Official title:

EFFECT OF A NUTRIGENETIC INTERVENTION ON BLOOD LIPID MARKERS AND BODY COMPOSITION OF ADULTS WITH OVERWEIGHT AND OBESITY

STUDY PROTOCOL

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- **Hypothesis**

A nutrigenetic diet administered to adults with genetic polymorphisms related to obesity and dyslipidemias will provide a greater decrease in plasma lipids, inflammatory markers and improvement in body composition, compared to the administration of a standard diet.

- **Objective:**

To evaluate the effect of a nutrigenetic intervention on blood biochemical and inflammatory markers and body composition in overweight and obese adults.

- **Design:**

Study type: Interventional

- **Interventional Study Model:**

Randomized controlled clinical trial, parallel assignment, with 2 study groups.
Two arms.
Masking: Single (participant).
Intervention period: 8 weeks.
Research site: ITESO and Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, México.

- **Methods:**

Participants

The recruitment of the population was carried out from the month of May to the month of September of the current year in the CD of Guadalajara, Jalisco and neighboring municipalities. The dissemination of informative flyers was done digitally through various social networks and also verbally.

Inclusion criteria

-Men and women
-18-50 years old
-Mexican- mexican ancestry (3 generations at least)
-Live in Guadalajara, Jalisco or metropolitan area
-Being overweight or obese (BMI 25>40)
-Waist circunference:
Women: > 80 cm
Men: > 94 cm
-Availability to attend virtual nutritional consultations.

Exclusion criteria
-Not meeting the inclusion criteria
-Pregnancy or breastfeeding
-Gastrointestinal disorders
-Endocrinopathies
-Cardiovascular events
-Diagnosed psychiatric illnesses
-Diagnosed diabetes
-Take lipid-lowering drugs
-Autoinmune diseases
- Covid +

Dependent variables:
Height, body weight, BMI, total body fat, fat mass index, skeletal muscle mass, visceral fat, waist circumference, hip circumference, total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglycerides, C-reactive protein (CRP), glucose and insulin, total energy, macronutrients, micronutrients.
Independent variables:
Sex, age, presence or absence of polymorphisms, and intervention group.

INTERVENTION
All the subjects will give their written informed consent before inclusion in the study.
The present study was approved by the ethical committee for human research of Instituto Tecnológico y de Estudios Superiores de Occidente (ITESO) (0001DRC) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Randomization:
Participants will be randomly assigned, using a computerized random number generator (STATA software), to one of two treatments: nutrigenetic diet or conventional diet. Randomization will be stratified according to age, BMI and sex. The allocation ratio will be 1:1

Genotyping of gene polymorphisms related to dyslipidemias in Mexicans
Based on a previous systematic review, 11 gene polymorphisms associated with dyslipidemias in Mexicans were identified and were taken into account to perform genotyping in the study population.

The gene polymorphisms under study are:
ABCA1 rs9282541

PPARG rs1801282
LIPC rs1800588
CETP rs708272
LPL rs13702
FABP2 rs1799883
APOA5 rs662799
APOC3 rs5128
APOA1 rs670
APOE rs429358 and rs7412

A blood sample will be taken from the participants, then DNA will be extracted from leucocytes using a commercial extraction kit (High Pure PCR Template Preparation kit, *Roche).* Polymorphisms will be detected by a real-time RT-PCR system using allelic discrimination (TaqMan ®) in a 96-well format and will be read by a Roche LightCycler 96 system (Roche, Mannheim, Germany). PCR conditions will 40 cycles of amplification at 95°C for 10 min, and annealing/ extension at 60°C for 60 seconds.

The experimental phase will last 8 weeks.
Participants will attend 3 three visits to the clinical unit (Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara): on the starting day of the intervention period (baseline), after 4 weeks (1st month) and after 8 weeks of intervention (final visit). Participants will also receive remote nutritional counseling every 15 days (5 consultations in total).

Anthropometric measurements
Anthropometric parameters will be measured three times during intervention period (baseline, week 4, week 8).
Measurements will be performed after 8- 10 hours of fasting. Height measurements will be taken using a stadiometer (Rochester Clinical Research, Rochester, NY, USA). Tetrapolar electrical bioimpedance will be used to assess weight and body composition (InBody 120 body composition analyser; Biospace, Seoul, Korea). BMI will be calculated as weight in kilograms divided by height in meters squared (kg/m2). The waist (narrowest diameter between the xiphoid process and the iliac crest) and hip (the widest diameter over the greater trochanters) circumferences will be measured (Lufkin anthropometric tape W606PM). Blood pressure will be measured, while seated, in the non- dominant arm using a digital automatic BP monitor (Model M3 Intelligence, Omron, Healthcare Company, Limited).

Biochemical measurements
Biochemical measurements will be performed three times during intervention period (baseline, week 4, week 8).
Venous blood samples will be taken after overnight fasting, and serum will be immediately separated by centrifugation. Measurements of total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), glucose, very low density lipoprotein (VLDL) and C- reactive

protein will be performed using a Vitros-250 dry chemistry analyser (Ortho- Clinical Diagnostics, Johnson & Johnson Services, Inc., Rochester, NY, USA). LDL-C levels will be calculated using the Friedewald formula. The homeostatic model assessment of insulin resistance (HOMA-IR) will be calculated as described by Matthews et al. (1985). Insulin levels will be determined by an Immunoassay Kit (LIAISON®).
The inflammation markers IL-1, IL-6, IL-10 and TNF-α, will be determined using ProQuantum High-Sensitivity Immunoassays technique

Diets

As above-mentioned, patients will be randomly allocated to one of two diets for a period of 8 weeks.
NUTRIGENETIC DIET: Weekly, personalized meal plans (menus) will be provided every 2 weeks, prepared based on the anthropometric needs of the patients, with a caloric reduction (-500 kcal). These weekly menus (Sunday to Monday) consist of 5 meal times (breakfast, snack, lunch, snack and dinner). The distribution of the macronutrients: carbohydrates, proteins and fats, as well as the percentages of polyunsaturated, monounsaturated and saturated fatty acids, will be established according to certain nutrigenetic recommendations identified in the reference bibliography.
CONVENTIONAL DIET: weekly, personalized meal plans (menus) will be provided every 2 weeks, prepared based on the anthropometric needs of the patients, with a caloric reduction (-500 kcal). These weekly menus (Sunday to Monday) consist of 5 meal times (breakfast, snack, lunch, snack and dinner). The target macronutrient composition of this treatment is: 50% carbohydrates, 20% proteins and 30% fats in accordance with the recommendations made by the WHO (World Health Organization), the AHA (American heart association) and the NOM (Official Mexican Standards) for the treatment of obesity and dyslipidemias.

The percentages of macronutrients and fatty acids of all the menus will be adjusted using Foodprocessor nutrition software ®

All participants will receive remote (virtual) nutritional counseling every 15 days (5 individual sessions) with the dietitian to explain the diet, solve doubts, provide nutrition strategies and to complete the nutritional medical history, which includes physical activity record (IPAQ- short version) and questionnaires of frequency of food consumption to monitor adherence to nutritional intervention. Records will be analyzed with a computer-based data evaluation system (Foodprocessor nutrition software ®). National composition food tables will be also used as reference.