University of Guadalajara
Health Science University Center

Department of Physiology

# STUDY PROTOCOL AND STATISTICAL ANALYSIS

## Appendix 1: Informed consent form

PROTOCOL TITLE

**EFFECT OF FUCOXANTHIN ON METHABOLIC SYNDROME, INSULIN SENSITIBITY AND INSULIN RESISTANCE**

**(English version)**

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**PROTOCOL TITLE:**

# EFFECT OF FUCOXANTHIN ON METHABOLIC SYNDROME, INSULIN SENSITIBITY AND INSULIN RESISTANCE.

## BACKGROUND

Metabolic syndrome (MetS) is a constellation of risk factors, which mainly includes abdominal obesity, hyperglycemia, dyslipidemia, and elevated blood pressure (BP), which predispose the individual to developing type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD).[1] Together, these two pathological entities are responsible for 30% of deaths in Mexico.[2]

It is estimated that the global prevalence of MetS is between 20 and 25% in the adult population.[3] Despite the fact that in other countries the prevalence of MetS has been maintained or reduced thanks to the reduction of dyslipidemia, atherosclerosis and arterial hypertension, in Mexico MetS has increased, in response to the increase in the prevalence of obesity and the related comorbidities.[4] The most recent estimate of the prevalence of MetS in the Mexican population is 54.0%, however, this value may vary depending on the diagnostic criteria used.[5]

There is still no consensus that defines the criteria for the appropriate diagnosis of MetS. Organizations and authorities in health have proposed different diagnostic criteria, but most agree in considering body weight, lipid profile, BP, and blood glucose levels among the evaluation variables. The differences between these criteria lie in the parameters established for their verification.[6]

MetS is of multifactorial etiology, among them, lifestyle is the predominant factor.[2] The most accepted pathophysiological cause is the development of insulin resistance (IR).[7] The most accepted IR mechanism suggests that increased pancreatic β cell activity added to a proinflammatory state results in alterations in glucose regulation and lipid metabolism; however, the interaction between IR and the other components of the MetS is still under study.[1]

Changes in lifestyle such as increased physical activity and reduced calories in the diet decrease body weight and improve the lipid profile, which is why it represents the first line of treatment.[3] Pharmacological therapy constitutes the second line and may include hypoglycemic, lipid-lowering, antihypertensive, and weight-reducing drugs, subject to the components of the MetS that are altered.[1]

Currently, pharmacological therapy for MetS focuses on treating each risk factor separately, but currently much of the current research is directed towards the development of nutritional pharmacological therapies that intervene in more than one factor simultaneously, such as the use of phytopharmaceuticals, nutraceuticals and other natural therapies capable of modifying more than one component of MetS simultaneously.[8,9]

Fucoxanthin is a phytopharmaceutical of marine origin, it is the most abundant of the group of carotenoids to which anti-inflammatory, antioxidant, anti-obesity and anti-diabetic biological properties are attributed. These effects have attracted the attention of the food industry due to their unique mechanism of action. Animal and human studies confirm that fucoxanthin supplementation may be beneficial in the treatment of MetS.[10]

Seaweed is a vital component in the diet of the populations of Southeast Asia, its nutritional characteristics have been well studied, however, some of its most important components such as fucoxanthin have become popular for their activities in the treatment of obesity, T2DM and anticancer properties.[11]

### Preclinical background

An experimental study conducted in 2010 by Woo et al. evaluated the effect of fucoxanthin supplementation in groups of 10 C57BL/6N mice for six weeks on a high-fat diet on plasma, liver, and fecal lipids, and blood glucose and HbA1c levels. The results show that the plasma TG concentration was significantly lower in the

group receiving fucoxanthin (1.10 ± 0.02 nmol/L vs 1.33 ± 0.08 nmol/L, $p < 0.05$), accompanied by a concomitant increase in fecal TG (85.13 ± 8.58 µmol/g vs 26.44 ± 3.36 µmol/g, $p < 0.05$), which could suggest an inhibition in lipid absorption from the diet. Plasma HDL-C concentrations were markedly higher in the group receiving fucoxanthin (0.67 ± 0.01 nmol/L vs 0.56 ± 0.04 nmol/L, $p < 0.05$). A significant decrease in plasma glucose and HbA1c (3.28 ± 0.10% vs 4.81 ± 0.08%, $p < 0.05$), insulin concentrations (777.09 ± 84.59 pg/mL vs 388.62 ± 53.84 pg /mL, $p < 0.05$) and resistin (1.87 ± 0.14 ng/mL vs 2.54 ± 0.08 ng/mL, $p < 0.05$) were reported in the same group. According to this study, fucoxanthin could play a key role as a lipid-lowering and hypoglycemic agent.[12]

Later, in June 2010, Jeon S.M. et al. performed a similar study in groups of 10 C57BL/6N mice with an extract of algae (Undaria pinnatifida) with 3.5% fucoxanthin with a high-fat diet. In addition to the effects on lipid metabolism; body weight and adipose tissue were evaluated. Differences were reported in TG levels (1.0 ± 0.1 mmol/L vs 1.3 ± 0.1 mmol/L, $p < 0.05$), total cholesterol (2.1 ± 0.1 mmol/L vs 1.5 ± 0.1 mmol/L, $p < 0.05$), body weight gain (0.28 ± 0.1 g/day vs 0.19 ± 0.01 g/day, $p < 0.05$) and significant reduction in white adipose tissue (WAT) compared with a control group. These results confirm its activity on TG and cholesterol levels reported by Woo, likewise, it indicates a significant reduction in body weight and white adipose tissue.[13]

The study by Lin et al., published in 2017, where the effect of fucoxanthin and low molecular weight fucoidan was compared in a C57BLKS/J mouse model with induced DM2 ($n = 8$), reported a significant increase in the levels urinary glucose only in the group in which both components were used simultaneously. In addition, a significant difference was indicated in the HOMA-IR index (130.02 ± 26.39 vs 93.88 ± 23.48, $p < 0.05$), a marginal difference in the HOMA-β, (97.69 ± 14.28 vs 108.02 ± 20.59, $p > 0.05$) and a difference in glucose levels (475.1 ± 73.0 mg/dL vs 381.6 ± 57.7 mg/dL, $p < 0.05$) and plasma insulin (110.0 ± 10.1 µU/mL vs 96.8 ± 16.5 µU/mL, $p < 0.05$) compared to a control group . These results suggest that fucoxanthin can modify insulin sensitivity and insulin secretion. [15]

Other preclinical studies conducted between 2011 and 2017 in which rodents were used to determine the activity of fucoxanthin on lipid metabolism, reduction in body weight and adipose tissue, and glycemic control, obtained comparable results, which revealed details of the mechanisms involved in its effects.[15,16]

## Clinical trials

Clinical study of its effects in humans is scarce. One of the most relevant was carried out in Russia, by Abidov et al. in 2010, in which the effect of Xanthigen [supplement containing 2.4 mg fucoxanthin and 300 mg pomegranate seed oil (ASG)] was evaluated on body weight, body fat, and lipid profile in premenopausal, obese women and with nonalcoholic fatty liver disease (NAFLD) and women without liver disease (SEH) ($n=36$) after 16 weeks of dosing. Demonstrated reduced WC (110.6 ± 1.6 cm vs 105.0 ± 5.6 cm, $p < 0.05$) in the NAFLD group, decreased body weight in the NAFLD group (94.1 ± 2.1 kg vs 87.2 ± 3.7 cm, $p < 0.05$) as in the group with SEH (94.5 ± 1.5 kg vs 88.2 ± 1.9 cm, $p < 0.05$). Serum TG concentration was reduced in women with NAFLD (195 ± 19 mg/dL vs 158 ± 21 mg/dL, $p < 0.05$), in addition, this same group reported a reduction in SBP (138 ± 6 mmHg vs 119 ± 6 mmHg, $p < 0.05$) and DBP (91.0 ± 4 mmHg vs 79 ± 3 mmHg, $p < 0.05$). Similarly, the activity of the Xanthigen components separately on resting energy expenditure (REE) in women with NAFLD was evaluated. Women who received > 1.6 mg of fucoxanthin significantly increased GEE compared to the placebo group, finding a greater effect in the group that was administered 8 mg (6.01 ± 0.19 kJ/24 h vs 7.37 ± 0.35 kJ/24 h, $p < 0.001$). [17] The increase in GEE could be related to the activity of fucoxanthin on UCP1, demonstrated in experimental studies.

Teas et al, in 2009, carried out a controlled clinical trial in which they used a lyophilized extract of the algae Undaria pinnatifida in patients with MetS ($n = 14$). 6 g/day of extract were administered for four weeks, which contained 12 mg of fucoxanthin among other nutritional components, in the results a significant reduction in CC was observed (94.2 ± 5.1 cm vs 91.0 ± 6.2 cm, $p < 0.05$). and in the SBP figures (128.1 ± 8.4 mmHg vs 117.6 ± 7.7 mmHg, $p < 0.05$) in the group that received the intervention. [18]

In 2012, Hashimoto T. et al. conducted a trial in healthy subjects, in which they administered 31 mg of fucoxanthin contained in an extract of Wakame seaweed (Laminaria japonica). The aim of this study was to obtain information on the pharmacokinetics of fucoxanthinol, the major metabolite of fucoxanthin, in humans. During the analysis of this study it is indicated that amaurocyaxanthin A (fucoxanthin metabolite

detected in other experimental studies) was not detected in the blood of any participant. On the other hand, Fucoxanthinol appears to be the most active metabolite in humans; It was determined that its maximum concentration, time to maximum concentration, and half-life were 44.2 nM/mL, 4.0 and 7.0 hours, respectively. This study suggests that 31 mg (0.52 mg/kg body weight) is safe and sufficient to induce health benefits. [19]

A clinical trial carried out in Japan and published in 2017 by Mikami N. et al., evaluated the effect of oil from the Akamoku algae (Sargassum borneri) enriched with fucoxanthin at a dose of 2.0 mg/day, administered for eight weeks in adults with normal and overweight (n = 20). In this study, a decrease in HbA1c (5.2 ± 0.1% vs 5.1 ± 0.1 p = 0.055) was observed in the group that received fucoxanthin, which was directly related to the serum level of fucoxanthinol. This study also determined that HbA1c levels decreased predominantly in subjects carrying the G/G allele of UCP1, than in carriers of A/A and A/G, which reveals that the activity on this protein is also present in human beings. humans. [20]

Also in 2017 Hitoe S et al. concluded a clinical trial conducted in Japan, in which the effect of fucoxanthin on weight, body fat, BP, and other biochemical parameters was examined in overweight patients (BMI ≥ 25 kg/m2) over a period of four weeks. (n=11). Two groups were studied in which 1.0 and 3.0 mg/day of fucoxanthin were administered, and a placebo group. The analysis reported a significant decrease in body weight (76.2 ± 3.2 kg vs 74.9 ± 3.1 kg, p > 0.05), BMI (26.9 ± 0.7 kg/m2 vs 26.4 ± 0.5 kg/m2, p > 0.05), CC ( 100.6 ± 1.5 cm vs 98.4 ± 1.1 cm, p > 0.05), visceral adipose tissue (22.3 ± 1.4 kg vs 21.5 ± 1.3 kg, p > 0.05), and SBP (118.8 ± 3.4 mmHg vs 114.2 ± 4.0 mmHg, p > 0.05), only in the group that received 3 mg of fucoxanthin. These results suggest that the anti-obesity effect is due to its activity in reducing visceral fat and corroborates the effect in reducing BP reported by Abidov in 2009. [21]

## RATIONALE

Metabolic syndrome (MetS) is a clinical condition with a multifactorial etiology and is a significant contributor to the development of atherosclerotic disease, T2DM, kidney disease, and other vascular complications.

As urbanization, food industrialization, sedentary lifestyles, and poor diets become more prevalent in the global population, mortality rates from MetS-related diseases are increasing, particularly in Western countries compared to the Eastern region. This discrepancy is associated with the daily consumption of marine algae, which are rich in nutrients and bioactive agents, in traditional Asian cuisine.

The initial treatment approach for MetS focuses on modifying risk factors such as obesity, physical inactivity, and unhealthy diets through lifestyle changes. While pharmacological treatments are available to control individual components of MetS, there is significant interest in developing new therapeutic approaches that target multiple components simultaneously, aiming to improve the cost-effectiveness and overall efficacy of treatment.

Fucoxanthin, a functional compound found in various species of marine algae traditional medicine. It has gained attention due to its beneficial effects on obesity, diabetes, and cancer. Animal studies have provided insights into its mechanism of action and safety; however, human research in this area is limited, and more studies are needed to establish the actual benefits of its consumption. The available information suggests favorable effects on weight control in overweight individuals, but its impact on glucose levels, lipid metabolism, and blood pressure appears inconsistent. Therefore, it is crucial to investigate the effects of fucoxanthin administration in patients with MetS.

Fucoxanthin represents an alternative worth studying, as it has shown anti-obesity, anti-diabetic, and antioxidant effects. It presents a natural and promising option for MetS therapy, but further research is required to establish its safety and effectiveness in this context.

## HIPÓTESIS

Fucoxanthin modifies components of the metabolic syndrome, insulin sensitivity, and insulin secretion.

## OBJECTIVE

To evaluate the effect of fucoxanthin on metabolic syndrome, insulin sensitivity and insulin secretion.

**Specific objectives**

- Determine and compare WC before and after the intervention with fucoxanthin and placebo.
- Determine and compare SBP and DBP before and after the intervention with fucoxanthin and placebo.
- Determine and compare TG concentration before and after the intervention with fucoxanthin and placebo.
- Determine and compare HDL-c concentration before and after the intervention with fucoxanthin and placebo.
- Determine and compare fasting serum glucose level before and after the intervention with fucoxanthin and placebo.
- Determine and compare insulin sensitivity before and after the intervention with fucoxanthin and placebo.
- Determine and compare the total secretion of insulin before and after the intervention with fucoxanthin and placebo.
- Determine and compare the first phase of insulin secretion before and after the intervention with fucoxanthin and placebo.

**Secondary objectives**

- Determine and compare body weight before and after the intervention with fucoxanthin and placebo.
- Determine and compare BMI before and after the intervention with fucoxanthin and placebo.
- Determine and compare body fat percentage before and after the intervention with fucoxanthin and placebo.
- Determine and compare insulin concentration before and after the intervention with fucoxanthin and placebo.
- Determine and compare post-load glucose concentration after oral administration of 75 g of glucose before and after the intervention with fucoxanthin and placebo.
- Determine and compare total cholesterol (TC), LDL cholesterol (LDL-c), and VLDL before and after the intervention with fucoxanthin and placebo.
- Determine and compare AST and ALT levels before and after the intervention with fucoxanthin and placebo.
- Determine and compare creatinine and uric acid levels before and after the intervention with fucoxanthin and placebo.
- Describe the tolerability of treatment with fucoxanthin and placebo.

## MATERIALS AND METHODS

### Design

Randomized, double-blind, and placebo controlled clinical trial.

### Universe

Men and women aged 30 to 60 with a diagnosis of MeS, residing in the metropolitan area of Guadalajara.

### Sample

The sample size was calculated using a formula for the difference in means in clinical trials. A confidence level of 95%, a power of 80%, the standard deviation, and the expected difference obtained from the response variables in patients with metabolic syndrome were considered.

$$n = 2\left(\frac{(Z_\alpha - Z_{1-\beta})(\sigma)}{\delta}\right)^2$$

- Zα corresponds to the value of statistical confidence, which was pre-set at a 95% confidence level, for a type I error of 5% expressed as a two-tailed α value of 0.05. For this confidence level, the Z value was 1.96.

- Zβ represents the statistical power and was pre-set at the Z1-β value of 80% for a type II error of 20%, which corresponds to a β value of 0.20. For this statistical power, the standardized Z score was 0.842.
- σ is the standard deviation of waist circumference (WC), insulin sensitivity, and insulin secretion in patients with MetS.
- δ is the expected difference in waist circumference, insulin sensitivity, and insulin secretion in patients with MetS.

A total of 28 patients, 14 for each study group, was the result obtained with the highest number of required patients, including an additional 20% to anticipate possible loss to follow-up.

## Criteria

Inclusion criteria

- Age between 30 and 60 years
- Diagnosis of metabolic syndrome (MetS) according to the IDF criteria:
  - Waist circumference (WC) ≥ 80 cm in women or ≥ 90 cm in men, plus two or more of the following elements:
    - Systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg
    - Triglycerides (TG) ≥ 150 mg/dL
    - HDL cholesterol (C-HDL) < 40 mg/dL in men or < 50 mg/dL in women
    - Fasting plasma glucose (FPG) ≥ 100 mg/dL
- BMI between 25.0 and 34.9 kg/m²
- No pharmacological treatment for the components of MetS
- Stable weight during the last three months (weight variation less than 10%)
- Female participants declared the use of a contraceptive method to prevent pregnancy during the study period
- Signed written informed consent form

Exclusion criteria

- Women who are pregnant or breastfeeding
- History of kidney, thyroid, or liver disease
- Consumption of medications or supplements with proven properties that modify the behavior of metabolic syndrome
- Glucose ≥ 126 mg/dL or diagnosis of type 2 diabetes mellitus
- Total cholesterol (TC) > 240 mg/dL
- Triglycerides (TG) > 500 mg/dL

Elimination criteria

- Non-compliance of medication intake exceeding 20%
- Intolerance to any of the intervention ingredients
- Meeting an exclusion criterion during the study
- Experiencing a serious adverse event
- Withdrawal of consent

## Outcome measures

*Primary outcome measures*

- WC
- DBP
- SBP
- TG
- HDL-c
- Glucose
- Insulin sensitivity

- First-phase insulin secretion
- Total insulin secretion

*Secondary outcome measures*

- Body weight
- BMI (Body Mass Index)
- Body fat percentage
- Lipid profile (Total cholesterol, LDL-c, VLDL)
- Post-load glucose
- Insulin levels
- Liver enzymes (ALT, AST)
- Creatinine levels
- Adverse events

## Measurement and evaluation procedures

### Demographic determinations

• Age: Expressed in completed years at the start of the intervention.
• Sex: Referring to the phenotypic sex at birth, it was recorded as male or female.

### Clinical determinations

- *WC:* It will be measured using a flexible measuring tape with the subject standing. The tape was placed parallel to the ground and without clothing at the measurement level. The tape was positioned at the midpoint between the costal margin and the iliac crests at the end of a normal exhalation.
- *Weight:* It will be measured with the participant standing in a bipedal position, wearing light clothing, without shoes, and with an empty bladder prior to measurement. A stationary bioelectrical impedance scale will be used.
- *BMI:* It will be obtained by calculating the ratio of body weight in kg to the square of height in meters.
- *Body Fat:* It was determined using scale that utilizes the bioelectrical impedance method. Through a low-frequency signal, it calculates body fat and body composition.
- SBP/DBP (Systolic Blood Pressure/Diastolic Blood Pressure): It was determined using a digital sphygmomanometer. The participant was in a seated position after at least 5 minutes of rest. The cuff will be placed 3 cm above the elbow fold of the left arm. Two measurements will be taken to obtain the average blood pressure.

### Clinical laboratory procedures

*Oral Glucose Tolerance Test (OGTT)*

To perform this test, each patient will be administered an oral solution containing 75 g of anhydrous dextrose in 300 mL of water after fasting. By temporarily placing a peripheral venous catheter, 5 mL of blood will be drawn into a sterile dry tube at 0, 30, 60, 90, and 120 minutes after the administration of dextrose.
The sample extraction procedure will be performed using sterile and disposable materials. The sample will be allowed to clot at room temperature, and then centrifuged at 3500 rpm for 15 minutes. The supernatant serum will be collected for subsequent clinical chemical analysis.

*Laboratory determinations*

The laboratory determinations will be performed using simple enzymatic-colorimetric techniques. For this purpose, the Erba Mannheim® XL100 automated clinical chemistry analyzer will be used, operated by a single chemist assigned to this study. The equipment will be routinely calibrated according to the laboratory logbook.

- *Glucose:* Glucose Oxidase/Peroxidase Method. The glucose present in the sample reacts with glucose oxidase, producing hydrogen peroxide, which then condenses with aminoantipyrine and phenol in the presence of peroxidase, forming the quinonimine chromogen. The chromogen is quantified in

proportion to the glucose concentration (COD 11538 Reagent, Biosystems S.A.). (Intra-assay coefficient of variation [CVa] = 0.6%, inter-assay coefficient of variation [CVe] = 0.8%)

- *TG:* Glycerol Phosphate Oxidase/Peroxidase Method. The triglycerides (TG) present in the sample are hydrolyzed by lipase, resulting in the formation of glycerol and fatty acids. In the presence of ATP and glycerol kinase, glycerol-3-phosphate is synthesized, which is then oxidized to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide, along with chlorophenol and aminoantipyrine, condenses in the presence of peroxidase, releasing the quinonimine chromogen. The complex is quantified in proportion to the triglyceride concentration (COD 11529 Reagent, Biosystems S.A.). CVa = 1.4%, CVe = 1.9%.
- *TC:* Cholesterol Oxidase/Peroxidase Method. Both free and esterified cholesterol in the sample are catalyzed by cholesterol esterase and cholesterol oxidase enzymes. The resulting product, hydrogen peroxide, along with chlorophenol and aminoantipyrine, condenses in the presence of peroxidase, releasing the quinonimine chromogen. The complex is quantified in proportion to the cholesterol concentration (COD 11505 Reagent, Biosystems S.A.). CVa = 1.7%, CVe = 2.1%.
- *HDL-c:* VLDL and LDL-c present in the sample precipitate in the presence of phosphotungstate and magnesium ions. The supernatant contains high-density lipoproteins (HDL), whose cholesterol is quantified using the coupled reactions described above in the CT section (COD 11557 Reagent, Biosystems S.A.). CVa = 1.6%, CVe = 2.0%.
- *AST:* Aspartate aminotransferase catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxaloacetate and glutamate. The catalytic concentration is determined by using the coupled reaction with malate dehydrogenase (MDH), based on the rate of disappearance of NADH, measured at 340 nm (COD 11531 Reagent, Biosystems S.A.). CVa = 0.5%, CVe = 1.4%.
- A*LT:* Alanine aminotransferase catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined using the coupled reaction with lactate dehydrogenase (LDH), based on the rate of disappearance of NADH, measured at 340 nm (COD 11531 Reagent, Biosystems S.A.). CVa = 0.6%, CVe = 2.0%.
- *Creatinine:* Jaffé Method. The creatinine present in the sample reacts with picrate in an alkaline medium, resulting in a colored complex (Jaffé method). The rate of formation of this complex is measured in short initial periods to reduce interference from other compounds. Serum and plasma samples contain proteins that react non-specifically; however, the results can be corrected by subtracting a fixed value. This correction is known as the compensated Jaffé method (COD 11531 Reagent, Biosystems S.A.). CVa = 0.4%, CVe = 1.0%.
- *Insulin:* For the determination of insulin, the enzyme-linked immunosorbent assay (ELISA) sandwich method was used. In this technique, samples and the conjugate reagent (biotinylated anti-insulin and HRP) are added to a streptavidin-coated surface. Insulin in the serum binds to the corresponding antigen pair, forming a sandwich complex, which immobilizes on the surface. After the addition of a substrate, a chromatic reaction occurs proportional to the insulin concentration. CVa = 3.1%, CVe = 4.5%.

## Calculations

C-LDL and VLDL concentrations:[22]

Formula de Friedewald: $\text{C-LDL} = \text{CT} - \left(\text{C-HDL} + \frac{\text{TG}}{5}\right)$ $\text{VLDL} = \frac{\text{TG}}{5}$

Glucose and insulin AUC: The calculation was performed using the mathematical formula proposed by Tai..[23]

$$\text{Area} = \frac{1}{2}\sum_{i=1}^{n} x_{i-1}\,(y_{i-1} + y_i)$$

## Indices

Matsuda Index: It is used to calculate insulin sensitivity using the following formula.:[24]

$$\text{Matsuda index} = \frac{10000}{\sqrt{(\text{Gluc}_0 * \text{Ins}_0) * (\text{Gluc}_{\text{mean } 0-120} * \text{Ins}_{\text{mean } 0-120})}}$$

Stumvoll Index: It is used to calculate the first-phase insulin secretion using the following formula.:[25]

$$\text{Stumvoll index}=1{,}283 + 1.829 \times \text{Ins}_{30} - 138.7 \times \text{Gluc}_{0} + 3.772 \times \text{Ins}_{0}$$

Total insulin secretion: It is used to calculate the first-phase insulin secretion using the following formula.[26]

$$\text{Total insulin secretion} = \frac{\text{AUC Insulin}_{0-120}}{\text{AUC Glucose}_{0-120}}$$

Other determinations

*Adherencia al tratamiento*

Each participant will be given an adherence and monitoring diary in which they will be asked to record each intake of the intervention capsules. This diary is designed to record the date and time of each intake for a period of 12 weeks.

During each visit throughout the intervention, the patient will be asked to return the assigned intervention bottle containing any capsules that have not been taken, in order to calculate and estimate the adherence percentage.

*Adverse events*

The patient will record in the adherence and monitoring diary any symptoms or adverse changes in their health status during the medication intake period. A direct contact telephone number with the investigators will be provided to consult and report any unforeseen events.

During each visit throughout the intervention, the patient will be interviewed and physically examined, if necessary, in search of changes in health status that may be related to the consumption of the formulation's ingredients.

**Grupos de estudio y administración de la intervención**

Two groups of 14 patients each will be formed:

- Fucoxanthin: Each patient will receive a capsule containing 12 mg of fucoxanthin every 24 hours. They will ask to take one capsule with the first bite of breakfast for a duration of 12 weeks.
- Placebo: Each patient will receive a placebo capsule (Magnesium stearate) that is indistinguishable from fucoxanthin. They will be instructed to take one capsule with the first bite of breakfast for a duration of 12 weeks.

**Allocation of intervention and blinding**

The treatment assignment will be performed through simple randomization, using the method of a simple random number table. By using computer software, each intervention will be randomly assigned an identification with a numerical code corresponding to an identified intervention within Group A or Group B. The patient and the researcher will not be informed of which pharmacological intervention corresponds to each numerical code.

## DATA COLLECTION AND STATISTICAL ANÁLISIS PLAN

All the results will be collected in a digital database designed for this study, where no confidential personal information not related to the study variables will be recorded. The information entered in the database will be transferred for analysis in the statistical software IBM SPSS Statistics® for Windows®. The statistical analysis will be conducted at the end of the total data collection once the intervention in the included patients has finished. All patients will be included in the intention-to-treat analysis. In descriptive statistics, measures of central tendency and dispersion will be used. Quantitative variables will be presented with means and standard deviations. Qualitative variables will be presented with frequencies and percentages.

The Shapiro-Wilk test will be performed to determine the normality of the sample distribution in each variable. If the sample distribution is found to be abnormal, non-parametric tests will be used. The analysis between groups (independent samples) will be analyzed using the Mann-Whitney U test for quantitative variables, and the X2 test or Fisher's exact test for qualitative variables. Intragroup analysis (two related

samples) will be performed using the Wilcoxon rank test for quantitative variables. A value of $p \leq 0.05$ will be considered statistically significant.

## ETHICAL CONSIDERATIONS

The project of this study was submitted for evaluation by the Research Ethics Committee of the University Center of Health Sciences of the University of Guadalajara. The approval of this committee was obtained in the record registered with the number CIE/467/2018.This study is classified as a study with greater than minimal risk, for which the voluntary signature of an informed consent was requested from each participant, in accordance with the Regulations of the General Health Law on Research for Health, in its Second Title, chapter I, article 17. In accordance with the guidelines of good clinical practice, all study participants were uniquely identified by alphanumeric code in a digital database. The data is kept confidential, as are the answers to the questionnaires and the results of the clinical and laboratory tests, to guarantee the privacy of the participants.

The procedures adhered to ethical standards, and strict respect was maintained for the individual integrity of each participant. In accordance with the Regulations of the General Health Law on Research and the principles emanating from the 18th Medical Assembly of Helsinki, Finland in 1964 and the modifications made by said Assembly in Tokyo, Japan in 1975, in Venice, Italy in 1983, in Hong Kong in 1989 and the 64th General Assembly, Fortaleza, Brazil, October 2013, where medical research (clinical research) is contemplated.

## FOUNDING INFORMATION

Founding in this project is parallel to other studies of the University of Guadalajara.

## AUTHORS DISCLOSURE STATEMENT

The researchers and personnel involved in conducting this study declare that they have no conflicts of interest.

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# APPENDIX I: INFORMED CONSENT FORM

## Protocol: Effect of fucoxanthin on metabolic syndrome, insulin sensitivity and insulin secretion.

*This document could contain words or terms that you do not understand, please ask the doctor in charge of the study and your interview to explain it to you in case such a situation exists. Sign this consent form only until all your doubts are satisfactorily clarified and you are convinced that you want to participate in the study.*

### Purpose of the study

The purpose of this study is to evaluate the effect of fucoxanthin administration on metabolic syndrome, insulin secretion, and insulin sensitivity, since there are studies that relate these alterations to the development of cardiovascular disease and diabetes, which in the long run term predispose to the development of serious complications.

Various studies have shown that fucoxanthin consumption can reduce body weight, however, in the present investigation we also want to evaluate its effect on the other components of the metabolic syndrome, that is, lowering blood glucose levels, cholesterol, triglycerides and blood pressure figures. Therefore, we consider that he could be a good candidate to participate in this project.

Due to the above, you have been invited to participate in this study, since you present some of the following characteristics: high blood glucose, overweight or obesity, high blood pressure, cholesterol or elevated triglyceride levels.

Like you, 27 more people who meet the criteria will be invited to participate in this study. Your participation is completely voluntary. Please read the information we provide and ask any questions you want before deciding whether or not to participate.

### procedures

This study lasts for 12 calendar weeks (3 months), which includes at least 4 appointments. If you agree to participate, the following will occur:

**A. Procedures that are routine in the care of patients in this service:**

1) You will come here with us, fasting for more than 8 and less than 12 hours, at 8:00 am
2) Clinical Procedures: A clinical history will be taken that includes a complete physical examination with the measurement of your blood pressure, weight, height, waist, hips, heart rate, and respiratory rate.
3) Laboratory procedures: We will take a blood sample from one of your arms, approximately 5 milliliters, to carry out some laboratory studies. Subsequently, you will be given a drink containing 75 grams of glucose diluted in 300 milliliters of water, with the purpose of measuring your blood glucose after oral glucose ingestion and thus determine if you have glucose intolerance. In addition, the laboratory studies that we will carry out include the measurement of your fasting glucose level, creatinine, HbA1c and a lipid or fat profile. For your safety and hygiene, all the material used in this study is sterile and disposable, and at the end of the planned analysis, the rest of the sample will be destroyed.

The purpose of carrying out the clinical and laboratory studies is to learn more about your general health conditions and your glucose levels, both fasting and glucose at 2 hours after a load of 75 g of glucose. It will take us approximately 2 hours to perform these clinical and laboratory tests. We will deliver the results of your laboratory studies within the next 3 business days.
Once this consent letter is signed, and if you meet the criteria to enter the study, you will be scheduled to fast for another day at 8:00 am, where the following specific procedures will be performed.

**B. Procedures specific to this investigation:**

1) Nutrition counseling will be provided by trained personnel.
2) Laboratory tests will be taken at the first visit and at the end of the study to compare the levels of cholesterol, triglycerides and glucose that you obtain upon admission with those that you will have upon leaving the study.
3) At the beginning of the study, you will be assigned a treatment, like each participant, which may contain fucoxanthin or a placebo (inert substance without effects). The assignment will be made at random and neither you nor the researcher will know which drug the treatment corresponds to, which will be provided free of charge and in sufficient quantity during the 12 weeks of the study. You should take 1 capsule of the medicine once a day with the first bite of breakfast.
4) You will be given a booklet which will be your adherence to treatment diary, in which you must record each time you consume the medication, as well as the signs or symptoms that you present during the study.
5) The visits with us will be every 30 days, in which we will take your blood pressure, weight, and waist circumference to observe the evolution you have had with the medication. Likewise, you will be asked if the capsules have been taken every day, if there has been a lack of intake at any time, as well as possible adverse events with the treatment.
6) After 12 weeks of administration of the drug that is assigned, you will be scheduled to fast again at 8:00 a.m. and the same procedures will be carried out as in the initial assessment, including final laboratory tests, and you will be questioned again about taking of the medication and possible adverse effects that it has presented, likewise, you will give us the adherence diary that you used.
7) In a period no longer than 7 days after the end of the study, a clinical summary will be made that includes the results obtained from the clinical measurements and laboratory tests that have been carried out during the study, as well as recommendations to continue your treatment, the which he will present to his usual doctor or specialist, who will continue his management.

The information resulting from this research will help to know and improve treatment schemes for patients with metabolic syndrome. In this case, it will allow the medical community to recommend the use of fucoxanthin not only to reduce body weight but also to modify the aforementioned components of the metabolic syndrome.

**Potential risks and discomfort**

*Discomfort or risks associated with clinical evaluation procedures.*

The measurement of weight, height, blood pressure, waist circumference and percentage of body fat are non-invasive clinical determinations, they do not cause pain, discomfort or any risk.

*Discomfort or risks associated with laboratory procedures*

Discomfort during blood sampling is minimal. On some occasions the procedure to take a blood sample may cause a little pain or slight discomfort, it is possible that you may develop a bruise.

*Discomfort or risk related to the consumption of fucoxanthin*

Currently there is no scientific evidence that mentions adverse effects of any kind that can be produced by the medication, even so, consider that it could cause mild intestinal discomfort.

**Possible benefits you will receive from participating in the study**

You will not receive payment or economic remuneration for your participation in this study, likewise, this does not imply any expense.

A possible benefit of your participation in this study is that the results of the clinical and laboratory tests that we will carry out will provide you with information about your state of health, in addition, you will be offered advice for weight control, which will have the strict supervision of specialized personnel.

Although the direct benefits for you may not exist, the results of this study will contribute to the advancement of knowledge about the beneficial effects of these treatments in patients with metabolic syndrome, and the results will be relevant for the better management of people like you, Despite the above, you will be provided with the results and a clinical summary of what was done in the study, so that your family doctor can use it to modify and monitor your treatment.

**Results or new information about treatment alternatives**

During the course of this study, we will tell you about any new findings (either good or bad) that are important to the decision to continue or end your participation; For example, if there are changes in the risks or benefits of your participation in this research. If we provide you with new information, we will again ask for your consent to continue in this study.

**Participation or withdrawal**

Your participation in this study is completely voluntary. If you decide not to participate, there is no problem, you are free to decide to participate or not. For the purposes of this research, we will only use the information that you have provided us from the time you agree to participate until the time you let us know that you no longer wish to participate.

**Privacy and confidentiality**

The information you provide us that could be used to identify you (such as your name, phone number, and address) will be kept confidential and separate from your responses to questionnaires and the results of your clinical tests, to ensure your privacy.

The research team, the people involved in your health care, and your regular doctor will know that you are in this study. However, no one else will have access to the

information you provided to us during your participation in this study, unless you wish. We will only provide your information if it is necessary to protect your rights or well-being (for example, if you suffer bodily harm or need emergency care), or if required by law. When the results of this study are published or presented at conferences, for example, no information will be given that could reveal your identity. Your identity will be protected and hidden. To protect your identity we will assign you a password that we will use to identify your data and we will use that password instead of your name in our electronic databases.

**Contact staff for questions and clarifications about the study**

If you have questions or want to talk to someone about this research study, you can contact:

[redacted]

**Declaration of informed consent**

By signing this consent, I acknowledge that I have been informed about the methods and routes of administration of study drugs, the procedures and tests to which I will be subjected; as well as the inconveniences, benefits and inconveniences that may arise.
I certify that I have read (or someone has read to me) the contents of this consent form, that I have had sufficient time to understand it, that all technical language used in the description of this research study has been explained to my satisfaction, and that I received an adequate and understandable answer to all my questions, that I have received a copy of this document, which I will keep safe for future reference. I understand that I am free to withdraw from the study at any time without loss of benefit or penalty. I give my consent to be included in this study.

Name of patient: _____
Name( s) Paternal surname Maternal surname

Home: _____
Street Number Colonia ZIP Municipality

Phones) _____

_____ _____
Signature Date

**Statement of the person responsible for obtaining informed consent**

I declare that I have explained the research study to the participant and have resolved the doubts that they have raised. I believe that you understand the information described in this document and freely consent to participate in this research study.

| Name of person responsible for obtaining informed consent | Signature of person responsible for obtaining informed consent |
|---|---|

Date: _____

**Witness 1**

Witness name: _____
Name( s) Paternal surname Maternal surname

Home: _____
Street Number Colonia ZIP Municipality

Phones) _____

| Signature | Date |
|---|---|

**Witness 2**

Witness name: _____
Name( s) Paternal surname Maternal surname

Home: _____
Street Number Colonia ZIP Municipality

Phones) _____

| Signature | Date |
|---|---|