e.g. schizophrenia, bipolar disorder).
- Patients with hepatic alteration defined as increase of ALT, AST, GGT more than 4 times the reference value.
- Patients with renal impairment defined as a glomerular filtration rate below 60 ml/min.
- Patients with type 1 or insulin-dependent DM, or DM2 on insulin therapy.
- Patients with obesity caused by endocrinological diseases (except type 2 DM).
- Patients with hemopathies.
- Cancer patients.
- Patients with active cardiovascular or cerebrovascular disease (heart rhythm disorders, recent infarction [<6m], unstable angina, decompensated heart failure, recent vascular accident [<6m]).
- Patients with gout.
- Patients with renal lithiasis.
- Patients with cholelithiasis.
- Patients with depression.
- Patients with electrolyte disorders.

- Patients with orthostatic hypotension.
- Patients with an altered or abnormal electrocardiogram.

7.5.3 Elimination criteria

- Patients who drop out and decide to stop participating in the study.

(The patient has the right to withdraw from the study at any time, without the need to offer any explanation and without affecting the patient's future care or health care).

- The patient may also be withdrawn from the study at any time at the discretion of the investigator or the sponsor, for administrative or behavioral reasons or for missing two consecutive follow-up visits.
- Patients who during follow-up present any of the following criteria: Any serious adverse event
- Incoercible vomiting and nausea that do not allow following an oral diet.
- Worsening of diabetes control in patients with type II diabetes mellitus or insulin resistance
- Uncontrolled hypertension (SBP > 150 mmHg or SBP < 90 mmHg in successive repetitions).
- The reasons for abandonment of the study must be clearly documented in the registration notes. Any adverse reactions will be immediately reported to the Pharmaco-Surveillance system in charge of Dr. Francisco J Nachón García Principal Investigator.

7.6 Variables

Variable name	Conceptual definition	Operational definition	Type of variable and measurement scale	Instrument for measurement
Clinical Variables				
Age	Life span of a person from birth to a given point in time	Age expressed by the patient during interrogation	Quantitative, Ratio, years.	Interrogation Registration form. Identification form section
Sex	Anatomical features that differentiate men from women	Sex expressed by the patient during interrogation	Qualitative Nominal, Male, Female	Interrogation Registration form. Identification form section
Weight	Mass of an individual expressed in kilograms	kg measurement with clinical scales	Quantitative, Ratio, Unit Kg, Minimum 20 Maximum 350	Data recording form, using In-Body electronic scales
Size	Distance from the floor to the crown of the head with the body resting on a flat surface and fully extended.	Standing height measurement, without shoes, with a calibrated stadiometer.	Quantitative, Ratio, Unit cm Min. 100, Max. 270,	Data recording form, using a
Blood Pressure	It represents the pressure exerted by the force of the heartbeat on the resistance of the arterial walls.	It is commonly measured with an ESFIGMOMANOMETER in the upper arm at the brachial artery.	Quantitative, Ratio, Unit mmHg. Min. 60-80, Max. 120-180	Data recording form, using a sphygmomanometer.
Heart Rate	Number of times the ventricles of the heart contract per unit of time, usually minute.	Number of beats per minute.	Quantitative, Ratio, Unit l/minute. Minimum 48, Maximum 120	Data registration form Measurement with digital monitor.
SaO2	Determination of O2 saturation in hemoglobin, through a non-invasive oximeter using pulse oximetry.	Oxygen saturation percentage	Quantitative, Ratio, % Saturation, Minimum 90%, maximum 150%.	Registration Form pulse oximetry using a noninvasive oximeter.

Body temperature	Property of bodies that determines the direction of heat flow produced by the energy released from the motion of atomic particles.	Measuring the heat level of a human	Quantitative, Ratio, Degrees Celsius Minimum 35.5 Maximum 42.0	Data Registration Form. Digital thermometer.
Body Composition Variables				
BMI	Indicator of body density determined by the ratio of body weight to body height.	BMI falls into these categories: below 18.5 (underweight); 18.5-24.9 (normal); 25.0-29.9 (overweight); 30.0 and above (obese).	Quantitative, Ratio, BMI = weight (kg) / height squared (m2). Unit Kg/m2, Minimum 11, Maximum 75	Data registration form,
Muscle Mass	Volume of total body tissue corresponding to muscle. ,	From the point of view of body composition, it corresponds to lean mass.	Quantitative, Ratio. Unit Kg. Minimum 10 Maximum 150	Data registration form Multi-frequency Bioelectrical Impedance Equipment
Fat Mass	Percentage of body weight made up of adipose tissue	Amount of fat in the body	Quantitative, proportion, Unit Percentage. Minimum 9 Maximum 80	Data registration form Multi-frequency Bioelectrical Impedance Equipment
Visceral Fat	Fatty tissue within the abdominal cavity, which includes visceral fat and retroperitoneal fat.	It is the most metabolically active fat in the body. Its increase is associated with metabolic complications of obesity.	Quantitative, Ratio. Unit Index. From 1 to 40	Data registration form Multi-frequency Bioelectrical Impedance Equipment
Bone Mass	Measurement of the amount of minerals (usually calcium and phosphorus) contained in the bones.	Skeletal weight	Quantitative, Ratio Unit Kg. From 1 to 7 kg.	Data registration form Multi-frequency Bioelectrical Impedance Equipment
Total body water	Amount of liquids, mainly water, found inside the body.	Percentage of the body that corresponds to water	Quantitative Proportion. Unit % and/or Kg. 0 a 150	Data registration form Multi-frequency Bioelectrical

				Impedance Equipment
Waist circumference	Measurement established at mid-armpit, at the point between the bottom of the last rib and the highest part of the hip.	Measurement made with a tape measure directly on the skin (without clothing). It will be measured at the height of the middle of the armpit, at the point between the bottom of the last rib and the highest part of the hip.	Unit Cm , Minimum 30 , Maximum 200	Registration form. Using tape measure
Hip circumference	specific anthropometric measurement for hip circumference measurement	Circumference of the widest part above the buttocks. Minimum 30, Maximum 200	Quantitative, Ratio Unit cm.	Recording format, measuring with tape measure.
Muscular Strength	The hand grip force measured by	Isometric muscle strength in the dominant hand	Quantitative Ratio Kg/Strength	Registration Form Digital dynamometer.
Blood Ketones	The blood ketone test measures the level of butyrate (ketones) in the blood.	Ketone bodies are substances produced by the body when cells do not receive enough glucose.	Quantitative, Ratio Unit mmol/liter Minimum 0.01 Maximum 25	Registration Form Abbott Ketone Test
Biochemical and Metabolic Variables				
Hb	Oxygen Transporting Protein in erythrocytes	Oxygen Transporting Protein in erythrocytes	Quantitative Ratio. Gr/l. minimum 10 maximum 16	Registration format. Clinical laboratory determination
Hto	Proportion of formed elements in the blood	Proportion of formed elements in the blood	Quantitative Ratio minimum 27 Maximum 50	Registration format. Clinical laboratory determination
Leukocytes	Number of white blood cells per unit volume in venous blood	Number of white blood cells per unit volume in venous blood	Quantitative, Ratio. Quantity per cc Minimum 4,000 Maximum 18,000	Registration format. Clinical laboratory determination

Neutrophils	Granular nucleated leukocytes participating in inflammatory processes.	Number of neutrophilic leukocytes	Quantitative, Ratio. Quantity per cc Minimum 4,000 Maximum 18,000	Registration format. Clinical laboratory determination
Lymphocytes	White blood cells formed by lymphatic tissue.	Number of lymphocytes in blood cell counts	Quantitative, Ratio. Quantity per cc Minimum 1,000 Maximum 8,000	Registration format. Clinical laboratory determination
Fasting Blood Glucose	*Glycemia* or *Glycemia* is the amount of glucose or sugar in the blood and is one of the sources of energy for our body, especially for the cells.	Blood sample for blood glucose with eight hours fasting time	Normoglycemia in fasting 70 to 100 mg/dl. Hyperglycemia higher than 100 mg/dl in fasting. Diabetes 126 mg/dl of fasting blood glucose.	Registration form. Blood tests
HbA1c	*Measurement of glucose concentration in blood plasma level.*	Glycosylated hemoglobin is considered: HbA1c ≥ 6.5% (48 mmol/mol) for diagnosis of DM2.	Categorical, Ordinal (%), 6% very low, 7% moderate, 8% high, 9% very high, 10% risk.	Registration form. Blood tests Glycosylated hemoglobin
Insulin	*the amount of this hormone insulin in the blood.*	Blood sample for insulin with eight hours fasting time	Hyperinsulinemia >6µIUI/ml	Registration form. Blood tests
Creatinine	*Muscle protein degradation product*	test that measures the level of creatinine in the blood	Quantitative, Ratio. mg/dl Quantity per cc Minimum 0.4 Maximum 1.3	Registration form. Blood tests
Urea	*A substance formed by the body during the metabolism of proteins and nitrogenous compounds in the liver.*	test measures the amount of urea in the blood	Quantitative, Ratio. mg/dl Quantity per cc Minimum 8 Maximum 54	Registration form. Blood tests
BUN	*Amount of nitrogen from protein metabolism in the blood.*	Test that measures the amount of urea nitrogen in the blood.	Quantitative, Ratio. mg/dl Quantity per cc Minimum 8	Registration form. Blood tests

			Maximum 54	
Uric Acid	*Uric acid is an organic compound consisting of carbon, nitrogen, oxygen and hydrogen that is formed when purines are broken down by metabolism.*	A test that measures the amount of uric acid in the blood.	Quantitative, Ratio. mg/dl Quantity per cc Minimum 2.4 Maximum 9.0	Registration form. Blood tests
Sodium	Electrolytes are minerals present in blood and other body fluids that carry an electrical charge. They affect the amount of water in the body, the acidity of the blood (pH), muscle activity and other important processes.	Determination of the amount of sodium in blood	Quantitative, Ratio. mEq/l Quantity per cc Minimum 135 Maximum 145	Registration form. Blood analysis
Chlorine	Electrolytes are minerals present in blood and other body fluids that carry an electrical charge. They affect the amount of water in the body, the acidity of the blood (pH), muscle activity and other important processes.	Determination of the amount of chlorine in blood	Quantitative, Ratio. mEq/l Quantity per cc Minimum 90 Maximum 110	Registration form. Blood análisis
Potassium		Determination of the amount of Potassium in blood	Quantitative, Ratio. mEq/l Quantity per cc Minimum 3.5 Maximum 5.5	Registration form. Blood análisis

Calcium		Determination of the amount of Calcium in the blood	Quantitative, Ratio. mg/dl Quantity per cc Minimum 8.5 Maximum 10.5	Registration form. Blood análisis
Phosphorus		Determination of the amount of phosphorus in blood	Quantitative, Ratio. mg/dl Quantity per cc Minimum 2.5 Maximum 4.5	Registration form. Blood análisis
Magnesium		Determination of the amount of magnesium in blood	Quantitative, Ratio. mg/dl. Quantity per cc Minimum 1.5 Maximum 2.5	Registration form. Blood análisis
Albumin	Acute phase protein synthesized by the liver.	Amount of this protein in the clear liquid part of the blood.	Quantitative, Ratio. mg/dl. Quantity per cc Minimum 3.4 Maximum 5.4	Registration form. Blood analysis
Direct Bilirubin	A liquid of an oily nature, formed during the destruction of red blood cells, it is secreted by the liver whose function is to digest fatty foods.	Portion of bilirubin conjugated with glucuronic acid, moves freely in the bloodstream.	Quantitative, Ratio Normal value less than 0.3 mg/dl (0 less than 5.1 mcMol/l)	Registration form. Blood analysis

Indirect Bilirubin		Protein-bound portion that requires passage through the hepatocyte.	Quantitative, Ratio unit mg/dl Normal value Minimum 0.1 Maximum 0.5	Registration form. Blood analysis
Total Bilirubin		Sum of Direct B. and Indirect B. Indirect	Quantitative, Ratio unit mg/dl Normal value Minimum 0.1 Maximum 1.2	Registration form. Blood analysis
SGPT Transaminase	Aminotransferases, a group of enzymes that transfer amino groups from one metabolite to another, located mainly in the liver.	Alanine aminotransferase, predominantly hepatic, is released into the bloodstream when the liver is damaged.	Quantitative, Ratio unit IU/l Normal value Minimum 10 Maximum 41	Registration form. Blood analysis
SGOT Transaminase		Aspartate Amino Transferase, an enzyme found in the liver and muscle, is released into the bloodstream when the liver is damaged.	Quantitative, Ratio unit IU/l Normal value Minimum 5 Maximum 37	Registration form. Blood analysis
GGTP		Primarily hepatic enzyme released into the circulation when there is liver damage or biliary tract disease.	Quantitative, Ratio unit IU/l Normal value Minimum 11 Maximum 50	Registration form. Blood analysis

Serum Iron	Essential mineral for the production of hemoglobin, necessary for growth and development.	Test that measures the amount of iron in the blood.	Quantitative , Ratio minimum 60 mcg/dl Maximum 170 mcg/	Registration form. Blood analysis
Total Cholesterol	They are the most common type of fats in the body, coming mainly from saturated fats.	Test designed to measure the amount of free and protein-bound cholesterol in the blood.	Quantitative, Ratio minimum 100 mg/dl Maximum 200 mg/dl	Registration form. Blood analysis
Triglycerides	They are the most common type of fats in the body, coming mainly from saturated fats.	Test designed to measure the amount of triglycerides in the blood.	Quantitative, Ratio Minimum 100 Maximum 500 mg/dl	Registration form. Blood analysis
C HDL	Combination of fats and proteins, necessary for the transport of lipids through the bloodstream.	High Density Lipoproteins	Quantitative, Ratio Greater than 40 mg/dl	
C LDL		Low Density Lipoproteins	Quantitative, Ratio Less than 100 mg/dl	Registration form. Blood analysis

C VLDL		Very Low Density Lipoproteins	Quantitative, Ratio Less than 130 mg/dl	Registration form. Blood analysis
CPK	Enzyme that acts mainly in muscle tissue, brain and heart.	A test that measures the amount of creatinine kinase in the blood is used to diagnose and follow up on muscle injury or damage.	Quantitative, Ratio Muscle damage value less than 200 IU/l	Registration form. Blood análisis
Prot. C Reactive	Circulating plasma protein that increases in levels in response to inflammation, is directly related to IL-6 elevation.	Acute phase reactant protein released into the plasma as a consequence of inflammation.	Quantitative, Ratio less than 10 mg/l	Registration form. Blood análisis
TSH	Thyroid profile is the set of determinations of hormone levels related to thyroid function.	Thyroid stimulating hormone	Quantitative, Ratio 0.3 to 3 IU	Registration form. Blood análisis
T3		Triiodothyronine	Quantitative, Ratio 0.8 to 2 ng/ml	Registration form. Blood analysi
T3 L		Non-protein-bound triiodothyronine	Quantitative, Ratio 2.3 to 4.4 pg/ml	Registration form. Blood análisis
T4		Thyroxine	Quantitative, Ratio 5.4 to 11.5 ng/dl	Registration form. Blood analysis

T4		Thyroxine	Quantitative, Ratio 0.75 to 1.85 ng/dl	Registration form. Blood analysis
Vitamin D	Nutritional co-factor necessary for calcium absorption	cholecalciferol is measured in blood as 25-OH Vit D	Quantitative, Ratio 25 to 90 ng/ml	Registration form. Blood analysis
Gasometric Variables				
PaO2	Partial pressure of oxygen is the measurement of dissolved oxygen particles in the blood expressed in mmHg.	Determine the amount of dissolved oxygen in plasma.	Quantitative, Ratio Unit mmHg Minimum 75 Maximum 100	Registration form. Blood analysis
HCO3	Bicarbonate ion plasma concentration	amount of bicarbonate in blood	Quantitative, Ratio Unit mEq/l Minimum 21 Maximum 30	Registration form. Blood analysis
Arterial pH	Indicates the amount of hydrogenions contained in the blood.	Determines the degree of acidity or alkalinity of the blood.	Quantitative, Ratio minimum 7.35 maximum 7.45	Registration form. Blood analysis
Lactic Acid	Substance produced by muscle tissue and blood cells. Lactic acid levels increase when oxygen levels decrease.	Acidic substance that accumulates in the blood and causes a decrease in pH, its ionized form is lactate.	Quantitative, Ratio 4.5 to 19 mg/l or 0.5 to 2.2 mmol/liter.	Registration form. Blood analysis
Anion Gap	Difference between anions and cations measured in the serum	Difference between anions and cations measured in serum	Quantitative, Ratio unit mEq/l minimum 8 max. 12	Registration form. Blood analysis
Excess Base	the amount of acid required to return the pH of an individual's blood to a normal value	the amount of acid required to return the pH of an individual's blood to a normal value	Quantitative, Ratio Minimum -2 maximum + 2	Registration form. Blood analysis

Satisfaction and Adherence				
Satisfaction Survey	Positive assessment of the different dimensions of the medical care received	Interrogation Very Satisfied to Very Dissatisfied	Categorical Nominal	Registration form.
Adherence to treatment	Taking medication according to the dosage of the prescribed schedule; and persistence, taking medication over time.	Interrogation Complied or did not comply with the program.	Categorical Nominal	Registration form. adherence to treatment

7.7 Data collection.

All data will be captured and recorded digitally in each of the queries through the Google Forms platform to consolidate the information in Google Sheets.

In the consultation, medical history data will be collected in the form of interrogation, weight will be determined through electronic scales, height through calibrated stadiometers, All measurements will be performed according to the WHO Physical Measurement Guide 2014 (www.who.int/surveillance /steps/Part3_Section4) Annex 1. In addition, a capillary determination of glucose and ketones will be performed at each clinical consultation. Once patients are drawn by lot they will be assigned to one of the control centers distributed in Mexico City, they will always have to go to the same control center (VIME). The consultation visits will be weekly for weight control and clinical check-up and every two weeks alternately for physical and psychological counseling.

Blood samples will be collected in Specialized Clinical Laboratories according to NOM-253-SSA1-2012 and the laboratory sample collection manual in force in each laboratory. A venous sample will be taken to determine Complete Blood Biometry, Blood Chemistry of 27 elements, Basic thyroid profile, Glycosylated

7.8 Measuring instruments.

With all the data, a database will be built in Excel MS ®. Patient satisfaction will be measured with the Likert Scale, which consists of 5 possible answers per item: very satisfied, satisfied, indifferent, dissatisfied and very dissatisfied. (attached)

7.9 Statistical analysis.

Descriptive statistical tests will be performed establishing general and group measures, determining variability measures such as standard deviation and Confidence Interval, comparative inferential tests will be performed within and 25 between groups, for quantitative variables it is proposed as an initial T-test, which will be contrasted according to the conditions of normality and homoscedasticity of the sample. For qualitative variables, *X2* is initially proposed, which will be contrasted according to the same conditions; one-way ANOVA and repeated measures ANOVA will also be used. Microsoft Excel 365 and SPSS V 21

7.10 Description of treatments

7.10.1 Low-fat normoprotein Controlled Ketogenic Diet.

Patients randomly assigned to this group will undergo a standardized weight loss program under a nutritional intervention, physical exercise plan and emotional support.

The nutritional intervention is based on commercial preparations adjusted to 6 different stages.
Frank ketosis: the initial diet will consist of a very low calorie ketogenic diet (between 650 and 730 kcal/day) in 5 meal times, based on commercial and vegetable preparations with low glycemic index, an average of 1.2 g of protein/kg of ideal weight/day, 20 g/day of lipids based on essential fatty acids and less than 60 g/day of absorbable carbohydrates. Patients will also receive vitamin and trace element supplements (sodium chloride, magnesium oxide, calcium carbonate) to cover the daily requirements recommended for this type of diet. There is no stipulated time for this nutritional

intervention, the advance to the next stage is proposed as soon as the patient has lost 50% of his or her excess weight.

Mixed Ketosis: in this stage, one or two intakes of commercial preparations will be progressively replaced by proteins of natural origin (meat, fish, eggs, etc.), which will discreetly increase by 100 to 150 Kcal/day, without the patient 26.

Leaving the state of ketosis, the supplementation of vitamins and minerals will continue until the patient reaches 70% loss of excess weight.

Transition Stage: In this stage simple carbohydrates based on fruit and some complex carbohydrates in the form of cereals are added to the previous program, which allows the exit from the state of ketosis to enter a controlled hypocaloric diet of approximately 1300 to 1500 kcal/day to metabolically readapt to the consumption of macronutrients in an approximate proportion of 30 to 35% protein, 25% fat and 40 to 45 % carbohydrates. Patients will receive vitamin supplements to cover daily needs. Weight loss is not expected at this stage due to its short duration.

Integral and maintenance phase: during this stage the patient will follow a hypocaloric diet adapted to his measured energy expenditure, which may vary between 1300 and 2250 kcal/day, with a macronutrient distribution of 50% carbohydrates, 25% proteins and 25% fats, according to the Diogenes study (17), in this stage the process of dietary reeducation will be completed with a regimen in which the loss of 20 or 30% of the remaining excess weight is expected to be completed.

7.10.2 Hypocaloric Balanced Diet

Balanced hypocaloric diet (caloric intake 20% below basal metabolic expenditure measured by Multifrequency Bioelectrical Impedance or calculated according to the FAO/WHO/UN formula (FAO/WHO/UNU (1985). Energy and Protein requirements. Technical Report Series No 724, World Health Organization, Geneva). The usual caloric intake of a balanced hypocaloric diet is between 1,200 and 1,400 kcal per day with a macronutrient distribution of 50% carbohydrates, 25% proteins and 25% fats, according to the Diogenes study (17). 27

8. ETHICAL CONSIDERATIONS

In the present project, the ethical principles for medical research on human beings, established in the Declaration of Helsinki of the World Medical Association, in the General Health Law (Official Gazette of the Federation, 2015), in the Regulations of the General Health Law on Health Research and in the Mexican Official Standard NOM-012-SSA3-2012, will be specifically addressed. The research is considered with minimal risk according to the Regulation of the General Health Law on Health Research. It will be submitted to the Research Committees of the Institute of Health Sciences, and to the Research Ethics Committee. The informed consent process will be carried out by the researcher responsible for the project, requesting the patient's authorization for his/her participation. In the process of this project it is considered that the benefit will be greater than the risk, as well as the confidentiality of the patients will be kept at all times.

8.1. Informed Consent

Prior to inclusion in the study, all investigators must provide the patient with information about the study by means of the Patient Information Sheet (Appendix), propose participation in the study, answer any questions and ask the patient to fill in the Informed Consent Form (Appendix), which will be kept in the patient's own file.

8.2. Evaluation by an Ethics Committee

All materials from this study will be submitted to an accredited Clinical Research Ethics Committee (IRB) for evaluation. The study will be initiated once IRB approval has been obtained.

8.3. Confidentiality

The study data will be entered into an automated file owned by the promoter. The analysis of the results of the study will be made from the dissociated database, without personal data, so that no subject can be identified or identifiable.

9. Resources

9.1 Material and Financial Resources Zele Products	$2,484,000.00
Appointments	$729,000.00
In.Body	$510,000.00
Computer Equipment	$75,000.00
Dynamometers	$2,260.00
Lab tests	$572,184.00
Diagnostic reagents	$35,000.00
Other	$10,000.00
Total	$4,417,444.00

9.2 Human Resources

In addition to the researchers, 4 clinical nutritionists, 4 psychologists, 4 physical activity advisors, and a group of external auditors from the Instituto Tecnológico de Estudios Superiores de Monterey will participate in the study.

10. Timeline (Gant scheme)

	Meses 2021										
Activity	Jun	Jul	Ago	Sep	Oct	Nov	Dic	Ene	Feb	Mar	Abr
Writing and revision	■										
Financial approval	■										
Research Committee		■									
Ethics Committee		■									
Staff training		■	■								
Convocatoria - Call for proposals		■									
Participant recruitment			■	■							
Protocol			■	■	■	■	■	■	■		
Partial data capture and analysis			■	■	■	■	■	■	■	■	
Follow-up analysis								■	■	■	■
Initial results						■					
Writing the first paper							■				
Publication of initial results								■			

11. Bibliography.

1. Organización Mundial de la Salud. Obesidad y Sobrepeso. Disponible: https://www.who.int/es/news-room/fact-sheets/detail/obesity-and-overweight. Último acceso: 20 enero 20201.
2. Regional Overview of Food Insecurity Latin America and the Caribbean. 2015. http://www.fao.org/3/a-i4636e.pdf
3. D.R. Secretaría de Salud. México Gobierno de la República. Estrategia Nacional para la Prevención y el Control del Sobrepeso, la Obesidad y la Diabetes 2018
4. Cano-Rodríguez I et al. Dietas bajas en hidratos de carbono frente a dietas bajas en grasas. Endocrinol Nutr 2006;53:209-17
5. WHO. Obesidad y sobrepeso. Nota descriptiva N°311. Enero 2015. http://www.who.int/mediacentre/factsheets/fs311/es/
6. Seis estrategias para la perdida de peso. Jun 19, 2020. https://www.mayoclinic.org/es-es/healthy-lifestyle/weight-loss/in-depth/weight-loss/art-20047752.
7. Pirozzo S, et al. Should we recommend low-fat diets for obesity? Obes Rev 2003; 4: 83-90.
8. Lindner PG and Blackburn GL. Multidisciplinary approach to obesity utilizing fasting modified by protein-sparing therapy. Obesity/Bariatric Med. 1976; 5:6 //Blackburn GL, Greenberg I. Multidisciplinary approach to adult obesity therapy. Int J Obes. 1978;2(2):133-42.
9. Dessanzo V et al. Morbid Obesity. An integral, intensive and ambulatory approach. Results after 3 years in the treatment of extreme obesity. Int J Obes. 2002; 26, S101:381.
10. Tschochner et al. Body weight and life-style modification after a weight reduction program. Int J Obes. 2002; 26, S101:384
11. Elfhag K, Rössner S. Initial weight loss is the best predictor for success in obesity treatment and sociodemographic liabilities increase risk for drop-out. Patient Educ Couns. 2010 Jun;79(3):361-6.
12. Handjieva-Darlenska T, Handjiev S, Larsen TM, van Baak MA, Jebb S, Papadaki A et al. Initial weight loss on an 800-kcal diet as a predictor of weight loss success after 8 weeks: the Diogenes study. Eur J Clin Nutr. 2010 Sep;64(9):994-9.
13. Handjieva-Darlenska T1, Holst C, Grau K, Blaak E, Martinez JA, Oppert JM et al. Clinical correlates of weight loss and attrition during a 10-week dietary intervention study: results from the NUGENOB project. Obes Facts. 2012;5(6):928-36.
14. Nackers LM1, Ross KM, Perri MG. The association between rate of initial weight loss and long-term success in obesity treatment: does slow and steady win the race? Int J Behav Med. 2010 Sep;17(3):161-7.
15. Blackburn GL. Weight of the nation: moving forward, reversing the trend using medical care. Am J Clin Nutr. 2012 Nov;96(5):949-50.
16. Hemmingsson E1, Johansson K, Eriksson J, Sundström J, Neovius M, Marcus C. Weight loss and dropout during a commercial weight-loss program including a very-low-calorie diet, a low-calorie diet, or restricted normal food: observational cohort study. Am J Clin Nutr. 2012 Nov;96(5):953-61.
17. Larsen TM1, Dalskov SM, van Baak M, Jebb SA, Papadaki A, Pfeiffer AF et al. Diets with high or low protein content and glycemic index for weight-loss maintenance. N Engl J Med. 2010 Nov 25;363(22):2102-13