

Study protocol

México CDMX. a January 20th 2016

Effect of Acute or Chronic Ingestion of Sucralose on Serum Insulin in Young and Healthy Adults: A Randomized, Double-Blind, Placebo-Controlled Trial.

Background

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Materials and Methods

Ethical considerations

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Background

Since the last 30 years, the prevalence of overweight and obesity have been considerably increasing worldwide (1); this is directly associated with a greater incidence of non-transmissible chronic disease such as Type 2 Diabetes Mellitus (DM2) (2, 3). Consequently, in recent years the consumption of Non-Caloric Sweeteners (NCS) has increased in young and healthy adults as a healthy alternative to reduce the ingestion of calories with the sweet taste in food or beverages (4). One of the NCS (No Caloric Sweetener) more consumed in the world is the sucralose, because is the 600 times sweeter than sugar but without calories intake (5, 6).

Nevertheless, there are some studies that suggest a correlation between high consumption of NCS and high levels of blood glucose, insulin, and metabolic hormones as C-peptide, Gastric Inhibitory Polypeptide (GIP) and Glucagon-Like Peptide-1 (GLP-1), these systemic inflammatory factors can have an impact role on the development of DM2, obesity and cardiovascular diseases (7). On the other

hand, a study in animal models shown that the chronic exposure to sucralose for 10 weeks increases the values of area under the curve (AUC) of glucose tolerance test, which was attributed to a change of the intestinal microbiota (8). However, these studies did not take in to consideration the effect of acute or chronic exposure to NCS over the systemic inflammatory response; which has been repeatedly documented as a previous step in the development of glucose intolerance in the obese patient (9-11).

Furthermore, it has not been studied until now whether the acute or prolonged consume of NCS such as sucralose is capable to cause a systemic inflammatory state and if this is related to the development of glucose intolerance in healthy individuals. This has generated in the scientific community a polemical discussion about the possible association between the consume of sweeteners and the development of glucose intolerance; which is characterized by the elevation in the AUC of insulin, glucose, C peptide, glucagon, GIP and GLP-1, as well as the rise of systemic inflammatory factors. Whence the aim of this research was measure the effect of the acute and chronic exposure to sucralose on blood glucose, insulin, C-peptide, GIP and GLP-1 levels and the reduction of interleukins 4, 10, 13 in young and healthy adults.

Relevance of the study

Until now, the international agencies safety assessment of non-caloric sweeteners has been enough to recommend their use in humans. This confidence has led to a mass production and use of these additives for the western population of all ages, complemented by the absence of epidemiological evidence focused on trade

adversities to the consumption of these substances. However, recent studies have revealed some studies that relate a state of glucose intolerance and insulin resistance with the chronic consumption of some of these available in the market products, as in the case of sucralose. Unfortunately, the results of these studies have been criticized for the lack of methodology robustness used and for omitting the presence of other markers that are directly involved with metabolic dysfunctions; the only consideration was the symptom of sucralose on the intestinal microbiota. Therefore, it is necessary to determine if the acute or chronic consumption of sucralose is capable of inducing a glucose intolerance and insulin resistance state in humans, through the performance of a dependable robust prospective, randomized, double blind study and placebo controlled, which allows us to issue reliable recommendations on the habitual use of non-caloric sweeteners in our population.

Hypothesis

In healthy, young volunteers, subjected to an oral glucose tolerance curve of 180 minutes, acute or chronic exposure to 48 - 96 mg of sucralose in a single dose or 10 weeks daily intake, respectively, causes an increase in the average levels of the area under the curve of insulin, glucose and other intolerance to this carbohydrate markers when compared with water as a placebo.

Principal outcome

To evaluate the effect of acute and chronic exposure to 48 - 96 mg of sucralose in a single dose or for 10 weeks, respectively, on the average levels of the area under

the insulin curve in healthy, young volunteers, subjected to an oral glucose tolerance curve at 180 minutes, in a randomized, double-blind, placebo-controlled study.

Materials and Methods

This is a randomized, double-blind, placebo-controlled trial in young and healthy adults, the study at the General Hospital of Mexico, Dr. Eduardo Liceaga from April 2016 to June 2018, and the present study meets with the criteria of CONSORT.

To evaluate the acute effect of sucralose, the experimental groups will receive 48 mg / 60 ml and 96 mg / 60 ml of fasting sucralose in a single shot, while the control group will receive 60 ml of water as a placebo, before undergoing a CTOG with 75 g of glucose during 180 min. During this procedure, 4 ml of blood will be taken at -15 (sucralose intake or placebo) and 0 (75 g glucose intake) as a base and then at 15, 30, 45, 60, 75, 90, 105, 120 and 180 minutes post-load glucose for the quantification of insulin, glucose, C-peptide, glucagon, GLP-1, and GPI levels. Subsequently, to evaluate the chronic effect of sucralose, the experimental group will continue with the daily intake of 48 – 96 mg / 60ml of sucralose for 10 consecutive weeks, while the control group will receive 30 ml of water daily as a placebo during the same period of time. At the end of 10 weeks of treatment with sucralose or placebo, a second CTOG (75g / 180 min) will be carried out, taking again 4 ml of blood at -15 and time zero, and subsequently 4 ml at 15, 30, 45, 60, 75, 90, 105, 120 and 180 minutes after glucose loading. To evaluate the acute exposure to the sweetener, the area under the curve (AUC) of the blood glucose levels obtained during the CTOG in each participant will be calculated and the average differences

between the sucralose groups versus placebo will be compared, adjusting for BMI. In the case of chronic exposure to the sweetener, the AUC values between the sucralose groups versus placebo will be compared after 10 weeks, and the AUC values will be compared at the beginning and end of the study in the group that received sucralose, adjusting in both cases for BMI. In addition to the determination of glucose levels during the two CTOGs, the circulating levels of insulin, glucagon, C peptide, GIP, GLP-1, TNF- α , pCr, IFN- γ , IL-1 β , IL-4, IL-6, IL-10, IL-12, IL-13, IL-17, IL-23, and inflammatory monocytes CD14hiCD16 + CD11c + CCR2hiCX3CR1lowCD206-, with the purpose of comparing their values in the acute exposure scheme and chronic to sucralose, adjusting for BMI. The samples of excrement from acute and chronic exposure will be processed to extract the DNA and run them in PCR in real time and the percentage of each bacteria present in feces will be compared at the beginning and at the end. The difference between the two samples will be analyzed by means of square chi.

Ethical considerations

This study will be carried out in compliance with the guidelines of the protocol, with the ethical principles of the Declaration of Helsinki in its most recent version approved during the 64th Assembly of the World Association of Physicians, in Fortaleza Brazil, in October 2013, in accordance to the principles that govern human research in the General Health Law and in accordance with the ethical and research guidelines of the General Hospital of Mexico "Dr. Eduardo Liceaga.

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