

Statistical Analysis Plan

México CDMX. 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.

The statistical analysis will help us to provide accurate and quantifiable data in order to increase the documented information about the sucralose; this analysis will also help us to have a better reliability on the study results.

Estimation of sample size

Considering the results on the difference of the averages of the areas under the curve for plasma insulin in obese volunteers exposed to sucralose and water, before an oral glucose load, as reported by Pepino et al., [1], the estimation of the Sample size was:

t tests - Means: Difference between two independent means (two groups)

Analysis: A priori: Compute required sample size

Input:

Tail(s)	=	One
Effect size d	=	0.419231264
α err prob	=	0.05
Power (1- β err prob)	=	0.8
Allocation ratio N2/N1	=	1

Output:

Noncentrality parameter δ	=	2.5153876
Critical t	=	1.6556552
Df	=	142
Sample size group 1	=	72
Sample size group 2	=	72
Total sample size	=	144
Actual power	=	0.8046976

For the estimation of the sample size the program GPower version 3.1.9.2 (University of Düsseldorf, Germany) was used. The difference between two

averages was estimated by means of a test of t, of an address, with an alpha value (0.05), assuming unequal variances, for a power of proof of 80%, a value of Cohen of 0.419231264, the sample size is 72 volunteers per group, the total sample size is left in 144 volunteers.

The demographic analysis will be assessed with descriptive statistics, ensuring the two groups are balanced in their moments for the main variables.

The statistical analysis of the presence or occurrence of any inflammatory process during the the study will be carried out by comparing the before / after differences between the sucralose and water groups for the whole sample, adjusting for BMI, these differences will be compared for each analyte related to chronic inflammation such as C-reactive protein, tumor necrosis factor-alpha, interleukin (IL) 6, IL-12, IL-10 and inflammatory monocytes CD11c + CCR2High.

In order to calculate the area under the glucose tolerance test curve will be assessed using the trapezium method [2] for glucose, insulin, C-peptide, glucagon GPI and GLP-1.

The statistical analysis of the oral glucose tolerance curves will be carried out by means of an analysis of variance test for repeated measurements and for the area under the curve of each of the metabolic analytes.

For each of the differences between the area under the initial and final curve of glucose, insulin, C-peptide, glucagon, GPI and GLP-1 between sucralose and water, an ANCOVA will be made, taking the BMI as a covariate.

For the analysis of the behavior of the intestinal microbiota, a comparison of the percentages of the flora between the first and the last sample of excrement will be carried out, and a chi square test will be applied.

The correlation between the initial HOMA-IR value and the glucosylated hemoglobin will be explored.

- 1 Pepino MY, Tiemann CD, Patterson BW, *et al.* Sucralose affects glycemic and hormonal responses to an oral glucose load. *Diabetes Care* Published Online First: 2013. doi:10.2337/dc12-2221
- 2 Allison DB, Paultre F, Maggio C, *et al.* The use of areas under curves in diabetes research. *Diabetes Care* Published Online First: 1995. doi:10.2337/diacare.18.2.245