Beneficial Effects of Quinoa (Chenopodium Quinoa Willd) in the Prevention of Type 2 Diabetes Mellitus

version 1.
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Study protocol

This study was a pilot clinical trial according to a cross-over design developed in IDIBAPS-Hospital Clinic de Barcelona.

Participants

Subjects included, from whom informed consent was obtained for experimentation and study was performed in accordance with The Code of Ethics of the World Medical Association, were males and females ≥65 years old with glucose levels between 100 and 125 mg/dL and without a previous diagnosis of diabetes. Participants were excluded from the study if they did not consume a diet with daily presence of grains or cereals derivatives, tubers or/and legumes, or they presented any other health problem that the research staff considered contraindicated.

Nine subjects were included and all participants provided written informed consent, and the study protocol was approved by the Ethics Committee of Hospital Clinic de Barcelona.

Measures

- Anthropometrics: Subjects were weighed and measured without clothing and shoes. BMI was calculated as weight (kg)/height (m)^2, and waist circumference was measured at the midpoint between the last rib and the iliac crest and hip circumference at the widest point of the gluteus.
- Blood pressure was taken after individuals sat quietly for 5 min in a clinical examination room. The mean of three different measurements, obtained every 3 min using an OMRON M6 AC sphygmomanometer, was recorded.
- Fasting blood samples were collected by a nurse and they were analyzed by the Biomedical Diagnosis Centre (CDB) in Hospital Clinic de Barcelona.

- Nutritional patterns were measured using a 14-days dietary record revised corrected by a nutritionist and analyzed by the validated DIAL nutritional calculation program.
- Glucose fluctuations were measured by FreeStyle Libre® Flash Glucose Monitoring System (Abbott Laboratories) which measures interstitial fluid glucose concentrations. The sensor was applied by researchers on to the back of the upper arm of subjects using the applicator and participants were trained to obtain electronically all the glucose records concentrations every 15 minutes so they had to scan at least once every eight hours.

Study design

The study was a cross-over pilot clinical study consisting of two periods. The first period was only an observational and monitoring phase where participants just continued with their regular diet (RD), for this reason all participants initiated this period and wash-out term was no needed. Subsequently, with the data of the first phase obtained, the subjects began the second period in which they had to undergo a nutritional intervention with a quinoa diet (QD).

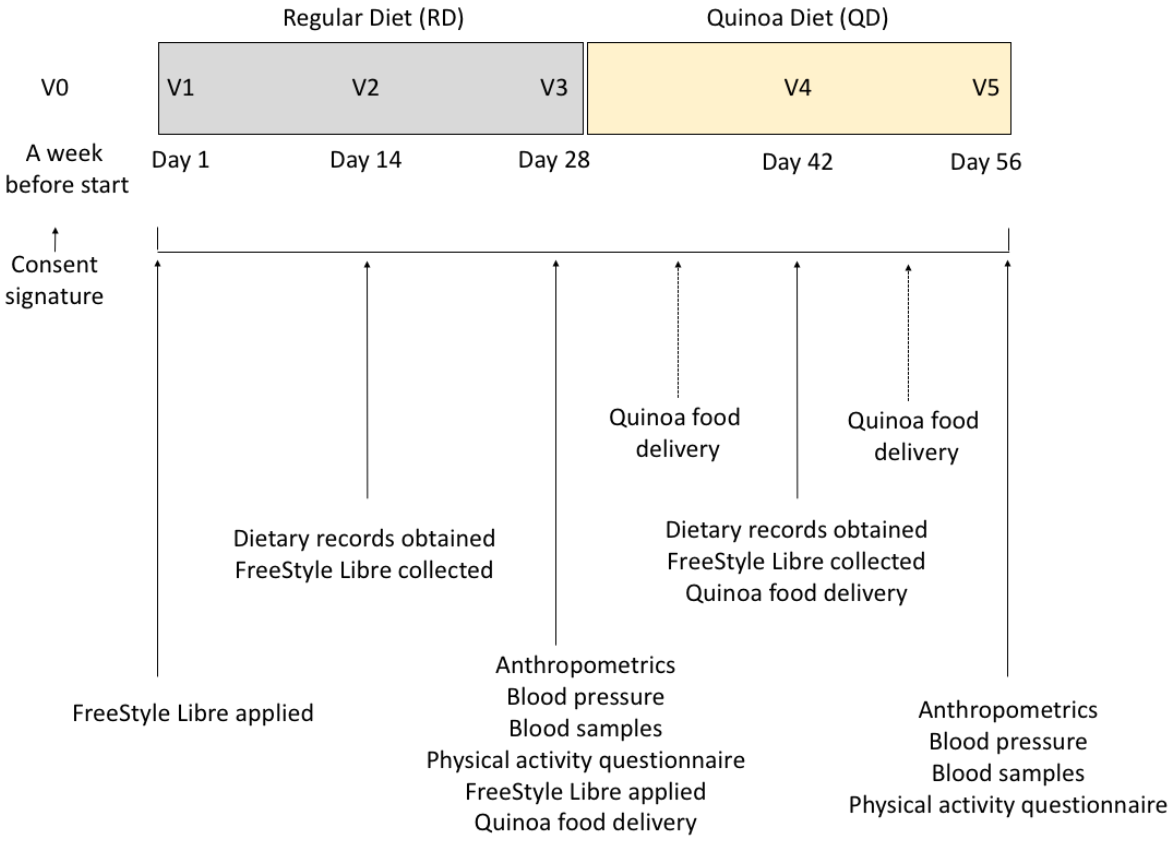
A total of six visits plus two quinoa products collection days were programmed (**Figure 1)**. After the pre-study visit (V0) which took place a week before start nutritional intervention and where researchers obtained signed informed consent, participants were summoned for a first visit (V1) where they were explained how they should fill in the dietary records and they were applied with the FreeStyle Libre®.

Subjects then began RD, a period of 4 weeks during which only their normal life was monitored. After the first 14 days of this, a second visit (V2) was made where the dietary record was collected that would serve to account for their usual consumption of cereals, flours, tubers and legumes, the FreeStyle Libre® sensor was also collected.

The last day on RD period, on day 28, they were cited (V3) in consultation where blood samples after an 8 hours fast, anthropometrics measurements and blood pressure measure where obtained and for the placement of the new FreeStyle Libre® sensor. Participants were asked about their physical activity and exercise practice during those past 4 weeks by a short questionnaire adapted from the Minnesota Leisure Time Physical Activity Questionnaire for individuals of advanced age (VREM questionnaire) and a new empty 14-day dietary record was given. In addition, the volunteers received the first foods with quinoa to initiate QD the next day. Products were delivered weekly, for conservation reasons but also to ensure that they followed an adequate consumption, they had to go through consultation to pick up the product and gave the researchers the empty packs where quinoa products had been.

On the next visit the day 42 (V4) the Freestyle Libre® sensor was collected and the filled dietary record was collected. Finally, after 28 days of quinoa diet they were summoned for the last visit the day 56 (V5) where all the determinations were repeated identically as V3.

Figure 1. Study design and visits



Study food

With the premise that the products created replaced not only grains, legumes or tubers, but also farinaceous commonly consumed by the participants and that only the cereal fraction was modified, similar products based on quinoa flour were created. The creation of these products was necessary, after conducting a market search where it was observed that there was not enough food to replace those consumed since these had percentages of quinoa flour not exceeding 20-30%.

Thus, apart from delivering quinoa, quinoa flakes and quinoa flour to the participants, they were given products created with ≥70% quinoa flour and were biscuits, crackers, brioche, sponge cake, baguette bread, sliced bread and pasta (**Table 1**). Moreover, a quinoa-based recipe was delivered with eight commonly consumed recipes that replaced the tuber, legume or grain of the recipe. Each subject received the equivalent of what they

consumed according to their RD dietary records. Thus, only if the volunteer had indicated that he consumed sponge cake was the quinoa-based product delivered to him.

Table 1. Comparison of nutritional values of products marketed in a regular diet with similar ingredients than products based on quinoa for the study.

	Energy		Proteins		Fats		Carbohydrates	
	RD	QD	RD	QD	RD	QD	RD	QD
Cookie	456	366,43	6,14	9,11	21,05	16,15	63,1	49,5
Cracker	400	352,83	10	9,75	10	16,9	65	35,54
Brioche	205	278,69	7,69	9,72	2,56	7,87	38,46	39,23
Sponge cake	388	300,14	6,25	11,49	16,25	18,18	53,75	24,55
Sliced bread	344	202,87	6,25	6,86	12,5	7,66	50	24,94
Baguette bread	263	210,35	7,02	6,95	1,75	4,96	50,88	29,63
Pasta	357,14	323,45	12,5	12,12	1,79	12,3	75	35,68
gr/100gr of product								

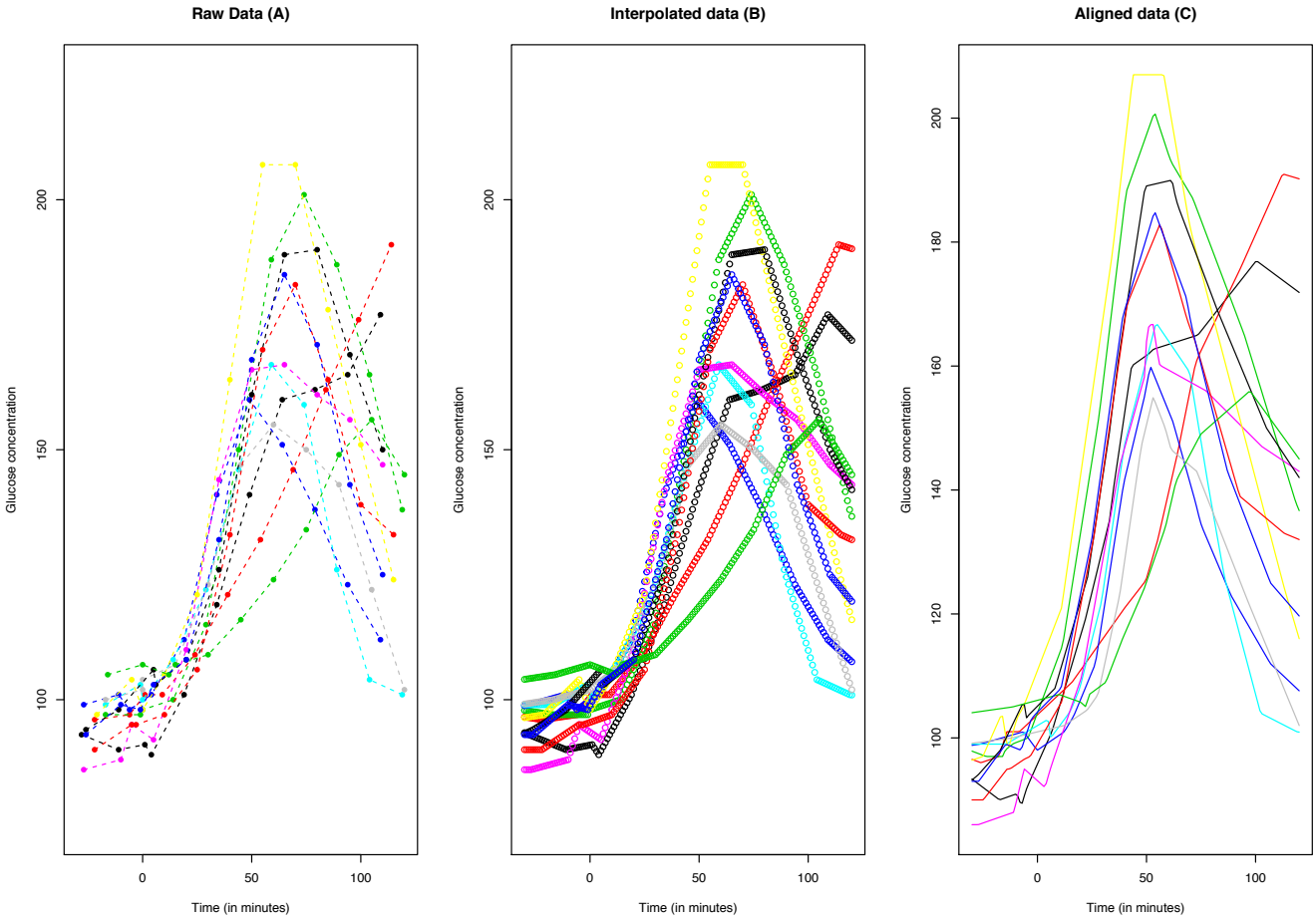
Statistical analyses

Calculations and Statistical Analyses

Descriptive data are presented as the mean and standard deviation (SD) or median and interquartile range (IQR) for continuous variables, and the frequencies and percentages (%) for categorical variables. Anthropometric measurements, blood test variables and dietary intake were compared at different times using the non-parametric Wilcoxon signed rank test because normality and equality of variance could not be assumed due to small sample size (n=9). In order to compare variables related to dietary patterns, mean value for dietary intake, including all meals, was considered for each participant.

The glucose level monitoring sensor takes measurements at discrete time points for each patient (**Figure 2 A**). Therefore, firstly the glucose curves have been linearly interpolated in order to have observations for each patient at equal time points (**Figure 2 B**). A first sight to the glucose curves over the day shown that they were more homogeneous around breakfast than around other later meals intakes. Therefore, the glucose concentration values corresponding to the breakfast were considered as a function of time in minutes over the interval t= [-30,120], that begins half an hour before the start of breakfast and ends two hours later. Before constructing a functional model, the functional data were time aligned in order to reduce the differences between different patients and/or different days (for instance, some patients could mark the starting time of breakfast systematically before than others, or spend systematically more time in breakfast than the average) (**Figure 2 C**). The time alignment has been done by warping functions, using the function WFDA in the R package fdapace.

Figure 2. Glucose concentrations of patients (A) at discrete time points (B) at equal time points with interpolation (C) as aligned curves



Once the glucose level curves have been synchronized, a functional regression analysis was conducted to model the effect of diet type, patient and nutrient intake on monitored glucose levels. Three different explanatory variables have been considered: diet type with two categories (regular and Quinoa diets), patient indicator (categorical variable with nine levels) and the contents in different nutrients. The breakfast glucose curves are handled as the functional response variable. To study the relationship between these variables function on scalar regression (fosr) models were used.

Firstly, the univariate effect of the diet type on glucose curves have been analyzed and then more complex fosr models with two factors (diet and patient factors) and scalar variables (nutrients) have been constructed. The most complex model including all effects of the independent variables is defined by the equation

$$g_{ij}(t) = \beta_0(t) + I_R^j \beta_R(t) + I_Q^j \beta_Q(t) + \alpha_i(t) + \sum_{k=1}^{K} x_k^{ij} \beta_k(t) + \varepsilon_{ij}(t),$$

where i indicates the patient ($i = 1, \dots, 9$), j indicates the day of observation ($j = 1, \dots, 28$), $g_{ij}(t)$ is the glucose curve observed the day j for patient i, I_R^j takes value 1 for regular diet and 0 for Quinoa diet, $I_Q^j = 1 - I_R^j$ is the indicator of Quinoa diet, x_k^{ij} is the content of nutrient k in the breakfast that patient i takes in the day j ($k = 1, \dots, K$) and $\varepsilon_{ij}(t)$ is a random noise function with expected value 0 for all t and independent from noise function corresponding to other days or patients. According to this model, the overall shape of glucose curves is described by the functional coefficient $\beta_0(t)$, the diet effect is summarized by the functions $\beta_R(t)$ and $\beta_Q(t)$ (by construction, they verify that $\beta_R(t) = -\beta_Q(t)$), functions $\alpha_i(t)$ measure the individual effects (by construction, $\sum_{i=1}^{9} \alpha_i(t) = 0$) and $\beta_k(t)$ function shows the effect of every nutrient k. The functional

regression models have been fitted by penalized flexible functional regression, as implemented in the function pffr of the R package refund.

Informed consent

INFORMED CONSENT

Beneficial effects of quinoa (Chenopodium quinoa Willd) in the prevention of type 2 diabetes mellitus

Research project: Dietary intervention study to investigate the beneficial effects of quinoa consumption in the prevention of Type 2 Diabetes mellitus in a population over 65 years of age.

Department: Endocrinology and Nutrition, Hospital Clínic, IDIBAPS.

Objectives: We request your participation in this research project, whose main objective is to delve into dietary factors that can help in the prevention of type 2 Diabetes in a population over 65 years of age, and who are at risk of developing said disease.

Participation in the study: Participation in this study is completely voluntary and it is possible to leave the study at any time, without implying a change in your future medical care or in your relationship with the doctor who normally treats you.

Before starting the study, the doctor will ask you about your medical history to find out if you meet the necessary requirements to be part of the study and assess your eligibility.

Benefits: There may be no direct benefit from your participation in this study. However, the identification of foods (such as quinoa) capable of providing a direct benefit in the prevention of type 2 diabetes mellitus could benefit other patients at high risk of suffering it in the future, and contribute to an improvement in the prevention and treatment of this disease.

Study procedures: The study will have a total duration of 8 weeks, the first 4 weeks you will continue with your usual diet, from week 5 to 8 you will be prescribed a diet with the same calories that you normally consume but with quinoa. The study will be structured in several visits as explained below.

Visit (-1)

The researchers will assess your eligibility to participate in the study. If you meet all the criteria to participate, the whole study will be explained to you, and if you agree to participate, you will have to sign the informed consent.

Subsequently, a detailed medical history and physical examination will be carried out, and the nutritional intervention that will continue for 8 weeks will be explained.

It may be a healthy diet for your physiological situation. Therefore, you will have a team of nutritionists who will explain well how to carry out the diet correctly.

Visit 0

It will take place in the 2 weeks after visit -1, you will receive a nutrition education with a group session. Your good understanding of the diet will be assessed. It is very important that you get involved with the recommendations that have been given to you. Good compliance is crucial to assess the possible beneficial effect of quinoa in the prevention of Type 2 Diabetes mellitus.

First week (1: start of study):

Blood samples will be taken from you. For this reason, you will have to go to the center on an empty stomach (8 hours of fasting). You will also be given questionnaires on your general health status, on the physical activity you practice, and questionnaires on your diet for 3 days, which you must bring filled out to the next visit (week 3). During this visit, the patients will receive sterile material for the collection of fecal samples that they must bring in week 4 of the study. In this visit, a glucose sensor will be placed, which will be changed every 2 weeks, that is, the change will be made in the visit of week 3.

Weekly:

You will be called by phone on a weekly basis to find out if you are following the diet correctly and whether this diet is causing any harmful effects on your health. A visit with a nurse or physician may be scheduled in connection with this call.

Every 2 weeks (weeks 3, 5, 7):

Your glucose sensor will be replaced. The sensor will be removed in week 8.

The diet questionnaires that you were given to fill out in weeks 1, 3, 5, and 7 will be collected.

Anthropometric data (weight, height, waist and blood pressure) will be taken.

Adverse clinical data will be recorded, and if necessary, a detailed physical examination will be performed. Adherence to diet will be considered. The nutritionist will control the correct understanding and monitoring of the dietary education of the patients. In addition, the patient will also be encouraged to adhere to the diet and any specific difficulties related to the quinoa diet will be evaluated.

Weeks 1, 4 and 8.

Blood samples will be taken for analysis. For this reason, you will have to go to the center on an empty stomach (8 hours of fasting). In weeks 1 and 4 the patient will be given a sterile stool sample bottle. Fecal samples will be collected at week 4 and 8.

Discomforts and possible risks:

Taking blood samples may cause a burning sensation where the water enters the skin and cause a small bruise or a mild infection that clears up within a few days.

More rarely, dizziness may appear at the time of blood collection. The rest of the tests are not invasive. In principle, following the diet with quinoa should not produce any harmful effect on your health.

Place of analysis:

Hospital Clinic of Barcelona.

Right to revoke consent:

Your participation in the study is completely voluntary, and if you decide not to participate, you will receive all the medical attention you need, and your relationship with the medical team that treats you will not be affected.

At any time during the study, you can voluntarily decide to stop participating, without this decision affecting your future relationship with the medical team and your future medical care.

However, the doctors involved in the study may decide not to continue in case of:

• Lack of cooperation or compliance with the diet.

• Appearance of a serious illness.

• Your own decision that the doctor who considers that the continuation of the study would not be appropriate for you.

Implications of the information obtained in the study:

If you decide to participate in the study, it is possible that the analysis of your biological samples will obtain information relevant to your health, or that of your family. In addition to the analyzes carried out in the context of this study, samples will be stored as a collection in the IDIBAPS Diabetes and Obesity Laboratory, which could be used for future biomedical research studies, always under strict control of anonymity and ethical principles .

In accordance with current legislation, you have the right to be informed of the data obtained in the course of the study. If you want to know the relevant data for your health that is obtained, inform yourself through your doctor about the implications that this information may have for you and your family. This information will be communicated to you if you wish, in the event that you prefer not to be informed, your decision will be respected.

In accordance with Law 15/1999 on the Protection of Personal Data, the personal data obtained will be those necessary to cover the purposes of the study. In none of the study reports will your name appear, and your identity will not be revealed to any person except to fulfill the purpose of the study, and in case of medical emergency or legal requirement.

Any information of a personal nature that may be identifiable will be kept by computerized methods under secure conditions for Hospital Clínic. Access to this

information will be restricted to personnel of the Hospital Clínic designated for this purpose or to other authorized personnel who will be obliged to maintain the confidentiality of the information.

In accordance with current law, you have the right to access your personal data, likewise, and if justified, you have the right to rectification and cancellation. If you wish, you should ask the doctor who treats you in this study.

Analysis results:

You will be informed of the results related to your metabolic control throughout the study.

Questions:

If you have any doubts or questions regarding the study, do not hesitate to tell your doctor or the medical team. They will be ready to answer any questions or concerns you may have throughout the study. If you have questions regarding your rights as a study participant, ask the main researchers responsible for the study: Dr. Diana Díaz Rizzolo, Dr. Ramón Gomis, Dr. Felicia Hanzu (phones: 34.933 129 411 ext. 9411).

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INFORMED CONSENT

I,_____

_____ (First and last name).

- I have read the information in the attached document that was delivered to me
- I agree to participate in the study
- I have been informed of all the details and I have answered all my doubts about it.
- I have been informed by Dr. .....
- I understand that my participation is voluntary
- I understand that I can leave the studio: or whenever I want, without having to give explanations, not affecting my medical care as a patient. –
I understand that my personal samples and all my data will be treated anonymously
- I understand that any residual material in the study will be destroyed I freely give my consent to participate in this study:

_____ _____

Signature of participant **Date**

_____ _____

Signature of doctor **Date**