Serum Insulin Response After Acute and Chronic Sucralose Ingestion in Healthy Volunteers With Variable Body Mass Index

INFORMED CONSENT TO PARTICIPATE IN THE RESEARCH STUDY

Principal Investigator.- Dr. Guillermo Meléndez Mier
Institution.- General Hospital of México "Dr. Eduardo Liceaga"
Dr. Balmis 148, Col. Doctores, Delegación Benito Juárez, CP 06726
México CDMX

This consent form may contain words that you do not understand. Please ask the researcher or study staff to explain any words or information you do not clearly understand.

INTRODUCTION

Your participation in this study is voluntary. It is important that you read and understand the following explanation of the procedures. This form describes the objective, procedures, benefits, known risks, discomforts and precautions of the study, including the duration of your participation. It also describes your right to leave the study at any time.

In order to enter to the study, as a volunteer, you must sign and date this consent form and put your initials and date on the bottom of each page.

You are being invited to participate in a research protocol that will study what happens when a healthy person is exposed acutely or chronically to sucralose (a sweetener found in foods and beverages) on blood levels of insulin and glucose in young, healthy adults.
Insulin is a substance that the pancreas produces and is necessary for the body to be able to use glucose; the glucose is a sugar form that circulates in the blood. This sugar is the basic fuel for the body cells, in the other hand, insulin takes this sugar from the blood and introduces it into the cells. Sucralose is a flavoring that has no calories and is used to replace sugar in foods and beverages. It has been seen that sucralose could make difficult for insulin getting sugar into the cells, which results to a high insulin necessity for the body to introduce the same amount of sugar into the cells.

You are receiving this invitation because you are considered as a candidate to participate in this study. Please read this letter carefully before signing it. You can ask any questions you have about the study; and be completely honest with the research team regarding your medical history.

STUDY OBJECTIVES

Serum Insulin Response After Acute and Chronic Sucralose Ingestion in Healthy Volunteers With Variable Body Mass Index

This study will test whether sucralose (an intense non-caloric sweetener), in different doses; 48 and 96 milligrams (the equivalent of the content found in 1 and 2 soda cans respectively), hinders or prevents the insulin to take the sugar from the blood and introduce it into the cells after taking that same dose daily for 10 weeks. When sugar levels rise in blood after a meal, even moderately, the body reacts by releasing insulin and other substances in order to introduce the sugar into the cells keeping it normal in the body. Insulin is a substance that rises when any normal person ingests some food or drink with sugar, insulin ensures the sugar utilization as energy by the different organs of the body and maintain it at normal levels in the blood. Although sometimes, for situations that are not known, insulin rises more than it should to get the sugar from the blood (this is when the body needs more insulin than normal to put the same amount of sugar circulating in the blood to the heart and muscles) as is the case in this study. The other substances that we are going to study in the blood are hormones and cells that indicate that there is moderate inflammation and that can be related to insulin resistance.

INCLUSION CRITERIA

In order to participate in this study, you must:

1. Be between 25 and 35 years old.
2. Being healthy, this means that no doctor has diagnosed any acute or chronic disease.
3. Take a balanced diet that becomes a light and moderate physical activity.
4. Do not smoke more than 3 cigarettes a day, in the last 15 days.
5. Not having drunk more than one glass of wine or another alcoholic beverage per week in the last 15 days.
6. Do not consume diet foods made with intense sweeteners other than sugar table.
7. It is not recommended to drink diet drinks that contain intense sweeteners.
8. Do not get used to chewing gum without sugar.
9. You, your parents and grandparents must be from the Mexico City metropolitan area.

EXCLUSION CRITERIA

You are not able to be in the study if:

1. You have taken antibiotics in the last 4 weeks
2. You have taken corticosteroids in the last 3 months
3. You have taken some type of non-steroidal anti-inflammatory in the last 4 weeks
4. You refuse to stay in the Clinical Pharmacology unit for as long as it is required.

5. You refuse to attend weekly follow-up visits for 10 weeks.
6. You have a night job.
7. You refuse to abstain from consuming industrialized foods that contain non-caloric sweeteners during your participation in the study
8. You refuse to abstain from consuming beverages containing non-caloric sweeteners during their participation in the study 9. You refuse to abstain from consuming sugar-free gum while participating in the study
10. You refuse to abstain from consuming alcoholic beverages three months before and during their participation in the study
11. You refuse to abstain from smoking three months before and during the study
12. You have been or will undergo surgery to lose weight three months before or during the study
13. You refuse to sign the informed consent letter to participate in the study
14. In the case of Women, be certain or suspect that you are pregnant, you are breastfeeding a newborn and do not use any contraceptive method to avoid pregnancy during the study.

STUDY PROCEDURES

If you agree to participate in the study, you will be presented with a list of procedures so that we can evaluate whether sucralose has an effect on your insulin, sugar and other hormones and inflammation markers for 10 weeks. The procedures to which you would submit yourself would be the following:

Procedures:

VISIT 1 (DAY 1)

1.- If you have been interested in participating in the study, you will be invited to the Clinical Pharmacology Unit of the Direction of Investigation, requesting to go with two people you trust in. You will be presented and explained this informed consent form, the study and its objectives will be explained, and if you voluntarily agree to participate in the study, you will be asked to sign the consent form in the presence of two witnesses.
2.- Once you have signed the informed consent, you will be asked some questions about your medical background and it will be verified if you correctly meet the inclusion and exclusion criteria.
3.- Afterwards, a nutritional history will be made, applying a weekly food frequency questionnaire and another questionnaire to know what you ate the previous day.
4.- You will be asked questions of another questionnaire to ensure that you have not consumed non-caloric sweeteners.

5.- You will be taken body measurements such as weight, height, fat percentage, waist circumference, this is what we call antropometric measures and you will be taken your vital signs such as pulse, blood pressure, breaths and temperature.
6.- You will have a physical exam.
7.- You will be summoned the next morning at 7 in the morning, with a fast of at least 8 hours for baseline laboratory tests.

VISIT 2 - (DAY 2)

1.- You will go to the Central Laboratory of the General Hospital of Mexico "Dr. Eduardo Liceaga" at 7 o'clock in the morning where our chemists will take two blood samples.
2.- The staff of the research team will verify that they were fasting for at least 8 hours.
2.- 8 milliliters of blood will be extracted to process the following analysis:
a) Blood chemistry
b) Insulin
c) Blood count (red blood cells, white blood cells and platelets)
d) Glycated Hemoglobin (is the hemoglobin of the red blood cells that is marked with sugar)
3.- Written instructions will be given to you so that he can go the next day to the revision of the laboratory results

VISIT 3 - (DAY 3)

1.- Your laboratory results will be checked, verifying that each test is within normal limits.
2.- It will be verified that you have not taken antibiotics or anti-inflammatories.
3.- Your HOMA-IR will be calculated, this is the relationship between insulin and blood sugar, verifying that it is less than 3.8.
4.- If your HOMA-IR is lower than 3.8, you can continue with the study processes
5.- If your HOMA-IR is equal or superior to 3.8, you will be explained why you cannot continue in the study.
6.- If you continue in the study, you will be given verbal and written instructions to go the next day without having eaten for at least 12 hours, to practice the oral glucose tolerance curve
7.- You will be provided with a container to bring a sample of your fecal material with you.

VISIT 4 - (DAY 4)

1.- You will go to the Clinical Pharmacology Unit clinic at 7 o'clock in the morning.
2.- It will be verified that you have remained fasting for 12 hours.
3.- It will be verified that you have not taken antibiotics or anti-inflammatories.

4.- The fecal sample will be received and sent to the Laboratory on ice.

5.- You will be explained the procedures and why you will remain in the laboratory for an approximate period of 4 hours so that we can carry out the 180 minutes oral glucose tolerance curve.

6.- It will be verified that during the previous 8 days, you have maintained a free and stable diet, without restriction of carbohydrates (pasta, potatoes, rice, bread, fruit, etc.), but without having eaten food or drinks with non-caloric sweeteners (including chewing gum with sweeteners) and doing regular physical activity.

7.- You will be taken to the short stay room of the Clinical Pharmacology unit, where you will be reclined to insert into a vein in your arm a small plastic tube called punzocath from which the blood samples will be taken. The oral glucose tolerance curve procedure will be started as follows:

8.- At the Clinical Pharmacology Unit you will have a first extraction of 4 ml of blood at the beginning of the test and you will be given to drink 30 mL of a solution containing sucralose or water. Neither You nor the researchers will know what you are drinking.

9. Fifteen minutes after taking the first blood sample and taking the sucralose or placebo, a second blood sample will be taken and immediately afterwards you will be given a load of 75 g of 50% glucose. , considering this takes the beginning of the tolerance curve to this carbohydrate.

10.- We will obtain more blood sample after the ingestion of sucralose and after 15 minutes you have ingested the load of 75g of glucose.

11.- You will remain at rest during the whole time of the test, without eating or drinking anything. The total of samples that will be extracted will be 11 samples, being as follows: at -15, 0, 15, 30, 45, 60, 75, 90, 105, 120 and 180 minutes. Each glucose sample will be processed immediately, the rest of the samples will be processed to obtain serum and from them the levels of insulin, C peptide, glucagon, GIP and GLP-1 will be quantified, covering the 11 points of each curve. Inflammatory monocytes and pro and anti-inflammatory cytokines will be quantified twice, using samples -15 and 180 minutes from the initial OGTT. Another member of the team will simultaneously keep a log of each blood sample taken, noting the date, time and conditions of the volunteer in each shot.

As mentioned above, in each blood sample the following analysis will be determined:

Of the 11 blood samples that were extracted to carry out the initial OGTT, the following tests will be processed:

a) Glucose
b) Insulin
c) Peptide C
d) Glucose-dependent insulinotropic polypeptide (GIP)
e) Glucagon,
f) Glucagon-like peptide-1 (GLP-1)

Inflammatory / anti-inflammatory markers analyzed at the beginning of the OGTT:

g) protein C-reactive (pCr)

h) Tumor necrosis factor alpha (TNF-α)

i) Interferon gamma (IFN-γ)

j) Interleukin (IL) 1α, IL-4, IL-6, IL-10, IL-12, IL-13, IL-17 and IL-23

k) CD14, CD16, CD11c, CCR2, CX3CR1 and CD206 in circulating monocytes

12.- At the end of the initial oral glucose tolerance test, you will be offered a balanced snack.

13.- Once you have consumed your food, you will be asked to show up at the Clinical Pharmacology office 8 days after you have been tested for oral glucose tolerance test.

14.- You will receive a package with the 9 bottles that contain the substance under study and you will have to take a bottle daily for 7 days, and keep 2 bottles of reserve in case you lose one.

15.- You will take a fresh drink with the substance under study until the day before the next visit.

VISITS 5 AT 14 (DAYS 12, 20, 28, 36, 44, 52, 60, 68, 76, 84)

1.- You will go to the clinic of the Clinical Pharmacology unit at the time you have been cited in the morning

2.- You will be asked if in the last 8 days you have taken antibiotics or anti-inflammatories

3.- The vital signs and weight will be taken.

4.- The jars that were given to you in the previous visit will be collected and the number of empty containers that you have consumed since your previous visit (8 days before) will be counted and the number of full containers that you will be delivering and will be recorded in your file .

5.- You will be asked about the number of times you ate different foods during the last 8 days.

6.- You will be asked in more detail about what you ate in the last 24 hours

7.- You will be asked if you ate or drank food or drinks with non-caloric sweeteners or if you chewed some gum without sugar in the last 8 days.

8.- You will receive again a package with 9 bottles with the test solution and you will be asked to take the first bottle of that week in the presence of the researcher.

9.- You will take 8 full bottles to your home and you will be asked to take one bottle daily until the day before your visit to the clinical pharmacology unit.

10.- You will be cited for an appointment in 8 days.

VISIT 15 - (DAY 92)

1.- You will go to the Clinical Pharmacology unit clinic in the morning.

2.- You will be asked if in the last 8 days you have taken antibiotics or anti-inflammatories.

3.- The vital signs and weight will be taken.

4.- The jars that were given to you in the previous visit will be collected and the number of empty containers that you have consumed since your previous visit (8 days before) will be counted and the number of full containers that you are delivering will be recorded on the sheet. of attachment.
5.- You will be asked about the number of times you ate different foods during the last 8 days.
6.- You will be asked in more detail about what you ate in the last 24 hours
7.- You will be asked if you ate or drank food or drinks with non-caloric sweeteners or chewed gum without sugar in the last 8 days.
8.- You are given a bottle with the test solution and you are asked to take it in the presence of the researcher.
9.- You will take 8 full bottles to your home and you will be asked to take one bottle daily until the day before your visit to the clinical pharmacology unit.
10.- The Clinical Pharmacology Unit is cited at 7 a.m. in 8 days
11.- It is indicated that he has to come fasting for at least 12 hours
12.- You are given a container to bring a sample of excrement with you the day before your appointment to the Clinical Pharmacology Unit

VISIT 16 - (DAY 100)

1.- You will go to the clinic of the Clinical Pharmacology Unit at 7 o'clock in the morning.
2.- It will be verified that you have remained fasting for 12 hours.
3.- It will be verified that you have not taken antibiotics or anti-inflammatories.
4.- The fecal sample will be received and sent to the Laboratory on ice.
5.- You will be explained the procedures and why you will remain in the laboratory for an approximate period of 4 hours so that we can carry out the oral glucose tolerance curve of 180 minutes.
6.- It will be verified that during the previous 8 days, you have maintained a free and stable diet, without restriction of carbohydrates (pasta, potatoes, rice, bread, fruit, etc.), but without having eaten food or drinks with non-caloric sweeteners (including chewing gum with sweeteners) and doing regular physical activity.
7.- At the Clinical Pharmacology Unit you will have a first extraction of 4 ml of blood per minute -15 and you will be given to drink 30 mL of a solution containing sucralose or water.
8. Fifteen minutes after taking the first blood sample and taking sucralose or placebo, at minute 0 a second blood sample will be taken and immediately afterwards you will ingest a load of 75 g of glucose at 50 %, in approximately 10 minutes, considering this takes the beginning of the tolerance curve to this carbohydrate.
9.- You will have new blood extractions 15 minutes after ingesting the load of 75g of glucose.
10.- You will remain at rest during the test, without eating or drinking. The total of samples that will be extracted will be 11 samples, remaining as follows: at -15, 0, 15, 30, 45, 60, 75, 90, 105, 120 and 180 minutes. Each glucose sample will be processed immediately, the rest of the samples will be processed to obtain serum and from these the levels of insulin, glucose, C peptide, glucagon, GIP and GLP-1 will be quantified, covering the 11 points of each

curve. Inflammatory monocytes and pro and anti-inflammatory cytokines will be quantified twice, using samples -15 and 180 minutes from the initial OGTT. Another member of the team will simultaneously keep a log of each blood sample taken, noting the date, time and the conditions in which you are in each shot.

11.- The blood samples will be used to repeat the analysis already mentioned in the first glucose tolerance curve.

12.- At the end of the final OGTT, you will be given a balanced snack.

13.- After you have consumed your food, you will have a nutritional appointment to review your results.

14.- You will be thanked for participating in the study.

YOUR RESPONSIBILITIES AS A PARTICIPATING VOLUNTEER

If you decide to participate in this study it is very important you consider your responsibilities; which are the following: You must attend all study visits, comply with taking the test substance from the study daily and return the empty and full bottles of the test substance at each visit, as well as inform your study doctor about any medication you have taken. before each visit and any unexpected medical conditions that you have presented during your participation in this study.

POSIBLE RISKS AND DISCOMFORTS

1) STUDY SUBSTANCE.

Sucralose is a sugar molecule to which 3 hydrogen atoms are exchanged for 3 of chlorine, this change gives it a sweet taste that is approximately 600 times sweeter than the sugar.

Long-term studies about intake of sucralose in humans have shown that when 125 milligrams per day are ingested for 3 weeks; then increased to 250 milligrams for seven weeks and finally increased to 500 milligrams per day for 12 weeks, no. adverse events clinically detectable experiences appear in healthy volunteers, nor were blood tests, biochemical tests or general urinalysis, nor the electrocardiogram during the 13 weeks that the study lasted. Sucralose was well tolerated by volunteers at doses up to 10 milligrams per kilo per day. Based on these studies and the extensive experience in laboratory animals, it is concluded that the long-term exposure of sucralose to the previous maximum levels does not affect the health of healthy volunteers.

In addition, safety assessments of sucralose in animals and in humans were been initiated since 1987, and until the last review in 2011 by the Scientific Committee on Food of the European Union and the Food and Drug Administration (FDA) of the United States. United concluded that sucralose is acceptable as a sweetener in foods in general and that the acceptable daily intake is between 5 and 15 milligrams per kilo of a person's weight, a higher doses than the one you will ingest daily for 10 weeks.

2) EXTRACTION OF BLOOD SAMPLES DURING THE STUDY

Great experienced nurses are carrying out the previously mentioned procedures exposed in this document, so normally an attempt to insert the catheter into the vein and extract the first blood sample will be sufficient , however, in some cases additional attempts may be necessary if the first is not successful. Rarely you may experience some pain and redness, bruising, lightness, and / or fainting and, in even more rare cases, an infection at the puncture site after taking out the blood.

Some people skin reacts to the adhesive tape with which the catheter is attached to the arm, but any skin irritation usually disappears when the adhesive tape is removed.

POTENTIAL BENEFITS

It is not possible to predict if there will be any personal benefit as a result of your participation in this study. Your participation in this study will help clarify whether sucralose has an effect on insulin, glucose and other metabolic and inflammatory substances after ingesting 75 grams of glucose and this can therefore benefit other healthy or sick people who go to consume in the future. You will receive the benefit of additional information and evaluation of your general health.

NEW FINDINGS

You will be informed by a member of the research team about any new significant findings that may develop during the course of this study and may affect your willingness to continue participating in this study. You can then use this information to make a decision about permanence in the study.

PREGNANCY

If you are a woman with the potential to have children, you must agree not to become pregnant and use adequate contraceptive methods during your participation in the study.
Acceptable methods of contraception are: double barrier methods, surgical sterility (hysterectomy and / or documented bilateral oophorectomy), oral contraceptives or postmenopausal state (defined as at least 2 years without menstruation). Dual barrier contraception is defined as two different barrier mechanisms used simultaneously each time you have sex. The accepted barrier mechanisms include: intrauterine device, male condom, female condom, diaphragm and cervical cap. Both the diaphragm and the cervical cap should be used together with a spermicidal cream or jelly.
Contraceptive methods that are NOT acceptable are: natural "rhythm" methods, withdrawal, abstinence, tubal ligation, partner sterility, and use of spermicides only.

In the event that you become pregnant during your participation in the study, the administration of sucralose will be suspended and follow-up visits will be made for the evaluation of pregnancy, baby and fetus. These evaluations will be carried out during the pregnancy period and until the birth. You can also have an evaluation visit after the birth that includes the baby. You also have the right to choose whether or not you would like to receive unblinded information from the researcher about what you were taking during the study.

WITHDRAWAL AND TERMINATION

Your participation in this study is strictly voluntary. If you want to suspend your participation, you can freely do it at any time. If you choose not to participate or withdraw from the study, your present and / or future medical care will not be affected and you will not incur any penalty or loss of benefits to which you may otherwise be entitled.
Also, your participation in the study may be suspended by the study doctor, without your consent, for any of the following reasons: (a) the study doctor determined it is the best for you, (b) you need additional medication which would interfere with the study, or (c) you violate the requirements of the study or you are uncooperative.
If you decide to suspend your participation in the study, the following steps should be taken:

(a) You must inform the doctor of this study, (b) you must return to the study doctor for a final examination within 72 hours after the suspension of the test substance and (c) you must return all the bottles used and not used that were provided to you during the last visit or those that you had pending to retun of previous visits.
If your participation in the study is terminated for any reason, the collected information during your participation in the study participation may continue being used and disclosed for the described purposes in this consent form.

COSTS, REIMBURSEMENTS AND PAYMENTS

There is no cost to you for your participation in this study, except for the cost of transportation to the hospital to attend the scheduled visits. The test substance, procedures and tests related to the study and study visits will be provided at no cost. There is also no refund or payment for stipends.

CONFIDENTIALITY / MEDICAL RECORDS

If you agree to be part of this research, the study doctor will collect and record information about your health and the effects of sucralose. This information will NOT contain your full name or address, but may contain other information about you such as your initials names and your birth date. Your name will not be disclosed outside the Hospital unless its required by law.
You authorize the results of this research study may be presented at meetings or in scientific publications. However, you will not be personally identified in any of those presentations or publications.

COMPENSATION AND TREATMENT FOR INJURY

If you become ill or injured due to an adverse event resulting directly from the test substance (sucralose) use, or from any study procedure during your participation in this study, you will be provided with the necessary treatment within the institution.

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VOLUNTEER DECLARATION

I acknowledge that I have had the opportunity to ask questions regarding this research study and these questions have been answered satisfactorily.

I understand that if I have additional questions or in the event of an illness or injury related to the study, I can contact Dr. Guillermo Melendez Mier at 50 04 38 43 or cell phone 044 55 13 53 24 74.

In case I have any questions regarding my rights as a research subject, I can contact the Ethics Committee at telephone number 27 89 20 00, extension 1330, with Dr. Estela García Elvira, who is the Chair of the Ethics Committee of the General Hospital of Mexico "Dr. Eduardo Liceaga."

By giving my consent, I acknowledge my participation in this research project is voluntary and I can refuse or withdraw my participation at any time without penalty or loss of benefits. My signature below means that I have read this consent form, understood its contents and that all my questions regarding this study have been answered by the doctor of the study and its staff. It also means that I agree that my personal health information may be used and transferred in the ways described in this informed consent form and that my personal health information may be added to research databases and used in the future by the research team to develop a better understanding of the effect of sucralose on insulin, glucose and other substances related to metabolism and chronic inflammation, develop a better understanding of the disease (s) included in the study and improve the efficiency, design and methods of study in future clinical investigations. I understand that by signing this consent form I will not lose any of my legal rights as a research subject. I will receive a copy of this fully signed and dated consent form.

Participant

_____	_____	_____
Name	Signature	Date

Witness 1

_____	_____	_____
Name	Signature	Date

_____

Address

_____

Relation with the participant

Witness 2:

Name	Signature	Date

Address

Relation with the participant

Person who has explained the document:

Name	Signature	Date