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Health Sciences University Center
INSTITUTE FOR TRANSLATIONAL NUTRIGENETICS AND NUTRIGENOMICS

Effect of Coffee Consumption on Appetite Traits in Overweight and Obese Woman

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. General information

a. Title

Effect of Coffee Consumption on Appetite Traits in Overweight and Obese Woman

b: Responsible researcher: Livier Nathaly Torres Castillo

2. Abstract

Background: There are studies suggesting that coffee could help in body weight control. The mechanisms by which coffee could help in weight control are unknown and it has been suggested that such effect is related to appetite control. In an investigation in which normal weight men participated, the effect of coffee consumption on the concentrations of peptide YY (an appetite suppressant) was analyzed and a higher concentration of this hormone was found 60 and 90 minutes after the ingestion of decaffeinated coffee, as well as a lower sensation of hunger during the 180 minutes after coffee consumption. By performing three different interventions: instant coffee with 3 mg/kg weight of caffeine, decaffeinated instant coffee and water (control), it was observed that, 180 minutes after the caffeinated coffee intervention, participants reported a lower desire to eat compared to the other two interventions. However, other studies have not found a decrease in energy intake and appetite sensations when consuming caffeinated coffee, decaffeinated coffee, or caffeine capsules in normal weight women. In spite of the scientific evidence, there are few studies regarding coffee and its effects on hunger and satiety and even fewer studies carried out in women with overweight or obesity.

Objective: To assess the effect of coffee consumption on hunger, satiety, appetite-regulating hormones, and dietary intake in women with overweight or obesity.

Methodology: This is a crossover randomized clinical trial. Subjective appetite is assessed through visual analogue scales, and appetite related hormones ghrelin and cholecystokinin are measured with ELISA kits. Diet intake is evaluated with 3-day food records and coffee and caffeine products frequency questionnaire. Biochemical and anthropometric variables are also measured.

Infrastructure: Is available the access to the “Instituto de Nutrigenética y Nutrigenómica” of the “Centro Universitario de Ciencias de la Salud” of the Universidad de Guadalajara. The institute has all the necessary equipment to perform biochemical determinations of appetite-regulating hormones (spectrophotometer, plate washer, plate shaker) and other analytes (centrifuge, 350 chemistry glassware, refrigerators at different temperatures, all the necessary consumables such as tips, tubes, cryo boxes, etc.). On the other hand, there is a room to carry on the session with the participants, give them breakfast, apply the visual analogue scales, and take the blood sample.

3. Background

There are studies that suggest that coffee could help in the control of body weight. To date, the mechanisms by which coffee could help in weight control are unknown and it has been suggested that such effect is related to appetite control (1). In an investigation in which men with normal weight participated, the effect of coffee consumption on the concentrations of peptide YY (an appetite suppressant) was analyzed and a higher concentration of this hormone was found in the 60 and 90 minutes after the ingestion of decaffeinated coffee, as well as a lower sensation of hunger during the 180 minutes after coffee consumption (2). In another study in men and women with overweight/obese, it was found that the consumption of coffee with 6 mg/kg weight of caffeine at breakfast resulted in a consumption of 550 kcal less during the rest of the day compared to the control group (simple water consumption), but no differences in hunger and/or satiety sensations were observed (3). By performing three different interventions: instant coffee with 3 mg/kg weight of caffeine, decaffeinated instant coffee, and water (control), it was observed that, 180 min after the caffeinated coffee intervention, participants reported a lower desire to eat compared to the other two interventions (4).

It remains uncertain which component of coffee influences appetite, but it has been observed that coffee abundant in chlorogenic acid has an effect on body composition. In a study conducted in overweight men and women who consumed instant coffee with 369 mg of chlorogenic acid and 37 mg of caffeine for 12 weeks, it was found a decrease in visceral fat area, total fat area, BMI, body weight and waist circumference, compared to coffee consumption with 35 mg of chlorogenic acid and 38 mg of caffeine (5). However, other studies have not found a decrease in energy intake, appetite sensations, or gastric emptying when consuming caffeinated coffee, decaffeinated coffee, or caffeine capsules (6); therefore, more studies are needed to clarify these effects.

Adding coffee as an extra ingredient of a food has shown effects on appetite. In a pilot study where the effect of cookies was analyzed, the main components were 5 grams of fiber and 1.9 grams of coffee grounds. It was observed that its consumption together with a breakfast of 1,160 kcal reduced appetite and food consumption at mealtime, hence, the researchers concluded that coffee grounds could have a positive impact on body weight control (7). Despite the scientific evidence, there are few studies regarding coffee and its effects on hunger and satiety and even fewer studies conducted in women with obesity.

4. Problem statement

The prevalence of obesity has increased in recent years. The World Health Organization (WHO) has expressed its concern about this increase since it is estimated that in 2016 about 13% of the world's adult population had obesity and in the last 40 years its global prevalence has almost tripled (8). Obesity negatively affects the physiological functions of the body and forms a major threat to public health as it increases the risk of developing multiple diseases such as diabetes mellitus, cardiovascular disease, several types of cancer, a variety of musculoskeletal disorders and poor mental health, all of which have negative effects on quality of life, work productivity, and healthcare costs (9,10).

In addition to these conditions, in obesity there is a reduced control of energy intake. The balance between energy intake and energy expenditure is controlled by complex neural networks within the central nervous system (CNS) that generate processes that inform about hunger and satiety, which are deregulated in people with obesity (11). Cholecystokinin (CCK), a hormone involved in these processes, is secreted by duodenal I cells and by central neurons in response to nutrient intake, mainly protein and lipid. On the other hand, in people with obesity, it has been observed that the ghrelin appetite suppression is affected and a dysregulated pattern in ghrelin secretion has been observed. Ghrelin is secreted by P/D1-type cells in the gastric fundus and increases appetite and food intake, but in people with obesity, reduced fasting ghrelin levels have been observed as a counter-regulatory mechanism for excess of body weight and, more importantly, subjects show uncontrolled postprandial ghrelin suppression (11).

In recent years, the effects of coffee consumption on the modulation of hunger, satiety, and energy intake have been studied. In this sense, it has been observed that this beverage has an effect in the reduction of appetite, therefore, this could help in weight control (12,13). Regarding the effects of coffee on hormones involved in hunger and satiety, there are few studies that evaluate ghrelin and CCK concentrations in response to coffee consumption. Two studies on the effects of coffee on ghrelin concentrations were found; in them it was reported that this beverage did not cause significant effects on ghrelin concentrations and there was no relationship with hunger (2,4); however, these studies were done only in men (14), so it is necessary to know its effect in women. Another study did find that ghrelin concentrations decreased with coffee consumption compared to the levels in the washout period of the study; however, the limitation of this study was that it did not include a control group (15). On the other hand, it has been reported that coffee induced the release of CCK (16), however, in this last study the effect on hunger or satiety was not measured. Coffee is a very popular beverage, and its worldwide consumption is estimated at 168 million 60-kilogram bags (17) and at the national level, Mexicans consume an average of two and a half cups a day (18). Nowadays, it is a challenge to find beverages that allow for successful nutritional interventions. Based on the above, the following research question has been proposed: What is the effect of coffee consumption on hunger, satiety, and appetite regulating hormones in women with overweight or obesity?

5. Rationale

In the last 40 years there has been an increase in overweight and obesity worldwide in children and adults. In 2016, 650 million people in the world presented obesity and it is estimated that if this continues by 2025, one in five adults worldwide will present obesity (19). In Mexico according to the latest National Health and Nutrition Survey of the Middle Way (ENSANUT-MC, 2018) the prevalence of obesity increased from 32.15% in 2012 to 35.35% in 2018, in addition 75% of the Mexican adult population presents excess weight. In the case of premenopausal women, the prevalence of obesity in the age range of 30 to 49 years was 89% (20). At the state level, Jalisco also showed an increase in the prevalence of obesity from 37.6% in 2012 to 39.8% in 2018 (20).

Obesity also imposes a significant cost on health systems. According to a study conducted by the Mexican Institute of Competitiveness, it was calculated that the total costs of diabetes along with obesity amount to be more than 85 billion pesos per year; of this, 73% corresponds to medical treatment expenses, 15% to loss of income due to absenteeism from work and 12% to loss of income due to premature mortality. The cost of various medical complications for a person with obesity and prediabetes, if he or she does not change his or her lifestyle habits, can reach 1.9 million Mexican pesos over 30 years. However, if the person modifies his or her lifestyle, in 30 years the total expenditure would decrease to only 92,860 Mexican pesos (10). Therefore, preventing and treating obesity early, as well as seeking and finding supplements that suit the patient to execute more effective nutritional interventions, has economic benefits for the individual patient and for the health system in general.

In the search of supplements to implement effective nutritional interventions to combat this problem, we found coffee. This beverage is highly consumed in Mexico since the average Mexican consumes two and a half cups a day (18). Recently, this beverage has caused great interest because there have been recent findings suggesting that coffee has an effect on appetite control which could help in weight control (3,4). Studying the effect of coffee on hunger and satiety in humans will contribute to generate knowledge for future nutritional treatments, will allow preventing the development of obesity, will provide a complementary option for a nutritional plan for patients who are already overweight or with obesity, and will be useful to give nutritional recommendations based on scientific evidence. According to our scientific search, so far only two studies have reported information on the effects of coffee in women with obesity. Both included the combination of overweight and obese women and did not measure any hormone involved in hunger or satiety, and in one of them they did not give coffee, but an extract of this beverage (3,13). For this reason, this study will be important to contribute to the knowledge gaps that exists regarding the effects of coffee on hunger, satiety, and appetite regulating hormones in women with overweight or obesity who are within one of the age ranges (30 to 49 years) with a high prevalence of obesity in Mexico (20).

6. Objective

a: General objective

To assess the effect of coffee consumption on hunger, satiety, appetite-regulating hormones, and dietary intake in women with overweight or obesity.

b: Specific objectives

1. To describe the demographic, anthropometric, biochemical, and dietary characteristics of the study sample.
2. To compare the sensations of hunger and satiety at times t=-30 min, t=0 min, t=30 min, t=60 min, t=90, t=120, t=150 min and t=180 min between the two interventions: coffee and simple water.
3. To compare the serum levels of cholecystokinin and ghrelin at times t=-30 min, t=0 min and t=180 min between the two beverage interventions (coffee and simple water).
4. To compare the change (t=180 - t=0) in hunger and satiety sensations between the two beverage interventions (coffee and simple water).
5. To compare the change (t=180 - t=0) in cholecystokinin and ghrelin concentrations between the two interventions (coffee and simple water).

7. Hypothesis

Coffee consumption decreases hunger and ghrelin, increases satiety and cholecystokinin concentrations, and changes energy intake in women with overweight or obesity.

8. Methodological design

a. Type of study

Randomized crossover clinical trial.

b. Research location

Instituto de Nutrigenética y Nutrigenómica Traslacional (INNUGET) of the Centro Universitario de Ciencias de la Salud of the Universidad de Guadalajara.

c. Study period

The scientific literature review phase began in September 2020 and the study is expected to conclude in April of 2023.

d. Inclusion and non-inclusion criteria

Inclusion criteria

- Women between 20 and 40 years old
- Body mass index between 25 to 40 kg/m^2
- Regular menstrual cycle of 25-32 days in the last 3 months
- Moderate coffee consumption (2 cups per day)
- Having the habit of eating breakfast

- Availability of time in the morning

Non-inclusion criteria

- Use of any type of contraceptives in the last three months
- Consumption of hypoglycemic, lipid-lowering, weight-loss, appetite altering and psychiatric medications
- Diagnosis of diabetes mellitus, hypertension, cancer, polycystic ovary syndrome, hypothyroidism, hyperthyroidism, infectious diseases (influenza, COVID-19, etc.), renal disease, dysgeusia, or gallbladder disease
- Weight loss ≥5% of their body weight in the last 6 months
- Consumption of more than 20 g of alcohol per day
- Smoking
- Being pregnant or breastfeeding
- Being vegetarian or vegan

e. Exclusion criteria

- Incomplete data and/or measurements
- Individuals that decide to leave the study

f. Sample size

The sample size was calculated using the formula for comparing two means with data previously reported by Douglas BR et al., who reported a significant difference ($p<0.01$) in CCK concentrations when drinking 165 ml of regular coffee versus drinking 165 ml of NaCl water (16).

$$n = \frac{\sigma 1^2 + \sigma 2^2}{(x1 - x2)^2}\left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2$$

n = sample size

α = 0.05 Z1-α/2 = 1.96

β = 0.2 Z1-β = 0.842

x1 = pmol/L of CCK when consuming regular coffee = 2.8

x2 = pmol/L of CCK when consuming water containing NaCl = 0.4

σ1 = standard deviation when consuming regular coffee = 2.2

σ2 = standard deviation when consuming water containing NaCl = 0.2

Substituting these values in the formula, an “n” of 7 subject per intervention was obtained.

$$n = \frac{2.2^2 + 0.2^2}{(2.8 - 0.4)^2}(1.96 + 0.842)^2 = 7$$

In addition, as reported by Horner et al., the sample size to detect a 20 mm difference in visual analog scales to assess appetite between meals (post-breakfast - pre-breakfast) in a paired design with an α=0.05 and a power of 80%, is 10 subjects (21).

g. Dependent and independent variables

Independent variable

- Coffee consumption

Dependent variables

- Hunger
- Satiety
- Ghrelin concentrations
- Cholecystokinin concentrations
- Dietary intake the day of the intervention

Confounding variables

- Score on the "Food restriction" section of the Three Factor Eating Questionnaire.
- Usual dietary Intake

h. Biosafety considerations

Biological-infectious waste (in this case blood) will be handled in accordance with Mexican Official Standard NOM-087-SEMARNAT-SSA1-2002, Environmental protection-health, environmental-biological-infectious hazardous waste-classification, and handling specifications (22). The blood obtained from the patients will be contained in red and purple vacutainer tubes. These are centrifuged and the plasma and serum are separated and stored at -80ºC for later use. During all handling, personnel wear lab coats, laboratory glasses and gloves. All vacutainer tubes are discarded in an airtight red container that complies with the characteristics specified in the aforementioned standard. Sharps should be deposited in a rigid red polypropylene container with a lid, in accordance with the specifications of the same official Mexican standard (22). Once the containers are filled to 80% of their capacity, they are taken to the appropriate containers at the university where a company collects them for incineration.

The laboratory techniques to be used include the determination of analytes in serum by dry chemistry, ELISA assays, and genomic DNA extraction (the latter for future use). The place where the techniques will be performed will be the INNUGET of the CUCS, which has a specific area to place the biological-infectious waste, showers, separate spaces for the different types of techniques, emergency telephones, evacuation route signs, and signs indicating the route for the transportation of the biological-infectious hazardous waste. Once the reagents have been used, they should be disposed of in an airtight red container that complies with the characteristics specified in NOM-087-SEMARNAT-SSA1-2002, and no reagents should ever be discharged into the sewage system (22).

The research team Livier Nathaly Torres Castillo, Erika Martínez López, Citlalic Saraí Rodríguez Reyes and Wendy Yareni Campos Pérez have the knowledge to handle and minimize waste, as they have received training in this regard. In the event of any contingency, personal protective equipment will be worn, and precautionary signs will be followed. If a spill occurs, the surface will

be cleaned with powdered soap with felt and placed in a special red bag and/or in a bucket with water and sodium hypochlorite, applying chemical disinfectant or germicide (0.5% sodium hypochlorite), and then mopping using a mechanical squeeze bucket or latex gloves. Finally, the area should be sprayed with a chemical disinfectant or germicide and then mopped and dried. If containers with sharps fall into the area, the waste should be removed mechanically (tongs or a brush and dustpan), deposited in a special container (never with hands), the area should be sprayed with a chemical disinfectant or bactericide (0.5% sodium hypochlorite), cleaned and dried. Finally, the materials and utensils should be washed and disinfected. In the event of an accident, personal protective equipment must be removed and the area where the accident occurred must be washed and disinfected, and medical attention must be sought immediately, or emergency numbers called.

i. Ethical considerations

This protocol is adapted to the ethical principles of the regulations of the General Health Law on Health Research and is classified as "with greater than minimal risk", according to article 17 of this Law, since a method of assignment to therapeutic schemes is used (23). In addition, the well-being, integrity, dignity, and rights of the participants will be respected in accordance with the Declaration of Helsinki (24). The standards of Good Clinical Practice (GCP), issued by the International Conference on Harmonization (GCP-ICH), are also considered, which protect and guarantee the rights, safety and well-being of the subjects participating in this research (25).

In accordance with the General Health Law on Health Research, each participant will be given a copy of the corresponding informed consent form (Annex 1) and another copy will be kept by the principal investigator. The informed consent will explain in detail the characteristics of the study, its objectives, the methodology for the collection of information, benefits and consequences of their participation (23) and the confidentiality and dissociation of the data they provide us with and those obtained through the analysis of the blood sample; the latter in accordance with articles 18, 19 and 21 of the Federal Law of Transparency and Access to Public and Governmental Information (26), and in accordance with the Mexican Official Standard 024-SSA3-2012, Electronic Registry Information Systems for Health. Health Information Exchange (27).

The international ethical guidelines for health-related research in humans developed by the Council for International Organizations of Medical Sciences, are also considered: randomization will be performed to apply the study beverage or the control beverage to avoid bias and ensure that the study results reflect the effects of coffee according to guideline 5 "Choice of control mechanism for clinical trials", the control beverage (bottled water) is considered as minimal risk, it is a single intake and does not cause any harm to health, ensuring the protection of the participant's health and well-being. According to guideline 12 "Collection, storage and use of data in health-related research", the confidentiality and protection of the data is guaranteed, these data will only be used for scientific purposes, the participant's name will be replaced by a folio assigned to keep it anonymous, all this will be informed to the participant by means of an informed consent. Participants will be duly compensated for the time invested as mentioned in guideline number 13 "Reimbursement and compensation for research participants". Our research group includes

women of childbearing age, not pregnant or lactating and without chronic diseases, their autonomy and low risk are guaranteed by receiving breakfast and test drinks according to guideline 18 "Women as research participants" (28).

The identification data of the participants will not appear in the databases, reports and publications derived from the project, only the identification number randomly assigned during the study to maintain and guarantee their anonymity. To guarantee the confidentiality and protection of the participants' personal data, this information is obtained in a format without identification folio and the clinical history format, the tubes for blood collection and processing, the format for body composition measurements, the visual analogue scales and all the questionnaires applied will contain only a randomly assigned folio and will not contain any personal data that could be used to identify the participant, whether it be by date of participation, address, name, etc. The information obtained will be kept and used for research purposes and only the research team will have access to it. In addition, each member of the research team will undertake not to disclose the data obtained by signing a letter of confidentiality.

The benefits for the participants will be a feedback of their body composition, results of their glucose levels and lipid profile and individualized recommendations regarding their dietary intake, at no cost to them. In addition, an economic aid ($200.00 two hundred Mexican pesos) will be given for transportation so that participants can cover their travel expenses to INNUGET on the days of the three sessions and reduce their exposure to public transportation and thus avoid a COVID-19 infection. This benefit will be provided in cash and is specified in the informed consent form. The data obtained will contribute socially to create evidence and information on the effects that coffee consumption has on hunger, satiety, and hormones involved in the control of these processes in women with overweight or obesity.

Storage of blood samples and clinical and nutritional information for future research projects

It should be noted that the serum, plasma, and DNA obtained from the blood collection will be stored for further research at the INNUGET of the Centro Universitario de Ciencias de la Salud at a temperature of -80°C, for a maximum period of 8 years or less in the case of the amount of sample were very small. The samples will be used for subsequent biochemical tests, immunoassays, and DNA analysis. The information obtained will be used for further studies in order to learn more about the relationship between these serum and genetic parameters along with the diet intake, with the objective of expanding knowledge in the area of Nutrigenetics in the Mexican population. This is mentioned to the participants in the informed consent (Annex 1) as indicated in article 12 of the World Medical Assembly (WMA) declaration on the ethical considerations of health databases and biobanks (29). The databases are stored in a computer owned by the Universidad de Guadalajara to which only the principal investigator has access to such information. Access to the databases and samples by other researchers and/or students will only be allowed with the prior authorization of the principal investigator of this project and once a confidentiality letter has been signed, reiterating that the identification data of each participant will be dissociated, and it will not be possible to identify who they belong to. Any of the participants

may request a copy of the results obtained from their biological samples and these will be delivered by the principal investigator in a sealed envelope. If the information or biological sample cannot be identified, the person will not be able to know what is done with their information or biological sample, nor will they be able to withdraw their consent.
Finally, the protocol was reviewed, approved, and registered by the Research, Research Ethics and Biosafety Committees of the CUCS of the Universidad de Guadalajara.

9. Methodology (procedures)

Selection of participants

Adult women between 25 and 40 years of age will be invited to participate in this study through social networks and personal invitations. Participants who meet the inclusion criteria and agree to participate will sign the informed consent form. Their participation involves their attendance to 3 sessions with a difference of one week between one and another. In the first session, they will be evaluated to determine whether or not they meet the inclusion criteria and will then be randomly assigned to the two types of interventions in the two subsequent sessions.

Anthropometry and blood pressure

Weight

Weight measurement is performed in the morning, after a period of 8 to 12 hours of fasting, with light clothing and with bare feet. An InBody model 370 electrical bioimpedance device with an accuracy of 0.1 kg is used. The subject is placed in the center of the scale without support and with his/her weight equally distributed on both feet.

Size

The size measurement is made with a Seca stadiometer which has an accuracy of 0.1 cm. The subject stands with feet together (heels together and toes slightly apart). The heels, buttocks, upper back, and occipital are in contact with the stadiometer. The head is placed in the Frankfort plane, without touching the equipment. The subject is instructed to take a deep, sustained breath in. To make the measurement, the square is placed firmly on the highest point of the skull, compressing the hair as much as possible. The measurement is taken at the end of a deep breath.

Waist circumference

Waist circumference measurement is performed with a Lufkin anthropometric tape, which has an accuracy of 1 mm. The subject keeps the arms crossed on the thorax, heels together with the toes a little apart and the view in the Frankfort plane. The measurement is taken at the narrowest level, between the edge of the lower costal (tenth rib) and the iliac crest. The subject breathes normally, and the measurement is taken at the end of an exhalation.

Hip circumference

Hip circumference measurement is performed with the same Lufkin brand tape and in the same position as described for waist circumference. The circumference is taken at the maximum posterior level of the buttock bulge.

Electrical bioimpedance

The measurements of fat mass, fat percentage, fat-free mass and bone mass are obtained through the InBody 370 equipment, under the same conditions described for the measurement of body weight.

Blood Pressure

Blood pressure measurement is performed in the morning, after 10 minutes of rest, with a LifeSource digital sphygmomanometer, the patient should be relaxed, seated, not stretched, with the back well supported by the back of the chair. The legs should be touching the floor, not crossed, and the hand relaxed, not clenched and in a resting position. The measurement is made on the reference or dominant arm resting at about the level of the heart. The subject's clothing does not cover the arm or fit snugly to the arm 25.

Biochemical determinations

Blood sampling

In the second and third sessions a blood sample is taken in the morning, after a period of 8 to 12 hours of fasting by venous puncture at times t=-30 min, t=0 min and t=180 min. Two vacutainer tubes, one red and one purple with 5 ml of blood, are obtained and centrifuged at 3500 rpm for 15 minutes at 4° C to obtain serum and plasma for biochemical tests.

Glucose and lipid profile determinations

The determinations are performed in a Vitros 350 Chemistry equipment by dry chemistry. A 10 µl biological sample (serum) is applied to a slide containing the reagents that allow the detection of the analyte.

LDL cholesterol is calculated with Friedewald's formula (30):

LDL cholesterol= total cholesterol - HDL cholesterol - triglycerides/5.

And VLDL cholesterol is also calculated with Friedewald's formula (30) as:

Total cholesterol= (LDL cholesterol + HDL cholesterol).

Ghrelin
Serum is obtained from the participants, from the same blood samples used to determine glucose and lipid profile. Ghrelin levels are determined using a RayBiotech kit, catalog number ELH-GHRL, and the plate is read on a Thermo Scientific Multiskan Sky spectrophotometer. The ghrelin assay is a sandwich ELISA assay using two specific, high-affinity antibodies.

Cholecystokinin
Serum is obtained from the participants, from the same blood samples. Cholecystokinin levels are determined using a RayBiotech kit, catalog number EIA-CCK-1, where the plate is read on a Thermo Scientific Multiskan Sky spectrophotometer. The cholecystokinin assay is a sandwich ELISA assay using two specific, high-affinity antibodies.

Dietary evaluation
Dietary Record
In the first session, participants will receive instructions on how to fill out the dietary record format to evaluate the individuals' diet, they are asked to record all food and beverages consumed during a 3-day period, being a weekday, a weekend day and the day prior to their second session. Nutritionist ProTM Diet Analysis software (Axxya Systems, Stafford, TX, USA) is used for diet analysis.

Frequency of consumption of foods with caffeine
A search of the nutritional information of foods and beverages with coffee and/or caffeine most consumed in Mexico was made in the food databases, the magazine “Revista del Consumidor”, a publication of the Mexican government, and in other sources where that information was available. This information was used to design a coffee and caffeine products frequency products and will be applied in each of the sessions to the participants.

Questionnaires
Clinical history
A clinical history will be taken during the first session to obtain information on pathological history, clinical data, nutritional aspects, sociodemographic and lifestyle aspects.

Visual analog scales
This evaluation format, which was previously reproduced and adapted to Spanish by our research group (manuscript in preparation), consists of questions that are asked before and after consuming breakfast, and subsequently at certain time intervals. The visual analog scales are composed of lines, usually 100 mm long, on which at one end is placed the term "None" or "Not at all" and at the other end the term "Yes, a lot" or "As much as I have never felt". The patient marks a point between these two extremes and quantification is done by measuring the distance from the left end of the line to the mark to which a score is given (31).

Physical Activity Questionnaire (IPAQ) short version
This instrument is a physical activity assessment format, that examines different dimensions of patient physical activity, such as time spent in walking, moderate and vigorous intensity activities

and time spending in sedentary activities. Weekly physical activity is measured by recording in METs (metabolic equivalent of task) per min per week. After calculating the physical activity index, whose value corresponds to the product of the intensity (in METs) times the frequency times the duration of the activity. The subjects are classified into 3 categories of high, moderate, and low physical activity (32). This questionnaire will be applied in the second and third sessions.

Three-factor eating questionnaire

This questionnaire consists of 51 items and is applied in the first session to assesses three dimensions of eating behavior: a) dietary restraint, consisting of 21 items intended to measure the cognitive dominance of food intake; b) disinhibition, containing 16 items assessing the tendency to overeat and loss of control overeating; and c) hunger susceptibility, with 14 items estimating subjective feelings of hunger and desire for food (33,34).

Breakfast

Participants will receive 2 breakfasts in two different sessions; one of them will consist of a breakfast that will include simple water as a beverage and in another session, they will have the same breakfast, but the beverage to be consumed will be coffee. The order in which the two types of beverages will be consumed will be random. Each breakfast will be offered 1 week apart and will consist of the following:

- Breakfast with coffee

  1 cup of coffee with 6 mg/kg of body weight of caffeine and a breakfast with 400 kcal, with a distribution of 50% carbohydrates, 30% lipids and 20% protein.

A Veracruz coffee will be used, which according to a study carried out by the Federal Consumer Protection Agency (Profeco) reports that for every 7 grams of this coffee there are 111 mg of caffeine (35); once the participant's weight is obtained, a calculation will be made to know the amount in grams of coffee to be used; finally, the amount of coffee in 250 ml of water will be prepared. Likewise, before the intervention, tests will be carried out to see the tolerance to the taste.

- Breakfast without coffee

  1 cup of simple water and a breakfast with 400 kcal, with a distribution of 50% carbohydrates, 30% lipids and 20% protein.

In the research carried out by Gavrieli et al. (3,4) and in a study by Beaudoin M et al. (36), simple water was used as a control beverage to analyze the effect of coffee on appetite regulating hormones. Based on this, it was decided to use simple swater as the control beverage for this study to make comparisons with the results obtained in other investigations.

Statistical analysis

For descriptive statistics, quantitative variables will be expressed as mean ± standard deviation or median and interquartile range, and qualitative variables as frequency or percentage. For inferential statistics, the distribution of the variables will first be analyzed with the Shapiro-Wilk test. In the event that the variable is not normally distributed, a logarithmic transformation will be

made, and it will be checked again if it is normally distributed; in the event that the variable continues with a non-normal distribution, non-parametric statistical tests will be used.
For comparisons between the two treatments, the mixed general linear model will be used. The data will be entered into an Excel spreadsheet to later be imported into the SPSS v.21 program for analysis, considering a value of $p<0.05$ as statistically significant. The graphs will be made with the Gradhpad software version 9.3.1.

10. Project viability

a. Funding

Financial resources are available to cover the expenses necessary to carry out the project, through the program "Support for the Incorporation of NPTC", report number 511-6/2020-8586 and through the PRO-SNI 2021 fund.

b. Infrastructure

Access to INNUGET of the University Center of Health Sciences of the Universidad de Guadalajara is available, which has all the necessary equipment to carry out this project.

11. References

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ANNEX 1

INFORMED CONSENT TO PARTICIPATE IN THE STUDY ENTITLED:

Effect of coffee consumption on hunger, satiety, appetite-regulating hormones, and diet intake in women with overweight or obesity

Project managers
Dra. en C. Livier Nathaly Torres Castillo y Dra. en C. Saraí Citlalic Rodríguez Reyes
Study location:
Centro Universitario de Ciencias de la Salud de la Universidad de Guadalajara
Date (dd/mm/yyyy): _____ / _____ / _____
Participant's name: _____

You are being invited to participate in this research study. Before you decide whether or not to participate, you should know and understand each of the following sections. This process is known as informed consent. Feel free to ask questions about anything that will help clarify your concerns.
Once you have understood the study and if you wish to participate, then you will be asked to sign this consent form. A signed and dated copy of which will be given to you.

PATIENT INFORMATION

JUSTIFICATION FOR THE STUDY
In Mexico, according to the latest National Health and Nutrition Mid-Track Survey (ENSANUT-MC,2018) 75% of the adult Mexican population is overweight. In the case of premenopausal women, the prevalence of obesity in the age range of 30 to 49 years was 89%. Jalisco also had an increase in the prevalence of obesity from 37.6% in 2012 to 39.8% in 2018. Preventing and treating obesity early, as well as seeking and finding complements that suit the patient to execute more effective nutritional interventions, has benefits for the individual patient and for the healthcare system in general. In the search for supplements to implement effective nutritional interventions to combat this problem, we find coffee. Recently, this beverage has ca used great interest since recent findings suggest that coffee has an effect on appetite control which could help in weight control. For this reason, this study will be important to contribute to the knowledge gap that exists regarding the effects of coffee on hunger, satiety, appetite regulating hormones and diet intake in women with overweight or obesity.

OBJECTIVE OF THE STUDY
You are being invited to participate in this research project which aims to learn about the effects of coffee on hunger, satiety, appetite regulating hormones, and diet intake in women with overweight or obesity.

STUDY PROCEDURE
If you decide to participate, you will be scheduled on three different occasions. In session 1 a nutritionist will evaluate your weight, height, waist circumference, percentage of fat, percentage of body water and amount of muscle, the last three will be done in an electrical bioimpedance equipment, which is similar to a scale and to get on this equipment you will have to do it with bare feet (this will not cause you any discomfort or

pain). You will also be asked a series of questions to fill out a medical history, similar to when you attend a consultation with a doctor, and you will be asked a questionnaire on eating behavior. In this first session you will be instructed to record your food and beverage intake for 2 days.
You will be scheduled for a second session corresponding to day 1 or 2 of the start of your menstrual cycle. At this second session you will need to attend with a fasting period of at least 8 hours. First a blood sample will be taken, then you will be given a breakfast (which may include simple water or coffee) and at 30 to 180 minutes after finishing your breakfast, your blood sample will be taken again. In addition, on an empty stomach, after consuming your breakfast and at 30, 60, 90, 120, 150 and 180 minutes you will answer questions to assess your hunger and satiety. One week later you will be scheduled for the third session where we will perform the same procedure as in session 2, with the only difference that, if in session 2 you received your breakfast with coffee, this third time you will be given the same breakfast, but with simple water or vice versa.

-About the storage of samples and clinical and nutritional information for future research projects
Your participation in this study also includes storing your DNA, blood serum and plasma for further research. Your samples will be stored in a laboratory within the University Health Sciences Center at a temperature of -80 degrees for a maximum period of 8 years or less if the amount of sample is very small. Your samples will be used for further biochemical tests and immunoassays (measuring substances naturally found in the blood) and analysis of your DNA. The information obtained from your samples will be used for further studies to learn more about the relationship between these components in your blood, the diet and the DNA, which will allow for more accurate health and nutrition recommendations for the Mexican population in the future. The researchers and/or students who participate in this study will be able to access all the times to the samples and results obtained, and they could also be shared with other Mexican researchers and researches in other countries, but at all times your information and personal data will be protected by a code, so that your data and information will have a folio instead of your name or any other identifying information, in this way we guarantee absolute confidentiality, i.e. no one can know that the sample is yours. In addition, in order for the researchers to have access to the information obtained in this study, they will sign a confidentiality letter where they commit to maintain the privacy of your personal data. The databases are stored in a computer owned by the University of Guadalajara to which only the principal investigator has access and the information contained therein may be shared with other researchers or students with whom they collaborate as long as the confidentiality letter is signed. Access to the databases and samples by other researchers and/or students will only be allowed with the prior authorization of the principal investigator of this project and once the confidentiality letter has been signed, reiterating that the identification data of each participant will be separated, and it will not be possible to identify who they belong to. You may request a copy of the results obtained from your biological samples and these will be delivered by the principal investigator in a sealed envelope. In the case that the information or biological sample cannot be identified, you will not be able to know what is done with your information or biological sample, nor will you be able to withdraw your consent.
It is worth mentioning that the information obtained here may be published in different scientific journals and scientific congresses in the future, but the confidentiality of your personal data will always be respected, as these will never be disclosed by any means.

RISKS
In accordance with the Regulations of the General Law of Health on Research for Health, this research is considered with a higher than minimal risk, because it is randomly assigned whether you will receive coffee or simple water in the first intervention.

VOLUNTARY

Your participation in this study is completely voluntary, so at any time you can stop participating in this study without having to give explanations to the investigators.

CONFIDENTIALITY

All information generated and personal data will be treated confidentially. Your identification data will not appear in the databases since only a participant folio will be shown. In the final report of the study, in the publication of results in scientific congresses, in publications in scientific journals and at all times, your identity will be kept anonymous. To guarantee the confidentiality and protection of your personal data, all the forms and questionnaires, as well as the tubes where the blood sample will be taken, will only have a folio (a number) assigned randomly and no personal data that could be used to identify you, not even by your date of participation, address, etc., will appear on them.

BENEFITS AND RESULTS

At the end of the session, you will receive free feedback on the interpretation of your weight, height, fat percentage, amount of muscle and your body mass index classification. You will also receive nutritional recommendations based on your dietary assessment, telling you how to improve your eating habits. Finally, participants will be given a financial assistance ($200.00 two hundred pesos) for transportation to cover their travel expenses to CUCS on the days of the three sessions and to reduce their exposure to public transportation to avoid COVID-19 infection.

DISCLAIMERS:

- Your decision to participate in the study is completely voluntary.
- There will be no unfavorable consequences for you if you do not accept the invitation.
- You may withdraw your participation at any time, even if you have already signed this consent form without explanation.
- You may request information obtained during the study, even if it might interfere with your decision to continue participating in the study.
- You will not have to pay any expenses during the study

CONTACT INFORMATION FOR CLARIFICATIONS OR DOUBTS

If you have any questions, doubts, comments, or concerns regarding the project, please contact any of the researchers responsible for the project at the addresses below, where you are guaranteed to receive a response to whatever your concern may be.

-Dra. en C. Livier Nathaly Torres Castillo (phone number 3310585200 ext. 34284) (nathaly.torrescas@academicos.udg.mx).
-LN Lisset Magaña de la Vega (phone number 3318409211) (lissma14@gmail.com)

LETTER OF INFORMED CONSENT

I, _____, declare that I have read and understood the above information and my questions have been answered in simple language, in a sufficiently clear and satisfactory manner. I have been informed and understand that the data obtained in the study may be published or disseminated for scientific purposes without my name being disclosed. I also understand that at any time and without giving any explanation (if I prefer), I may revoke the consent I now give. I therefore agree to participate in this research study for the proposed purposes. And I will receive a signed and dated copy of this consent form.

Guadalajara, Jalisco, _(day)__ of __(month)____ of _(year)__

_____

Participant's name and signature

Witness 1	**Witness 2**
_____	_____
Name and signature	**Name and signature**
_____	_____
Address	**Address**
_____	_____
Relationship with the investigator	**Relationship with the investigator**

This part must be completed by the investigator (or his/her representative):

I, _____, have explained to the participant the nature and purposes of the research; I have explained the objective of the study and the importance of their participation. I have answered questions to the best of my ability and have finally asked if there were any remaining doubts. I accept that I have read and know the corresponding regulations for conducting research with human subjects and that I, as well as my work group, adhere to them. Once the question-and-answer session was concluded, the present document was signed.

_____	_____
Name and signature of researcher and/or representative	**Witness name and signature**
Relationship with the investigator and/or representative:	_____