

**PROTOCOL clinictrials.org**

**Subjects and study design**

The calculation of the sample size in the present study has been done so that we ensure a power of 90%, in order to lower the conversion rate to DT2 up to 5% in the sardine group, compared to the 20% conversion in the control group. Thus, with a level of significance of 0.05 and using the statistical program STATA (STATA.11 software), it was calculated that 114 patients would be required per group. Considering a 10% abandonment rate, 125 patients would be required for inclusion visits. Recruited patients will come from three primary care centers in Barcelona (CAP Les Corts, Casanova and Borrell). After recruitment, the 250 participants had to be assigned to the sardine group (125 people) or the control group (125 people). (Figure 1)

Individuals aged 65 years old or more with a fasting diagnosis of altered basal glycemia (100-125 mg/dL) interested in joining the nutritional intervention could do it provided that the informed consent was obtained before starting any activity related to the study.

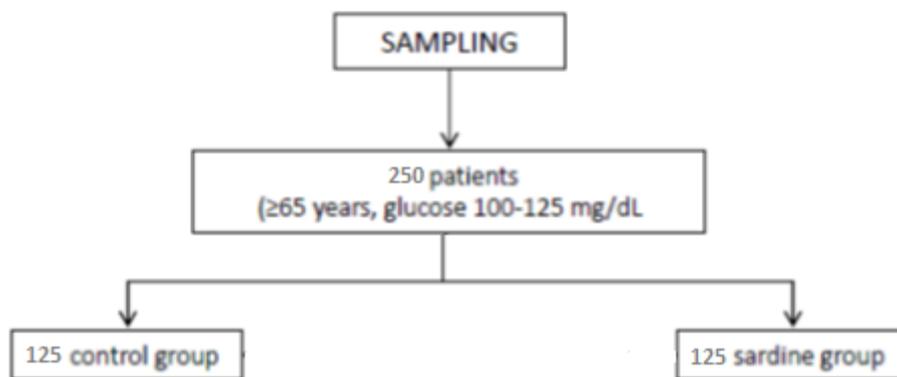
Otherwise, the exclusion criteria were the following: suspicion or consciousness of hypersensitivity to sardines, chronic treatment with oral steroids and/or AINEs, treatment with oral antidiabetic drugs or insulin, treatment with immunosuppressive drugs, diagnosis of an active neoplasia, diagnosis of VIH or SIDA, abnormal hepatic profile (>6-fold normal values), diagnosis of acute psychiatric syndrome, presence of acute concomitant disease which required more than 7 days for recovery, major cardiovascular event (ictus or myocardial infarction) during the previous month to randomization and any other condition considered as inopportune by researchers.

**Dietary assessment**

All the recruited individuals were randomly distributed among two groups: the control group (CG) and the sardine group (SG).

Both groups received the same nutritional education, based on ADA clinical guidelines. The nutritional recommendations included intaking carbohydrate from vegetables, legumes, whole grains or legumes, avoiding those with higher glycemic load. A dietary plan similar to Mediterranean diet was also recommended, emphasizing monounsaturated and polyunsaturated fats, and those foods rich in long  $\omega$ 3-FA such as nuts and seeds. Alcohol should have been limited. Emphasis was placed on the plate method (ADA) for the correct distribution of macronutrients. Group education was performed before starting the study and every four months, in order to reinforce nutritional knowledge.

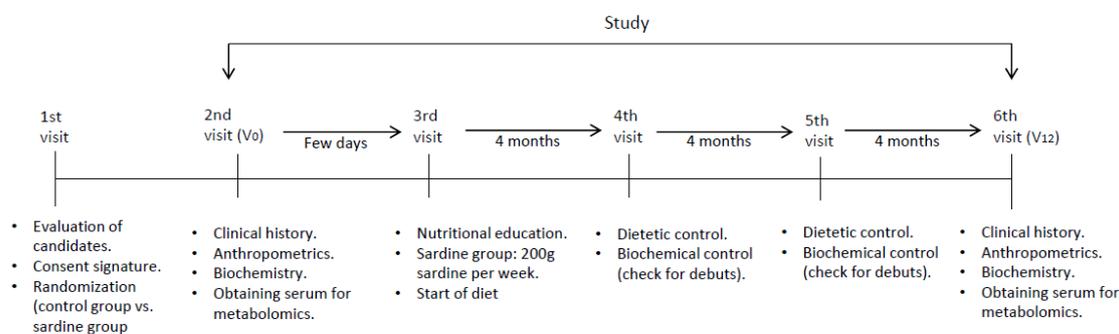
Besides, the SG received 200g of sardine per week. Sardines were canned in olive oil and, in order to obtain more accurate results, they were provided by IDIBAPS.



**Figure 1.** Overview of the recruitment process.

### Visits

As shown in Figure 2, a total of six visits were programmed during the year of the clinical study.



**Figure 2.** Overview of the 12 months visits programming.

The first visit was used to evaluate the candidates, to obtain informed consent and to do the randomization so in the second visit candidates could start the intervention. In order to perform gene expression analysis, serum was obtained at the second visit (V0) and at the last visit (V12).

### Ethical issues

The study will be carried out in compliance with the Declaration of Helsinki (current version, currently Fortaleza, Brazil, October 2013) and in accordance with the protocol and with the pertinent legal requirements, Law 14/2007 of July 3 on Biomedical Research.

Permission has been secured from all participants to use the aforementioned data in the present proposal, in the form of signed written consent, prior to the start of the project.

### Data management

The data we plan to collect and generate within the framework of our proposal will have the purpose of answering questions regarding the mechanisms underlying a sardine-rich diet's beneficial effects on T2D prevention.