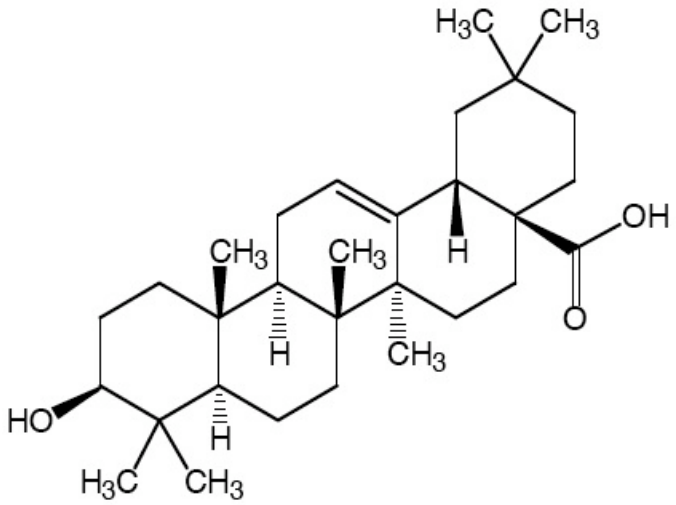
SUPPLEMENTARY APPENDICES

APPENDIX 1. CHEMICAL STRUCTURE OF THE OLEANOLIC ACID MOLECULE



APPENDIX 2. TYPICAL CHEMICAL COMPOSITION OF THE OLIVE OILS USED IN THE PREDIABOLE STUDY

component	
free acidity (% oleic acid)	0.37 ± 0.02
peroxide value (meq O_2/kg oil)	3.39 ± 0.02
k_{232}	1.82 ± 0.17
k_{270}	0.29 ± 0.00
fatty acids (%)	
palmitic (16:0)	10.4 ± 0.3
palmitoleic (16:1 n-7)	0.8 ± 0.0
estearic (18:0)	3.4 ± 0.1
oleic (18:1 n-9)	77.4 ± 0.1
linoleic (18:2, n-6)	5.8 ± 0.0
α-linolenic (18:3 n-3)	0.3 ± 0.1
arachidic (20:0)	0.4 ± 0.1
gadoleic (20:1 n-11)	0.7 ± 0.0
total phenolics (µg/g oil)	62.4 ± 0.7
hydroxytyrosol and derivatives	21.7 ± 0.2
tyrosol and derivatives	39.8 ± 0.2
lignanes	0.5 ± 0.0
flavonoids	0.3 ± 0.0
simple phenols	0.0 ± 0.0
total sterols (µg/g oil)	1409.3 ± 4.6
β-sitosterol	1223.0 ± 2.1
δ5-avenasterol	50.6 ± 0.9
campesterol	47.9 ± 0.1
stigmasterol	12.7 ± 0.0
clerosterol	15.5 ± 0.0
δ5,24-stigmastadienol	16.2 ± 0.7
δ5,23-stigmastadienol	9.9 ± 0.0
total tocopherols (µg/g oil)	270.8 ± 4.4
α-tocopherol	236.7 ± 0.7
triterpenoids (µg/g oil)	
erythrodiol	40.9 ± 0.1
uvaol	3.5 ± 0.7
oleanolic acid (control/enriched oils)	3.9 ± 0.1 / 604.7 ± 1.4
maslinic acid	4.6 ± 0.2
total chlorophills (µg/g oil)	1.3 ± 0.1
total carotenoids (µg/g oil)	1.4 ± 0.0

APPENDIX 3. PREDIABOLE TRIAL PROTOCOL

1. Hypothesis

The regular intake of an oleanolic acid-enriched olive oil is an effective strategy to prevent or delay the incidence of type 2 diabetes in prediabetic individuals.

2. Objectives

Main Objective

Demonstrate by mean of a randomized and controlled trial that a dietary intervention based on the regular consumption of an oleanolic acid-enriched olive oil reduce the incidence of type 2 diabetes in prediabetic patients (impaired fasting glucose plus impaired glucose tolerance), in comparison with those who consume the same commercial olive oil not enriched in the triterpene.

Secondary Objectives

1. Study the effect of this dietary intervention on overweight/obesity.
2. Evaluate its influence on the basic lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides).
3. Assess the effect on glucose homeostasis and insulin resistance.

3. Study design

The PREDIABOLE (PREvention of DIABetes with OLEanolic acid) Study is a parallel-group, randomized, controlled, double-blind, and multicenter trial, entirely performed in primary care from June 2010 to November 2016. Participants in the trial were assigned in a 1:1 ratio to one of the two study groups: diet with OA-enriched olive oil (intervention group or IG), or the same diet with not enriched olive oil (control group or CG). Internacional Standard Randomized Controlled Trial Number: ISRCTN 03372660.

4. Sample size

The sample size was calculated assuming a two-tailed alpha error of 0.05, a beta error of 0.20, and an expected incidence after 2 years of follow-up of 20% in the CG and of 5 % in the IG. A dropout rate of up to 20% was considered. The calculated sample size required was approximately 80 participants per group.

5. Participants

Eligible participants were community-dwelling patients who meet the following inclusion criteria:

1. Men or women with an age range between 30 and 80 years.
2. IFG, according to 2008 ADA's criteria: fasting glucose ≥ 100 mg/dl and <126 mg/dl, and not receiving treatment with antidiabetic drugs.
3. IGT, according to 2008 ADA's criteria: plasma glucose at 2 hours after a standard OGTT 75 g, comprised among 140 mg/dl and 199 mg/dl.
4. Body Mass Index in the range 25-40 Kg/m^2.
5. Acceptance of participating in the study and signing of the corresponding informed consent.

The following were established as exclusion criteria:

1. Diabetes Mellitus diagnosed according the 2008 ADA's criteria, at the time of recruitment: Fasting glucose ≥ 126 mg/dl or Glycemia 2 h at OGTT 75 g equal or higher than 200 mg/dl. To be in antidiabetic treatment (oral antidiabetics and/or insulin).
2. A history of prediabetes (IFG and/or IGT) longer than seven years
3. Syndrome of alcohol dependence or any other type of drug addiction.
4. Physical, mental or intellectual limitation to participate in a study of dietary intervention.
5. Difficulty or absence of predisposition to change eating habits.

6. Drugs or medical conditions that interfere with the diagnosis of Diabetes. Use of: systemic glucocorticoids; selective inhibitors of serotonin reuptake at the indicated dose for weight reduction; other drugs for weight reduction (orlistat and sibutramine); thyroid disease suboptimally treated; other endocrine diseases (such as Cushing's disease, acromegaly); fasting triglycerides higher than 600 mg/dl, despite treatment.
7. Conditions or circumstances that may affect the conduct of the trial: Inability to communicate with the researchers carrying out the intervention; not willing to accept the intervention assigned by randomization; participation in another project that could interfere with the present one; weight loss greater than 10% in the last 3 months for any reason, except that due to postpartum; inability to walk 400 meters continuously in 10 minutes.
8. Cancer in the last 5 years that requires treatment, unless the prognosis is good.
9. Blood Pressure: Systolic Blood Pressure ≥ 180 mmHg and/or Diastolic Blood Pressure ≥ 110 mmHg.
10. Women of childbearing age, if they are currently pregnant or within 3 months postpartum, breastfeeding, or pregnancy scheduled during the trial period.

6. Informed consent

Participants were thoroughly informed about the trial features, that their participation would be voluntary and that they would be free to withdraw from the study at any time. Participants were also informed that nonparticipation would not affect any health or medical services they might receive and that confidentiality would be maintained.

7. Recruitment strategy

Twenty-five Health Centers in the city of Seville (Spain) participated in the participant enrollment for the trial. Through an opportunistic recruitment, individuals with IFG were identified by their family physicians, and derived to the Study Coordination Center, where

they underwent a standard 75g oral glucose tolerance test (OGTT). Only those individuals meeting the inclusion criteria and none of the exclusion ones were randomly assigned to the study groups,

8. **Randomized allocation**

Randomization was performed following a concealed scheme based in four tables of random numbers, stratified by sex and age (cut-off 60 years), and managed by the trial coordinator. The study nurse requested by phone the assignment of participants to one of the two study groups at the time of admission (centralized randomization).

9. Intervention and follow-up

Participants were instructed to intake 55 mL/day of the assigned oil (OA-enriched or control olive oils), raw and freely distributed among the three main meals. All of them were follow-up for up to 30 months from recruitment, according to a programmed schedule of visits.

10. Compliance to allocation

Adherence to the dietary intervention was assessed through the self-reported compliance Haynes-Sackett test (31) and the return of the empty bottles presumably consumed. In addition, the OA plasma levels were analyzed in a randomized collection of blood samples corresponding to 0, 12, 18 and 30 months of follow-up, from 25 individuals per study group, applying the procedure by Rada et al. (GC-FID determination and pharmacokinetic studies of oleanolic acid in human serum. Biomed. Chromatogr. 2015; 29:1687–1692).

11. Ethics

The Ethics and Health Research Committee of the Primary Health Care District Seville (Andalusian Health Service, Andalusia, Spain) approved the protocol on May 20, 2008. All participants signed their consent, after being informed of the objectives and methodology

of the trial. The trial was conducted according to the recommendations of the Helsinki Declaration and the Good Clinical Practice Guidelines of the International Council for Harmonization (www.ich.org).

12. Study variables

12.1. End points

The primary outcome was new-onset diabetes, diagnosed according to the ADA criteria at 2008. Namely, fasting plasma glucose higher than 7.0 mmol/L or 2h-glycaemia above 11.2 mmol/L in a standard 75-g OGTT. In the absence of unambiguous hyperglycemia the values should be confirmed by repeating the test on a different day. In addition to the biannual controls, a fasting glucose test will be requested if symptoms suggestive of diabetes. Ascertain of the case was definitively done by the PREDIABOLE Clinical Event Committee, whose members were blinded to allocation in the study groups. New-diabetic individuals were informed and referred to their family doctors, and subsequently left out the study.

12.2. Other dependent variables

1. Body Mass Index (BMI = Weight in kg / height^2 in meters)
2. Circumference of the waist (abdominal perimeter, in cm)
3. Blood pressure (Systolic (SBP) and Diastolic (DBP), in mmHg)
4. Lipid profile: Triglycerides (mg/dl); Total cholesterol (mg/dl); HDL-cholesterol (mg/dl); LDL-cholesterol (mg/dl)
5. Fasting glucose (mg/dl)
6. Plasma glucose at 2 hours after standard OGTT (mg/dl)
7. Fasting insulin (µU/ml)
8. Homeostasis Model Assessment. HOMA-IR: fasting insulin (µU/ml) x fasting glucose /mmol/L)/22.5
9. Glycosylated hemoglobin (% and mmol/mol IFCC)

12.3. Basal variables

1. Family Antecedents of type 2 diabetes in first grade
2. Time at the prediabetes stage
3. History of arterial hypertension, ischemic heart disease, cerebrovascular disease and peripheral arterial disease
4. Consumption of tobacco and alcohol
5. Level of physical activity

12.4. Variables: definitions and types

variable	definition	type
allocation group	OA-enriched/control oil	dichotomous qualitative
age	years	discrete quantitative
sex	man/woman	binary qualitative
weight	Kg	continuous quantitative
height	cm	continuous quantitative
body mass index	Kg/m^2	continuous quantitative
	normoweight ($\leq$25 Kg/m^2) overweight (25$\leq$BMI<30 Kg/m^2) obese (30$\leq$BMI<40 Kg/m^2)	ordinal qualitative
waist circumference	cm	continuous quantitative
blood pressure	mmHg	continuous quantitative
fasting glucose	mg/dL	continuous quantitative
OGTT	mg/dL	continuous quantitative
HbA_{1c}	% and mmol/mol	continuous quantitative
basal insulinemia	µU/mL	continuous quantitative

HOMA-IR	-	continuous quantitative
triglycerides	mg/dL	continuous quantitative
total cholesterol	mg/dL	continuous quantitative
	normal (<200 mg/dL)	ordinal qualitative
	hypercholestherolemia (≥ 200 mg/dL)	
LDL-cholesterol	mg/dL	continuous quantitative
	normal (<150 mg/dL)	ordinal qualitative
	high (≥150 mg/dL)	
HDL-cholesterol	mg/dL	continuous quantitative
	low (≤40 mg/dL)	ordinal qualitative
	normal (>40 mg/dL)	
time at prediabetes	months	discrete quantitative
tobacco use	smoker/no-smoker/ex-smoker	polytomy qualitative
	cigarettes packages-year	discrete quantitative
alcohol consumption	standard drinking units	discrete quantitative
physical activity	yes/no	binary qualitative
	frequency (sessions/week)	discrete quantitative
history of hypertension	yes/no	binary qualitative
personal history of cardiovascular diseases (ischemic heart disease, cerebrovascular disease, obstructive arteriopathy of lower members)	yes/no	binary qualitative

13. Materials and procedures

13.1. Personalized clinical interview

After randomization, the study nurse performed personalized clinical interview to the patients for the obtaining of information about: 1) Family history of diabetes in the first degree (parents or siblings). 2) Time of evolution at prediabetes. 3) Consumption of alcohol. 4) Tobacco use. 5) Physical activity. 6) Personal history of hypertension, dyslipidemia, ischemic heart disease, stroke and peripheral arterial disease; and 6) Medication of habitual use.

13.2. Anthropometric determinations

1. Weight and height: with the subject barefoot and in underwear, using a calibrated scale and height meter.

2. Waist circumference: with the person standing, surrounding the waist in a horizontal plane (parallel to the floor) that passes through the upper edge of the iliac crests, with a tape measure adjusted to the skin but without compressing it. The measurement is made at the end of a normal expiration, not forced.

3. Blood pressure: A validated digital electronic device (OMRON M6 Comfort) with sleeve of adequate size to the circumference of the arm will be used. Two blood pressure shots will be taken, one at the beginning and the other at the end of the interview, and the obtained data will be averaged. The shots will be made in the dominant arm, whereas the individual is seated.

13.3. Diet and healthy lifestyle habits interventions

All participants received individual education sessions on a quarterly basis. In which the study nurse gave them verbal and written recommendations about diet and healthy lifestyle habits.

DIET. An energy-unrestricted traditional Mediterranean diet was recommended at each visit. The study nurse explained this type of diet with emphasis on improving dietary quality (i. e., focusing on food groups and their frequency of consumption).

HEALTHY LIFESTYLE HABITS. They were given recommendations on the moderate consumption of alcohol and abstinence from tobacco use, as well as on benefits of physical activity, adapted to the individual's age and, of light-moderate intensity, aerobic, 30 minutes a day, five days per week. No specific recommendations were made aimed at achieving a weight loss.

13.4. Blood Biochemistry

Sampling. Blood samples were withdrawn from the cubital vein after an overnight fasting, and analyzed for plasma glucose, insulin and glycated hemoglobin (HbA1c), hs-RCP, as well as for triglycerides, total cholesterol, HDL, and LDL. At each annual visit, an OGTT was done after a 12-hour fast, following the WHO recommendations.

For the blood sampling, sterile plastic tubes with vacuum system were used. The tubes were subsequently centrifuged at origin, to separate plasma from the blood cells.

Preservation and transport of samples. All blood tubes were transported al 4-7 ºC in insulated containers, within a gap of 4 hours, to the Laboratory of Analysis and Clinical Biochemistry of the Virgen del Rocio University Hospital of Seville.

Laboratory techniques. For plasma glucose determination, the enzymatic method with hexokinase and glucose-6-phosphate dehydrogenase was performed in a modular equipment Roche Diagnostics. Insulinemia was measured with an immunofluorescence-time-resolved method, using Europium as a marker, in an AutoDelfia equipment (Perkin-Elmer). Glycosylated hemoglobin (%) was analyzed by HPLC.

Determination of triglycerides was performed the lipase and glycerol kinase enzymatic method. Total cholesterol was determined by an enzymatic method with cholesterol esterase. LDL cholesterol was assessed using the Friedewald formula. When the triglycerides were higher than 400 mg/dl direct evaluation was made by homogenous enzymatic technique, previous selective micellar solubilization of LDL cholesterol with a non-ionic detergent. On the other hand, HDL cholesterol was quantified by a homogeneous enzymatic method with enzymes modified with polyethylene glycol (PEG-

cholesterol esterase and PEG-cholesterol oxidase). All these determinations were performed using a modular equipment Roche Diagnostics.

Hs-CRP was analyzed by an immunonephelometric method with intensifying particles, using monoclonal anti-CRP antibody, in a Dade-Behring BN-II nephelometer system.

13.5 Scheduled visits and their contents

Visit 0. Pre-randomization

Through an opportunistic recruitment, individuals with IFG were identified by their family physicians, and derived to the Study Coordination Center. These Pre-selected candidates were informed in detail of the trial features, and if they meet the inclusion criteria and none of the exclusion ones, were invited to participate in the study, signing the informed consent. Next, they were proposed to perform a standard OGTT to verify that it meets the IGT inclusion criterion, with glycemia values in the range 140-199 mg/dl, at 2 hours after the intake of 75g glucose. These individuals were randomized to one of the allocation groups by means of a concealed scheme based in four tables of random numbers, stratified by sex and age (cut-off 60 years), and managed by the trial coordinator. The study nurse requested by phone the assignment of participants to one of the two study groups at the time of admission (centralized randomization).

Afterwards, the participants were followed through a protocol of scheduled quarterly visits.

Visit 1

On this visit, the data referring to the anamnesis were filled in and a physical examination was performed, as previously described. Likewise, a sample of cubital venous blood was withdrawn for biochemical determinations. A personalized interview was also carried out to explain to the participants the form and frequency of the consumption of the oil provided and how to store it. They received free the amount of the olive oil assigned for a consumption of three months (23 bottles of 250 ml each quarter). They were also informed of the amount of oil to be consumed (55.5 mL/day). To facilitate the consumption of the exact amount of oil, patients were given a dosing graduate cylinder. This oil volume, in the case of the OA-enriched olive oil, provided 30 mg/day of oleanolic acid. The

interview also included recommendations about the benefits of a healthy lifestyle (Mediterranean diet, physical activity, smoking cessation if they smoke and moderate consumption of alcohol).

Visit 2. At 3 months of the visit 1

The anthropometric variables collected were weight, waist circumference and blood pressures. In the personalized interview conducted, participants were invited to follow the recommended lifestyle. They were also questioned about adherence to the assigned dietary intervention, applying the Haynes-Sackett self-reported compliance test. Adherence was checked too by returning the empty bottles presumably consumed. The participants received free the amount of the assigned olive oil assigned for the consumption of three next months.

Visit 3. At 6 months of the visit 1

The anthropometric variables collected were the same that those assessed at visit 2. Fasting blood samples were withdrawn for biochemical determinations, as in the visit 1. Personalized interviews with the same contents of the Visit 2 were also carried out. Adherence checking as in the Visit 2. Allotment of the assigned olive oil.

Visit 4. At 9 months of the Visit 1

The same contents as the Visit 2.

Visit 5. At 12 months of the Visit 1

The same contents as Visit 3 plus the realization of a standard OGTT.

Visit 6. At 15 months of the Visit 1

The same contents as Visit 2.

Visit 7. At 18 months of Visit 1

The same contents as Visit 3

Visit 8. At 21 months of the Visit 1

The same contents as Visit 2.

Visit 9. At 24 months of the Visit 1

The same contents as Visit 5.

Visit 10. At 27 months of the Visit 1

The same contents as Visit 2.

Visit 11. At 30 months of Visit 1

The same contents as Visit 5.

visit no.	0	1	2	3	4	5	6	7	8	9	10	11
month	-	0	3	6	9	12	15	18	21	24	27	30
baseline values and filiation		×										
lifestyle habits		×										
personalized interview*		×	×	×	×	×	×	×	×	×	×	×
anthropometric 1†		×										
anthropometric 2‡		×	×	×	×	×	×	×	×	×	×	×
follow-up blood biochemistry		×		×		×		×		×		×
OGTT	×					×				×		×
HbA_{1c}	×			×		×		×		×		×
compliance to allocation			×	×	×	×	×	×	×	×	×	×
oil allotment		×	×	×	×	×	×	×	×	×	×	

*personalized interview (recommendations on: diet, healthy lifestyle, physical activity, abstinence from tobacco and moderate alcohol consumption)
†anthropometric measurements 1 (height)
‡anthropometric measurements 2 (weight, waist circumference, and blood pressure)

14. Assessment of the safety of the dietary intervention

At December 2018, a family physician, researcher collaborator with the trial, blind to the allocation and not belonging to the trial staff, carried out a retrospective study of the medical histories in DIRAYA, the software used in the Health System of Andalusia as support for the electronic medical record. For each participant, the period from the inclusion to up to two years after completing was studied, collecting all the registered analytical values of serum creatinine as marker of kidney function, and of alanine and aspartate aminotransferase enzymes as markers of hepatic alteration.

The electronic medical records were also carefully analyzed for cerebral, cardiac and peripheral vascular events. In addition, any other adverse findings suffered by participants

were considered. The PREDIABOLE Clinical Event Committee validated the reported events.

15. Statistical analysis

The calculated sample size was 80 participants per group, assuming a two-tailed alpha of 0.05, a statistical power greater than 80%, and expected proportions of new cases of diabetes after 30 months' follow-up of 20% in the CG and of 5 % in the IG. A dropout rate of up to 20% was considered.

The trial was conducted according to the intention-to-treat (ITT) principle. Qualitative variables were expressed by their absolute and relative frequencies, whereas the quantitative ones with normal distribution did so by the mean and standard deviation and those with non-normal distribution by the median and interquartile range (IQR). Comparisons between study groups for qualitative variables were done with the Chi-square and McNemar's tests, whereas comparisons for quantitative variables were executed with the Student's t test and ANOVA. The homogeneity of the populations included in the allocation groups was evaluated using the Mann-Whitney-Wilcoxon U test.

Cox regression models were fitted to assess the relative risk of diabetes by allocation groups, estimating hazard ratios and 95% CI. The 'time' variable was the interval between recruitment and the date of last follow-up or diabetes diagnosis, whichever occurred first. Participants who were free of diabetes or lost during follow-up were censored at the date of the last visit. Interactions of the intervention with other variables were evaluated using the likelihood ratio test for multiplicative product terms introduced in fully adjusted Cox models. The survival analysis (probability of remaining free of diabetes during follow-up) was carried out comparing the Kaplan-Meier curves by the log-rank method. All p values were two-tailed at $\alpha=0.05$. Statistical analysis was performed with the SPSS 24 (IBM SPSS Statistics, New York, USA) software.

APPENDIX 4. INFORMED CONSENT (Spanish and English versions)

INFORMACIÓN AL PACIENTE SOBRE EL ESTUDIO PREDIABOLE

Título del proyecto: **Ensayo clínico aleatorizado y controlado sobre la eficacia de un aceite de oliva enriquecido en ácido oleanólico en la prevención de la diabetes mellitus tipo 2 en pacientes prediabéticos**

Investigador Principal: Dr. José Manuel Santos-Lozano

Centros participantes: 25 Centros de Salud. Distrito Sanitario Atención Primaria Sevilla.

Objetivo

Analizar el efecto de una intervención dietética, basada en el consumo de aceite de oliva enriquecido en ácido oleanólico sobre la prevención de la diabetes en pacientes prediabéticos.

Metodología y desarrollo del estudio

Usted ha sido preseleccionado por su médico de familia por tener valores de glucemia (azúcar en sangre) en ayunas comprendidos entre 100 y 125 mg/dL. Esto es un estado de prediabetes que se denomina Glucosa Basal Alterada (GBA).

Para saber si usted puede ser incluido en el estudio, se le realizará un test de sobrecarga oral con 75 gramos de glucosa ("curva de azúcar"), si es que no tiene realizada esta prueba en los últimos 6 meses. Si el valor de glucemia a las 2 horas de la ingesta del azúcar se encuentra entre 140 y 199 mg/dL usted también padecería Tolerancia Alterada a la Glucosa (TAG).

Tener GBA y la TAG implica que sus niveles de azúcar en sangre son algo más elevados de lo normal, aunque sin que usted llegue a ser diabético. Se sabe que las personas que tienen GBA y TAG tienen un mayor riesgo de desarrollar diabetes.

El Estudio PREDIABOLE requiere reclutar, al menos, 160 pacientes prediabéticos, diagnosticados de GBA y TAG. Por ello, usted solo podría participar en el estudio si tiene simultáneamente GBA y TAG.

El ensayo clínico se basa en una intervención dietética que consiste en tomar diariamente 55 centímetros cúbicos del aceite de oliva que se le asigne mediante un proceso de aleatorización. Este aceite se le suministrará libre de coste y en cantidad suficiente. Un grupo de participantes ingerirá un aceite de oliva comercial, y el otro grupo el mismo aceite de oliva, pero enriquecido en ácido oleanólico, un componente natural de la aceituna, el aceite de oliva y la hoja de olivo. En el aceite enriquecido, la concentración de ácido oleanólico se incrementa hasta los 600 mg/Kg.

Usted desconocerá el tipo de aceite que se le asigne. Lo debe ingerir preferentemente crudo, y libremente distribuido entre las tres comidas principales. Esta intervención dietética se aplicará durante 30 meses.

Una vez incluido/a en el ensayo, se le abrirá una historia clínica específica, y se le pedirá que asista a una serie de visitas programadas. En estas visitas, se le realizará una exploración física con medidas del peso, la talla, el diámetro de la cintura, y la presión arterial. Periódicamente, se le extraerá sangre (unos 40 mL) para un análisis bioquímico básico. También se le realizarán nuevos test de sobrecarga oral de glucosa cada 12 meses.

La enfermera del estudio, en coordinación con dietistas-nutricionistas, le hará también recomendaciones para promover una dieta saludable de tipo mediterráneo, así como sobre los beneficios de la reducción del peso corporal y la práctica regular de ejercicio físico.

En este estudio no se le administrará ningún fármaco específico, ni se le modificará el tratamiento que tenga. Si a lo largo del estudio fueran necesarias modificaciones en su tratamiento habitual, estas serán realizadas por el/los médico/s que le atienden habitualmente.

Beneficios y riesgos de participar en el ensayo clínico

Su participación en el estudio le puede ayudar a un mejor conocimiento de su estado de salud, de los factores de riesgo cardiovascular y de hábitos de vida saludables. También cabe la posibilidad de que usted no obtenga ningún beneficio directo por participar en el estudio. No obstante, la información que se obtenga podrá beneficiar en un futuro a otros pacientes y contribuir a un mejor conocimiento del efecto terapéutico del ácido oleanólico.

Su participación en el estudio no implica ningún riesgo para su salud. La cantidad de sangre que se le extraerá cada seis meses será ligeramente superior a la que le extraen para realizar una analítica habitual. Sin embargo, a veces, la extracción de sangre puede provocar una sensación de ardor en el punto en el que se introduce la aguja en la piel y puede ocasionar un pequeño hematoma que desaparece en pocos días. Más raramente puede provocar un mareo transitorio.

Al no tratarse de un estudio con fármacos, no es previsible ningún efecto adverso. Por otra parte, el enriquecimiento del aceite de oliva en ácido oleanólico no altera su olor, color o sabor.

Voluntariedad

Su participación en este estudio es totalmente voluntaria por lo que en cualquier momento puede retirarse del mismo, sin tener que dar explicaciones y sin que su relación con su equipo médico habitual se vea afectada.

Confidencialidad

Todos los datos de carácter personal obtenidos en este estudio son confidenciales y se tratarán conforme a la Ley Orgánica de Protección de datos de Carácter Personal 15/99.
Los resultados que se obtengan serán analizados por los investigadores del estudio, pero en las listas de trabajo no aparecerá su nombre y sólo constará su número identificador como participante en el estudio. Asimismo, la identidad de los participantes se mantendrá en el más absoluto anonimato en el informe final de resultados, y en las posibles comunicaciones realizadas a la comunidad científica.
Tal como prevé el Art. 5 de la Ley Orgánica 15/99 de Regulación del Tratamiento Automatizado de los Datos de Carácter Personal, le informamos de que éstos podrán ser objeto de tratamiento automatizado y que tiene derecho a consultar, modificar o eliminar del fichero sus datos personales. La responsabilidad de la tutela del fichero generado corresponde al Distrito Sanitario Atención Primaria Sevilla del Servicio Andaluz de Salud)
La información obtenida se utilizará exclusivamente para los fines específicos del estudio.

Compensación

No está prevista ningún tipo de compensación económica.

Investigadores de contacto

Si tiene alguna duda sobre algún aspecto del estudio o le gustaría comentar algún aspecto de esta información, por favor no deje de hacérselo saber a los miembros del equipo investigador, Dr. José Manuel Santos-Lozano, Dr. José Lapetra-Peralta, y Manuel Ortega-Calvo.
Teléfonos contacto: 955040453, 954994140, 954544601, 954544587 y 610948138.
Correos electrónicos:
josem.santos.lozano.sspa@juntadeandalucia.es
jose.lapetra.sspa@juntadeandalucia.es
106mayorque104@gmail.com

Ética

Los protocolos que rigen el presente estudio han sido aprobados por el Comité de Ética e Investigación Sanitaria del Distrito Sanitario Atención Primaria Sevilla en su sesión de 20 de mayo de 2008.

Si tras la lectura de esta información y la aclaración de las dudas le hayan surgido, decide aceptar participar en el estudio, deberá firmar el consentimiento informado que se adjunta.

HOJA DE CONSENTIMIENTO INFORMADO

Título del proyecto: **Ensayo clínico aleatorizado y controlado sobre la eficacia de un aceite de oliva enriquecido en ácido oleanólico en la prevención de la diabetes mellitus tipo 2 en pacientes prediabéticos**

Investigador Principal: Dr. José Manuel Santos-Lozano

Centros participantes: 25 Centros de Salud Distrito Sanitario Atención Primaria Sevilla.

Yo, (nombre y apellidos) ……..

Manifiesto haber leído las hojas informativas que acompañan este consentimiento. Asimismo, afirmo que he podido hacer preguntas sobre el estudio, y que he recibido suficiente información al respecto.

Se me ha informado que todos los datos recabados y los resultados obtenidos serán confidenciales y tratados conforme establece la Ley Orgánica 15/99 de Protección de Datos de Carácter Personal.

Se me ha informado igualmente que la información generada se utilizará exclusivamente para los fines científicos específicos del estudio.

Expreso mi deseo de (Si/No) ser informado de aquellos datos de carácter personal que se obtengan en el curso de la investigación, incluidos los descubrimientos inesperados que se puedan producir, siempre que esta información sea necesaria para evitar un grave perjuicio para mi salud.

He hablado con ……,

(nombre del investigador)

Y comprendo que mi participación es voluntaria. También entiendo que puedo retirarme del estudio cuando quiera, sin tener que dar explicaciones, y sin que esto repercuta en mis cuidados médicos.

Con mi firma doy libremente mi conformidad para participar en el proyecto

Fecha, nombre y firma del participante Fecha, nombre y firma del investigador

INFORMATION TO THE PATIENT ON THE PREDIABOLE STUDY

Project title: **Randomized and controlled clinical trial on the efficacy of olive oil enriched in oleanolic acid in the prevention of type 2 diabetes mellitus in prediabetic patients**

Principal Investigator: Dr. José Manuel Santos-Lozano

Participating centers: 25 Health Centers of the Primary Health Care District of Seville.

Objective

To analyze the effect of a dietary intervention based on the consumption of an oleanolic acid-enriched olive oil on the prevention of diabetes in prediabetic patients.

Methodology and development of the study

You have been preselected by your family doctor for having fasting glucose (blood sugar) values between 100 and 125 mg/dL. This is a prediabetes state called Impaired Fasting Glucose (IFG).

To know if you can be included in the study, an oral overload test (OGTT) will be performed with 75 grams of glucose ("sugar curve"), if you have not performed this test in the last 6 months. If the blood glucose value at 2 hours of the sugar intake is between 140 and 199 mg/dL, you would also suffer from Impaired Glucose Tolerance (IGT).

Having IFG and IGT means that your blood sugar levels are somewhat higher than normal, but without you becoming diabetic. It is known that people who have IFG and IGT have a higher risk of developing diabetes.

The PREDIABOLE Study requires recruiting at least 160 prediabetic patients, diagnosed with IFG and IGT. Therefore, you can only participate in the study if you have IFG and IGT at the same time.

This clinical trial is based on a dietary intervention that consists in the daily intaking of 55 cubic centimeters of the olive oil assigned to you through a randomization process. This oil will be provided free and in sufficient quantity. A group of participants will intake a commercial olive oil, and the other group will have the same olive oil, but enriched in oleanolic acid, a natural component of the olive oil and olive fruit and leaf. In the enriched oil, the concentration of oleanolic acid is increased to 600 mg/kg. You will not know the type of oil assigned to you.

You should intake the olive oil preferably raw, and freely distributed among the three main meals. This dietary intervention will be applied for 30 months.

Once included in the trial, a specific clinical history will be opened and you will be asked to attend a series of scheduled visits. During these visits, you will have physical examinations which include measurements of weight, height, waist diameter, and blood pressure. Periodically, blood samples will be drawn (about 40 mL) for a basic biochemical analysis. You will also perform OGTT every 12 months.

The study nurse, in coordination with dieticians-nutritionists, will make recommendations to follow a healthy Mediterranean-type diet, as well as reduce body weight and make regular exercise.

In this study, you will not be given any specific drug, nor will your treatment be modified. If during the study modifications were necessary in your usual treatment, these will be carried out by the family doctor who usually attend you.

Benefits and risks of participating in the clinical trial

Your participation in the study may give you a better understanding of your health status, cardiovascular risk factors and healthy lifestyle habits. There is also the possibility that you may not get any direct benefit from participating in the study. However, the information obtained may benefit other patients in the future and contribute to a better understanding of the therapeutic effect of oleanolic acid.

Your participation in the study does not involve any risk for your health. The amount of blood that will be withdrawn every six months will be slightly higher than that which is extracted to perform a routine analysis. However, sometimes, the extraction of blood can cause a burning sensation at the point where the needle is inserted into the skin and can cause a small bruise that disappears in a few days. More rarely, it can cause transient dizziness.

As it is not a drug study, no adverse effect is foreseeable. On the other hand, the enrichment of olive oil in oleanolic acid does not alter its smell, color or taste.

Willfulness

Your participation in this study is totally voluntary so that at any time you can withdraw from it, without having to give explanations and without your relationship with your usual medical team being affected.

Confidentiality

All personal data obtained in this study are confidential and will be treated in accordance with the Spanish Organic Law on Protection of Personal Data 15/99.

The results obtained will be analyzed by the researchers of the study, but in the work lists their name will not appear and only their identifier number will be included as a participant in the study. Likewise, the identity of the participants will be kept in the most absolute

anonymity in the final report of results, and in the possible communications made to the scientific community.

As provided for in Article 5 of the Spanish Organic Law 15/99 on the Regulation of Automated Processing of Personal Data, we inform you that these may be subject to automated processing and that you have the right to consult, modify or delete the file with your personal information. The responsibility for the protection of the generated file corresponds to the Sanitary District Primary Care Seville of the Andalusian Health Service)

The information obtained will be used exclusively for the specific purposes of the study.

Compensation

No type of economic compensation is provided.

Ethics

The protocols that govern this study have been approved by the Ethics and Health Research Committee of the Primary Health Care District of Seville in its session of May 20, 2008.

Contact researchers

If you have any questions about any aspect of the study or would like to comment on some aspect of this information, please do not hesitate to contact the members of the research team Dr. José Manuel Santos-Lozano, Dr. José Lapetra, and Dr. Manuel Ortega-Calvo

contact phones: 955040453, 954994140, 954544601, 954544587 and 610948138.

e-mails:
josem.santos.lozano.sspa@juntadeandalucia.es
jose.lapetra.sspa@juntadeandalucia.es
106mayorque104@gmail.com

If after reading this information and clarifying the doubts that have arisen, decide to accept to participate in the study, you must sign the informed consent that is attached.

INFORMED CONSENT FORM

Project title: Randomized and controlled clinical trial on the efficacy of olive oil enriched in oleanolic acid in the prevention of type 2 diabetes mellitus in prediabetic patients

Principal Investigator: Dr. José Manuel Santos-Lozano

Participating centers: 25 Health Centers of the Primary Health Care District of Seville.

I, (Name and surname) ……..

declare to have read the information sheets that accompany this consent. Likewise, I affirm that I have been able to ask questions about the study, and that I have received enough information about it.

I have been informed that all the data collected and the results obtained will be confidential and treated as established by the Spanish Organic Law 15/99 on the Protection of Personal Data.

I have also been informed that the information generated will be used exclusively for the specific scientific purposes of the study.

I express my wish (Yes / No) to be informed of those personal data that are obtained in the course of the investigation, including unexpected discoveries that may occur, provided that this information is necessary to avoid serious damage to my health.

I have spoken with ……,

(name of the researcher)

And I understand that my participation is voluntary. I also understand that I can withdraw from the study whenever I want, without having to give explanations, and without this having an impact on my medical care.

With my signature I freely give my agreement to participate in the project

Date, name, signature of the participant Date, name, signature of the researcher

APPENDIX 5. CONSORT 2010 checklist of information to include when reporting a randomised trial

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3,4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	Not applicable
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	5-7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	Not applicable
Sample size	7a	How sample size was determined	8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	Not applicable
Randomisation:			

Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	5
	11b	If relevant, description of the similarity of interventions	5, 6
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8, 9
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	8, 9
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	9
	13b	For each group, losses and exclusions after randomisation, together with reasons	9, 10, Figure 2
Recruitment	14a	Dates defining the periods of recruitment and follow-up	9, 10
	14b	Why the trial ended or was stopped	Not applicable
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	19
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	8, 9
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	10, 11, 20

	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	20
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	20
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	11, 21
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12, 13
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	11 - 14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	11 - 14
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	Electronic Supplementary Material. Appendix 1
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15