Study title: Randomized, cross-over study comparing the effect of two alcohol-free beers with different carbohydrates composition on lipid and glycemic metabolism in subjects with prediabetes and recently diagnosed diabetes and overweight or obesity.

NCT number: Not yet assigned.

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Title

Randomized, cross-over study comparing the effect of two alcohol-free beers with different carbohydrates composition on lipid and glycemic metabolism in subjects with prediabetes and recently diagnosed diabetes and overweight or obesity.

Brief title: Effect of two alcohol-free beers with different carbohydrates composition on lipids and glucose metabolism.

Objective:

Primary: To determine the effect of an alcohol-free beer with low glycemic index carbohydrates (isomaltulose) and a resistant maltodextrin, comparing to an alcohol-free beer with regular composition, on glycemic metabolism (glucose, glycated hemoglobin, insulin and HOMA index) in subjects with recently diagnosed diabetes mellitus and overweight or obesity.

Secondary: To determine the effect of an alcohol-free beer with low glycemic index carbohydrates (isomaltulose) and a resistant maltodextrin, comparing to an alcohol-free beer with regular composition, on: a) lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides and apolipoproteins A and B); b) body weight and waist circumference; c) inflammatory biomarkers (C reactive protein); d) intestinal microbiota in a subgroup of participants.

Inclusion criteria:

- Aged between 18 and 80 years.

- To provide informed consent in writing of voluntary participation in the study after having read the participant information sheet, and having consulted the doubts that may arise from it.

- Diagnosis of prediabetes or diabetes mellitus type 2 defined as: a) fasting glucose $\geq$ 100 mg/dL in the last 3 months; b) glycated hemoglobin $\geq$ 5.7% and $\leq$ 6.5%.

Exclusion criteria:

- Gluten intolerance.
- Taking lipid-lowering drugs.
- Taking antidiabetic drugs, except for metformin in a stable dose in the last 3 months.
- To be under treatment with insulin.
- Presence of uncontrolled endocrinological disease by including hypothyroidism.
- Regular intake of functional foods with plant sterols in the past 6 weeks.
- Intake of vitamin supplements.
- Hormone replacement therapy.
- High intake of alcohol (> 30 g ethanol) on a regular basis.
- Pregnancy or intention of pregnancy during the study since the proposed nutritional intervention may not be suitable for this situation.
- Serious illness of any type with less than 1-year life expectancy or if, in the opinion of the investigators, it would limit a stable diet throughout the study.
- To be under treatment with corticosteroids, hormonal treatment or antibiotics the 3 months prior to randomization.
- To take prebiotics, probiotics, vitamin supplements and any other drug that could influence the intestinal microbiota, in the 3 months prior to randomization.
- Any other circumstances which, according to researcher' s assessment, could interfere with the correct development of nutritional intervention (e.g.: frequent trips during the study, failure to attend visits by personal or business circumstances, etc.).

Brief Summary:

This a controlled, double-blind, randomized, cross designed study to determine the effect of an alcohol-free beer with low glycemic index carbohydrates (isomaltulose) and a resistant maltodextrin, comparing to an alcohol-free beer with regular composition, on glycemic metabolism (glucose, glycated hemoglobin, insulin and HOMA index) in subjects with recently diagnosed diabetes mellitus and overweight or obesity. We include 44 subjects who are randomized to consume for 10 weeks: a) two alcohol-free beers with regular carbohydrates composition per day; b) two alcohol-free beers with modified carbohydrates composition per day. Those subjects randomized to begin with A beer

during 10 weeks will change to B beer during the second phase for 10 weeks and vice versa. There is a 4-8 weeks wash-out period between two phases.

Study Detailed Description:

The study involves a nutritional intervention with a controlled, double-blind, randomized, cross design. It includes 44 healthy subjects with a total duration of 20 weeks with a "wash-out/stabilization" period for 2-4 weeks before randomization. Subjects are randomized in two groups: (1) those who begin drinking 2 alcohol-free beers (33 cl each one) that are enriched in a resistant maltodextrin and isomaltulose during 10 weeks and continue drinking 2 alcohol-free beers (33 cl each one) with regular composition during the next 10 weeks following; (2) those who will follow the same previous intervention but in reverse, beginning with standard alcohol-free beer for 10 weeks, followed by resistant maltodextrin and isomaltulose-enriched beer for the remaining 10 weeks. There is a washout period between both phases to facilitate adherence to nutritional intervention, since we consider that a period of 20 consecutive weeks could be difficult to assume for the participants. Participants and the research team are "blind" to the type of beer that subjects are taking in each phase. Only one person of the company that provide beers, which does not directly participate in the clinic visits or in the analysis of the data, is aware of this information. Clinical staff performed the randomization based on a computerized method.

Among those subjects included in the study, microbiota sub-study is proposed which require a specific authorization within informed consent. Among all subjects that are included in this substudy and that have completed the whole nutritional intervention, 10 subjects (5 from each randomized group) will be selected to microbiota determination.

Study visits:

1. Prescreening visit: Inclusion and exclusion criteria assessment and blood collection if glucose or glycated hemoglobin is not available in previous 3 months. Healthy dietary and physical activity counselling is provided by a nutritionist; it should be maintained during all study. Baseline visit is scheduled in 2-4 weeks after lifestyle stabilization.

2. Baseline phase 1 visit: Clinical, biochemical, dietary, physical activity and anthropometric data are assessed. Computer-based randomization is performed. Alcohol-free beer (A or B according to randomization procedures) for the next 10 weeks is delivered to the participant who has to consume two per day. A nutritionist emphasizes healthy lifestyle counselling to the participant. A feces sample is collected if subjects accept to be included in microbiota sub study.
3. Intermediate phase 1 visit: Clinical, biochemical, dietary, physical activity and anthropometric data are assessed. Satiety after alcohol-free beer consumption is measured by a validated questionnaire. A nutritionist emphasizes healthy lifestyle counselling to the participant.
4. End of phase 1 visit: Clinical, biochemical, dietary, physical activity and anthropometric data are assessed. Satiety after alcohol-free beer consumption is measured by a validated questionnaire. A nutritionist emphasizes healthy lifestyle counselling to the participant. A wash-out period of 4-8 weeks is scheduled before the next phase start. A feces sample is collected if subjects accept to be included in microbiota sub study.
5. Baseline phase 2 visit: Clinical, biochemical, dietary, physical activity and anthropometric data are assessed. Alcohol-free beer (A or B: if subjects consumed alcohol-free beer A, he/she change to alcohol-free B and vice versa) for the next 10 weeks is delivered to the participant who has to consume two per day. A nutritionist emphasizes healthy lifestyle counselling to the participant. A feces sample is collected if subjects accept to be included in microbiota sub study.
6. Intermediate phase 2 visit: Clinical, biochemical, dietary, physical activity and anthropometric data are assessed. Satiety after alcohol-free beer consumption is measured by a validated questionnaire. A nutritionist emphasizes healthy lifestyle counselling to the participant.
7. End of phase 2 visit: Clinical, biochemical, dietary, physical activity and anthropometric data are assessed. Satiety after alcohol-free beer consumption is measured by a validated questionnaire. A nutritionist emphasizes healthy lifestyle counselling to the participant. A feces sample is collected if subjects accept to be included in microbiota sub study.

Study variables:

- Clinical variables: Gender, age, medical records including diseases and medication, tobacco consumption and blood pressure.
- Anthropometric variables: Weight, height, body mass index, waist circumference, body composition analysis.
- Biochemical variables: Glucose, lipid and iron metabolism are assessed. Inflammatory biomarkers, including C reactive protein, are also determined.
- Microbiota: Feces samples are collected to microbiota analysis including *Bacteroides*, *Lactobacillus*, *Enterococcus*, *Prevotella* or *Roseburia*, among others.
- Dietary and physical activity assessment: Diet and physical activity are assessed by validated questionnaires.

Informed consent

All participants must sign a consent before entering the study. Informed consent is obtained by one of the researchers of the study after the patient has carefully reviewed the information sheet which explains the objectives, methodology, benefits and disadvantages of intervention. The participant is encouraged to ask for any doubt he/she has regarding the study before signing the informed consent. Informed consent includes a section where participant has to indicate if he/she wants to participate in the microbiota substudy. If they do not accept, they are included in the study if meeting eligibility criteria, but feces samples are not collected in corresponding visits.

The study, including protocol, informed consent, information sheet and other documents, has been approved by the Ethical Committee of Clinical Research of Aragon (CEICA).

Statistical analysis plan

The sample size was established by taking as main outcome variable the change of glucose concentration, whose clinical variability is estimated at 16 mg/dL in pre-diabetic population. This is the estimated value based on the epidemiological study carried out in Spanish population (PREDIMED) (Estruch R et al. N Engl J Med 2013;368:1279-90). It was expected to find a difference of 12 mg/dL in glucose levels between two intervention

arms. It was established a confidence level (1-α) of 95% (bilateral Zα = 1,960) and 90% of statistical power (1 – β) (bilateral Zβ = 1,282), by obtaining a sample size of 37 subjects. It was estimated a 25% loss rate, so final sample was 44 subjects.

Different alcohol-free beers effects on study outcomes will ve evaluated by repeated measures ANOVA or the Friedman test, as appropriate. When significant differences are detected, multiple comparisons will be made by using the Bonferroni correction for normally distributed variables or the Wilcoxon test for paired samples for variables with a skewed distribution. Treatment order will be entered in all models as an additional factor. Data will be presented as means (±SD) for continuous variables or medians and interquartile ranges (IR) for those with a skewed distribution). Statistical analysis will be processed using the software SPSS (version 24.0; SPSS, Chicago, Illinois, USA), using a significance level of $P < 0,05$.