Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2025, **16**, 6213

Exploring Galician phenolic-rich olive oil as a glycemic control strategy: the OILDIABET randomized trial†

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The rising prevalence of type 2 diabetes (T2D) demands effective dietary strategies. High-phenolic extra virgin olive oil (EVOO) has been proposed as a functional food with antidiabetic properties. This study evaluates the effects of a high-phenolic EVOO from native Galician olives on glycemic control (primary outcome), lipid profile, anthropometric and blood pressure parameters (secondary outcomes) in adults with T2D. A 24-week experimental, prospective, randomized, parallel, long-term controlled trial was conducted with 116 T2D subjects. Participants were randomly allocated either to a Control group advised to minimize consumption of EVOO (preferring refined olive oil blends) or an Interventional group receiving 30 mL day^{-1} of Galician phenolic-rich EVOO. Glycemic biomarkers, lipid profile, anthropometric indices, and blood pressure were assessed at baseline, 12 and 24 weeks. After 24 weeks, the Interventional group demonstrated significant reductions in insulin resistance (HOMA IR). No significant changes were observed in lipid profile or blood pressure in either group, while both groups exhibited modest reductions in body weight and body mass index (BMI). Although beneficial effects were particularly pronounced among individuals with obesity (reductions in fasting glucose, estimated average glucose and glycosylated hemoglobin (HbA1c)) and insulin-resistant participants (reductions in fasting insulin and HOMA IR), these subgroup analyses lacked sufficient statistical power and must be interpreted cautiously. These findings highlight the therapeutic potential of phenolic-rich EVOOs as a complementary dietary strategy for managing T2D.

Received 18th February 2025,
Accepted 3rd July 2025

DOI: 10.1039/d5fo00873e

rsc.li/food-function

Introduction

According to the latest update of the International Diabetes Federation (IDF), approximately 537 million adults (20–79 years) are living with diabetes around the world in 2021; this number is projected to rise 643 million by 2030 and 783 million by 2045.[1] Type 2 diabetes (T2D), which accounts for about 90% of all diabetes cases, is the most common form of the disease. Changes in diet and physical activity, driven by rapid development and urbanization, have led to a sharp rise in the number of people with T2D. Once a disease that predominantly affected older adults, it is now increasingly affecting children, adolescents and younger adults due to rising obesity rates, sedentary lifestyles and poor dietary habits. All evidence suggests that diabetes mellitus is the most rapidly expanding global public health issue, and early intervention together with lifestyle modifications can substantially mitigate the related hazards.

Diet is a key factor in the development, prevention and management of T2D. The Mediterranean diet (MedDiet) is a healthy dietary pattern whose main pillar is the consumption of olive oil.[2,3] Olive oil, and in particular extra virgin olive oil (EVOO), is the main source of fat in this dietary pattern. In terms of nutritional composition, EVOO has a high content of monounsaturated fatty acids (especially oleic acid) and minor compounds such as polyphenols (oleuropein, hydroxytyrosol-

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† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d5fo00873e

HTy and tyrosol-Ty) and/or squalene. These substances can be considered some of the key active ingredients found in this matrix.[4] The phenolic fraction of EVOO is known for its anti-inflammatory and antioxidant properties, establishing it as a key nutritional factor in combating neurodegenerative disorders, various cancers, metabolic syndrome and chronic diseases.[5]

In northwestern Spain, Galicia has steadily developed into a promising region for olive cultivation, particularly for producing EVOOs through the co-crushing of ancient autochthonous varieties. These oils are distinguished by their exceptional organoleptic, nutritional, and health-enhancing qualities, attributed to their high concentration of phenolic compounds, which exceeds 700 mg kg^{-1}.[6] In recent years, our research group has demonstrated that phenolic-rich extracts from native Galician EVOOs are more effective in inhibiting α-glucosidase than acarbose, a medication used to decrease glucose absorption in the small intestine, in the context of T2D management.[7,8] This inhibition slows down carbohydrate digestion and reduces postprandial hyperglycemia.[9]

The human intervention studies with olive oil showed an overall improvement in the antioxidant and inflammatory profiles of participants, as will be discussed in later sections. The beneficial effects were particularly pronounced in individuals diagnosed with metabolic syndrome or other chronic conditions and diseases.[10,11] Until now, the evidence from dietary interventions on the impact of olive oil phenolic compounds on T2D is limited and inconclusive,[12] attributable to the methodological design of the interventions, which encompassed a small number of participants and a relatively short period of exposure to the oil.[13,14] Further research is therefore needed to explore the potential therapeutic applications of EVOO phenolic compounds in the prevention and management of T2D.

In this work, a dietary intervention trial was conducted at the hospital in the city of Ourense (Galicia, NW Spain), aiming to evaluate the impact of native Galician EVOOs on primary outcomes related to glycemic control and secondary outcomes including lipid profile, anthropometric, and blood pressure measurements in a cohort of volunteers diagnosed with T2D over a 24-week period. The unique phenolic profile of Galician EVOOs could offer an unexplored opportunity to address glycemic control challenges in T2D management.

Materials and methods

Selection of EVOO and phenolic content analysis

Galician phenolic-rich EVOO obtained by milling autochthonous olives (collected during the 2020/2021 crop season in Ribas do Sil, Lugo, NW Spain) was selected for this study. The EVOO used in the intervention (600 L, 1200 amber glass bottles of 500 mL) was kept refrigerated in a cold chamber (4 °C) until it was distributed to the study participants. Physico-chemical parameters and sensory evaluation were performed to classify the olive oil into the highest category, as their quality and purity indices were within the legally established ranges[15] (Table S1 of ESI†). A series of analyses were conducted at regular intervals throughout the intervention period in order to verify the highest category.

The phenolic fraction was extracted from Galician EVOO using a liquid–liquid extraction protocol previously reported by Bajoub *et al.* (2016),[16] with minor modifications. LC-DAD/FLD/MS analysis of the phenolic extracts was performed according to the method described by Reboredo-Rodríguez *et al.*, (2021).[17] Moreover, the identification of the phenolic compounds was based on the use of pure standards (when commercially available), retention time data, high-resolution MS information, and the comparison of the MS/MS spectra with previously published results.[16] Calibration curves for each standard were constructed using different concentrations of the standard mixture solution and plotting peak areas *versus* concentration levels. When a pure standard was not available, the quantification was made using the calibration curve of a similar (or structurally related) compound: oleacein (DOA) was used for oleuropein aglycone (OlAgl) and related compounds; oleocanthal (DLA) was used for ligstroside aglycone (LigAgl) and related compounds; lignans were quantified in terms of pinoresinol (Pin); and luteolin (Lut) was used for diosmetin (Dios) quantification. The results were expressed in mg kg^{-1} of EVOO, as mean ± standard deviation (calculated from four extracts; $n = 4$).

Study design

The OILDIABET trial is an experimental, prospective, randomized, parallel-group, long-term dietary intervention study designed to evaluate the effects of a Galician phenolic-rich EVOO on biomarkers associated with diabetes (primary outcomes), dyslipidemia, anthropometric and hypertension status in T2D participants over a 24-week intervention period.

Inclusion/exclusion criteria for the participants

Inclusion criteria. (a) Subjects diagnosed with T2D (fasting glucose > 126 mg dL^{-1} and/or HbA1c ≥ 6.5%); (b) subjects previously diagnosed with T2D on treatment with oral or injectable non-insulin agents (biguanides, thiazolidinediones, α-glucosidase inhibitors, dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) agonists, sodium-glucose co-transporter 2 (SGLT2) inhibitors); (c) BMI ≥ 25 kg m^{-2} and <41 kg m^{-2}; (d) acceptance to participate in the study and signed the corresponding written informed consent.

Exclusion criteria. (a) Patients on treatment with insulin, sulfonylureas, or rapid-acting insulin secretagogues; (b) history of severe ketosis or hyperglycemic decompensation; (c) pregnancy, pregnancy planning, or breastfeeding; (d) BMI ≥ 41 kg m^{-2} or <25 kg m^{-2}; (e) difficulties or significant barriers to changing eating habits, or a low predicted likelihood of changing eating habits according to Prochaska and DiClemente's Stages of Change Model;[18] (f) severe medical conditions that may affect the ability of the individuals to participate in a dietary intervention study (*e.g.*, digestive disease with fat intolerance, malignancy, or significant neurological, psychiatric, or endocrine disease); (g) any other medical condition that is considered to limit survival to less than 1 year; (h) immunode-

ficiency or HIV positive status; (i) illicit drug use, chronic alcoholism, or problematic alcohol use with a total daily alcohol intake > 80 g day^{-1}; (j) participation in any drug trial or use of any investigational drug in the past year; and (k) institutionalized patients receiving chronic care.

Recruitment and randomization

Diabetic eligible participants were initially recruited in the division of Endocrinology of the University Hospital Complex of Ourense (CHUO, Ourense, NW Spain) and in various Primary Health Care Centers in the province of Ourense. A total of 10 subgroups (comprising between 8 and 16 individuals who potentially met the inclusion/exclusion criteria) of volunteers were invited to hospital recruitment visits to be informed about the study characteristics between April 2021 and February 2022. It should be noted that owing to the occurrence of multiple distinct waves of the coronavirus pandemic, the recruitment process was periodically disrupted. 116 participants were ultimately enrolled in the study after providing informed consent (the intervention period spanned from April 2021 to July 2022). Motivations for participation in this study included access to dietary assessments and involvement in dissemination programs or scientific support.

All participants were randomly assigned to a group — Control and Interventional group — through a simple randomization method using a random number generator.

Intervention

Following randomization, a parallel-group design was implemented, with each participant remaining in their assigned group for the duration of the trial. Subsequently, during the long-term intervention (24 weeks), participants in the Interventional group were requested to consume a daily dose of 30 mL of Galician phenolic-rich EVOO, distributed evenly across three meals (breakfast, lunch and dinner), with a dosage of 10 mL per meal. Participants in this group received dark, sealed 500 mL bottles of EVOO, along with a plastic measuring cup, provided free of charge. Conversely, participants in the Control group were not required to consume the daily dose; in fact, they were advised to minimize consumption of EVOO as much as possible, opting instead for refined olive oil blends characterized by lower phenolic compound concentrations.

All subjects were instructed to preserve their lifestyle, physical activity and dietary habits, following general recommendations aligned with a Mediterranean dietary pattern specifically adapted for diabetic individuals.

Throughout the study, brief telephone calls were conducted between scheduled visits to ensure adherence to the intervention protocol, emphasize the significance of participant involvement, and remind participants of upcoming clinical evaluations performed by the medical team. Telephone contact was also used to confirm logistical details, including appointment location, timing, and specific requirements such as fasting conditions.

Baseline demographic and clinical characteristics of the OILDIABET participants are summarized in Table 1.

Intervention adherence

To assess the level of adherence to the intervention, participants were instructed to return the containers on a four-week basis so that the daily amount of unconsumed EVOO could be measured and recorded. In addition, the presence of HTy metabolites in urine was determined. For this purpose, 24-hour urine samples from each participant were collected at baseline (T0) and after 24 weeks (T24) and they were stored at −80 °C until analysis.

To clean-up the biological matrix and isolate the phenolic metabolites, the urine samples were pretreated using microelution SPE plates (μSPE) according to Rubió *et al.*, (2014).[19] Briefly, OASIS hydrophilic-lipophilic balance (HLB) μElution plates 30 μm (Waters) were used and conditioned sequentially with 250 μL of methanol and 250 μL of Milli-Q water at pH 2 with acetic acid. Aliquots of 50 μL of 4% phosphoric acid and 50 μL of catechol, used as the internal standard (with a concentration of 1 mg L^{-1}, prepared in 4% phosphoric acid), were combined with 100 μL of human urine sample. The retained metabolites were then eluted with 2 × 50 μL of methanol.

The LC-MS sample analysis was done in an Elute series Ultra High-Performance Liquid Chromatography (UHPLC) coupled to the tims-TOF high-resolution spectrometer from Bruker Datonics. The chromatography was performed with a Waters AcQuity UPLC BEH Shield C18 column (1.7 μm, 100 mm × 2.1 mm id). To achieve the separation of the metabolites was necessary to use a mobile phase A consisting of Milli-Q ultra-pure water (0.1% formic acid) and acetonitrile (0.1% formic acid) as mobile phase B, with a flow rate of 0.4 mL min^{-1}. A lineal gradient elution was applied: 0 min, 90% A; 3 min, 79% A; 3.1 min, 70% A; 7.5 min, 43% A; 7.6 min, 5% A; 8.5 min, 5% A; 8.6 min, 81% A and at 10 min return to initial conditions. The injection volume was 10 μL. The mass spectrometer was equipped with an electrospray source (ESI) operated in negative polarity; the data were acquired in BBCID mode (within the range m/z 50–1100). Source parameters were adapted to the MS systems conditions as follows: 1.8 bar of nebulizer pressure, 6 L min^{-1} and 220 °C of drying gas flow and temperature, respectively, and +3000 V capillary voltage on. Broadband fragmentation was carried out to facilitate compound identification. Collision energy stepping factors varied within the range of 35 to 70 eV. The software controlling LC-QTOF MS was Compass® Hystar and QtofControl. Data treatment was done with Data Analysis 5.1 and TASQ2021 1.2.452 from Bruker Daltonics.

Table S2 of ESI† lists the target metabolites selected, hydroxytyrosol sulfate (sulfHTy) and hydroxytyrosol acetate sulfate (sulfHTyAc). The standards were commercially available (Toronto Research Chemicals), and both metabolites were quantified using matrix-matched calibration curves.

Monitoring the efficacy of dietary intervention. Data sampling

All participants received identical follow-up throughout the experimental intervention. Primary and secondary outcomes

Table 1 Baseline demographic and clinical characteristics of the OILDIABET participants

Parameter	Control Group (n = 49)	Interventional Group (n = 59)	*p*-value
Age (years)	67 (59–73)	66 (57–70)	0.23
Gender			
Female	29 (59.2%)	33 (55.9%)	0.73
Male	20 (40.8%)	26 (44.1%)	
Smokers			
Never smoked	21 (42.9%)	23 (39.0%)	0.69
Smoker	4 (8.2%)	5 (8.5%)	
Ex-smoker < 1 year	1 (2.0%)	0 (0.0%)	
Ex-smoker ≥ 1 year	23 (46.9%)	31 (52.5%)	
BMI (kg m^{-2})			
Individuals without obesity (BMI < 30)	26 (53.0%)	25 (42.4%)	0.27
Individuals with obesity (BMI ≥ 30)	23 (47.0%)	34 (57.6%)	
Arterial hypertension[a]	31 (63.3%)	38 (64.4%)	0.90
Dyslipidaemia[b]	42 (85.7%)	50 (84.8%)	0.89
Heart-related diseases			
Heart disease	5 (10.2%)	8 (13.6%)	0.59
Heart failure	1 (2.0%)	2 (3.4%)	0.67
Coronary heart disease	2 (4.1%)	6 (10.2%)	0.23
Acute coronary syndrome	1 (2.0%)	4 (6.8%)	0.24
Coronary revascularization	2 (4.1%)	4 (6.8%)	0.54
Nephropathy			
Urine albumin ≤ 30 mg per 24 h	32 (68.1%)	45 (76.3%)	0.35
Urine albumin > 30 mg per 24 h	15 (31.9%)	14 (23.7%)	

Age (quantitative variable) is expressed as median and interquartile range (IQR); *p*-value was derived from the Mann–Whitney test. The rest of variables (categorical) are expressed as absolute frequencies and percentages for each group in parentheses; *p*-values for examining associations between categorical variables were derived from the Pearson Chi-square test. [a] Diagnosis of arterial hypertension: >140/90 mmHg, according to the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension and the European Society of Cardiology.[43] [b] Diagnosis of dyslipidemia according to 2019 European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) Guidelines for the Management of Dyslipidaemias.[44]

were assessed at baseline (T0), after 12 weeks (T12) and 24 weeks (T24).

Biochemical measurements. Fasting blood and 24 h-urine samples were collected and immediately sent to the Laboratory of Analysis and Clinical Biochemistry at the University Hospital Complex of Ourense. The following parameters were conducted for each participant: (a) fasting plasma glucose, estimated average plasma glucose, HbA1c, fasting insulin and insulin resistance (calculated by the homeostatic models HOMA IR index) for glucose management (primary outcomes); (b) total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), very low-density lipoprotein cholesterol (VLDL-c) and triglycerides for lipid profile (secondary outcome). All parameters were assayed according to standard laboratory methods.

Anthropometric parameters and clinical data. A physical examination, blood pressure evaluation, and collection of clinical data (including medical condition and medication use) were conducted for all patients at each visit (T0, T12, and T24). Height and weight (secondary outcomes) were measured using cloths, but not shoes. Systolic and diastolic blood pressures (secondary outcomes) were recorded in the seated position following a five-minute period of rest. This was achieved by averaging two measurements, with a five-minute interval between each, using a standard automated device. BMI (secondary outcome) was calculated as weight divided by height squared (kg m^{-2}).

Ethical considerations

The protocol was approved on 18 July 2019, by the Regional Ethics Committee for Clinical Research of the Galician Health Service (Registration identifier: 2019/309). Prior to enrolment, all participants were required to sign an informed consent form, which detailed the objectives and methodology of the trial. The OILDIABET trial (ClinicalTrials.gov Identifier: NCT06757751) was conducted according to the recommendations of the Helsinki Declaration,[20] the CONSORT reporting guidelines[21] and the Good Clinical Practice Guidelines of the International Council for Harmonization.[22]

Sample size estimation

Sample size calculation was performed by Ene 3.0 software (Universitat Autònoma de Barcelona) to assess the minimum number of participants to be included in the study.

Based on reported literature, HbA1c level was considered to calculate the sample size. Indeed, the critical role of HbA1c in the prevention and management of T2D was substantiated in the EPIC (European Prospective Investigation of Cancer and Nutrition)-Norfolk study, which demonstrated that a 1% increase in HbA1c can elevate the risk of all-cause mortality by 28%.[23] Power calculations indicated that a sample size of 48 participants for Control group and 59 participants for Interventional group was adequate to provide a statistical power of 80% to detect a statistically significant difference of

0.5% between means of Control group and Interventional group at T24, considering a standard deviation (SD) of 0.85, a 5% level of significance, a proportion of the sample in the Control group of 45%, and a drop-out rate of 10%.

Statistical analysis

Appropriate descriptive statistical techniques were employed to characterize the participants under investigation: categorical variables were presented as absolute frequencies and percentages, quantitative variables as mean and SD for roughly symmetrically distributed variables, or as a median and interquartile range for variables whose distribution is heavily skewed. The Kolmogorov–Smirnov test was performed to examine normality of the data set when sample size was $n \geq 30$, when $n < 30$ Shapiro Wilk test was employed.

Comparative analyses of outcomes at Baseline, 12, and 24 weeks (T0, T12, and T24) between the Control and Interventional groups were conducted utilizing the Mann–Whitney test for non-parametric data and the Student *t*-test for parametric data concerning quantitative variables, along with the Chi-square test or Fisher exact test for categorical variables. To estimate the evolution and change of the outcomes at T0, T12 and T24 of follow-up, within each group, the repeated measures ANOVA test or Friedman test were used in the case of quantitative variables, depending on the type of distribution they followed. Regarding qualitative variables, Cochrane *Q* test was executed. Additionally, *post-hoc* tests were performed using the Bonferroni correction adjustment method for multiple pairwise comparisons.

Analyses were conducted with a 95% confidence level using IBM Statistical Package for Social Sciences (SPSS) version 29 in Spanish.

Results

Characterization of the phenolic composition of the selected Galician EVOO

A total of 23 phenolic compounds were detected in the Galician EVOO (Table 2). The phenolic compounds were categorized based on their chemical structure and grouped into several families, including secoiridoids (derivatives of oleuropein and ligstroside), simple phenols, organic acids, flavonoids, and lignans.

The group of secoiridoid derivatives were the main phenolic group in the selected EVOO. This group was divided into: oleuropein derivatives, including decarboxymethyl oleuropein aglycone (DOA, also known as oleacein), dehydro oleuropein aglycone (DH-OlAgly), hydroxy oleuropein aglycone (Hy-OlAgl), and four oleuropein aglycone isomers (OlAgl (Is I), OlAgl (Is II), OlAgl (Is III), and OlAgl (Is IV)); and ligstroside derivatives, including decarboxymethyl ligstroside aglycone (DLA, also known as oleocanthal) and three ligstroside aglycone isomers (LigAgl (Is I), LigAgl (Is II), and LigAgl (Is III)). The total concentration of oleuropein derivatives (quantified in terms of oleacein-DOA) was 319.96 mg DOA per kg oil, meanwhile ligstroside derivatives (quantified as oleocanthal-DLA) was 641.50 mg DLA per kg oil. Both subgroups (oleuropein derivatives and ligstroside derivatives) constituted 32% and 64%, respectively, of the total phenolic compounds.

Regarding oleuropein derivatives, DOA emerged as the predominant phenolic compound, with a concentration of 196.02 mg DOA per kg oil, followed by OlAgl (Is III) at 102.73 mg DOA per kg oil. For ligstroside derivatives, LigAgl (Is III) exhibited the highest concentration with 400.81 mg DLA per kg oil, followed by DLA with a concentration of 201.35 mg DLA per kg oil.

The last four groups made up just 4% of the total phenolic compounds present in the Galician EVOO sample:

- Simple phenols, constituting 3.4% of the composition, encompass oxidized hydroxytyrosol (O-HTy), hydroxytyrosol (HTy), hydroxytyrosol acetate (HTy-Ac), and tyrosol (Ty). The concentration of HTy was the most prominent with a concentration of 20.38 mg kg^{-1} oil, followed by Ty at 11.59 mg kg^{-1} oil and oxidized HTy at 1.41 mg kg^{-1} oil.
- Flavonoids (0.4%) comprise Lut, apigenin (Api), and Dios. Lut was identified as the major flavone, with a concentration of 2.94 mg kg^{-1} oil, followed by Api at 0.57 mg kg^{-1} oil, and Dios at 0.30 mg kg^{-1} oil.
- Phenolic acids, constituting 0.033% of the composition, include a hydroxybenzoic acid (vanillic acid, Van) and a hydroxycinnamic acid (*p*-coumaric acid, *p*-Cou). These compounds were quantified at relatively low concentrations, with total amounts of 0.03 mg Van per kg oil and 0.30 mg *p*-Cou per kg oil, respectively.
- Lignans, comprising 0.13% of the phenolic content, include Pin as well as its acetylated derivative, with respective concentrations of 1.11 and 0.13 mg Pin per kg oil.

Baseline characteristics of the OILDIABET participants

Out of 155 subjects evaluated for eligibility, 116 were eventually allocated into two groups according to EVOO supplementation: the Control group and the Interventional group. Eight participants left the study, with 2 unable to comply and 6 citing personal reasons. As a result, the final study population consisted of 108 participants—49 in the Control group, which included 29 females and 20 males (47% of whom were people with obesity), and 59 in the Interventional group, composed of 33 females and 26 males (57% of whom were people with obesity). Consequently, at the end of the intervention, the OILDIABET trial achieved high compliance rates (93.1%) and low dropout rates (6.8%). Fig. 1 provides a visual representation of the study following the CONSORT flow diagram.

The baseline demographic and clinical characteristics of the OILDIABET participants are described in Table 1. The baseline characteristics of the study volunteers were largely comparable between groups, with no statistically significant differences. The median age of participants in both groups was 66–67 years, and the gender distribution was comparable, with a marginally higher number of females than males in each group. Smoking habits, obesity prevalence, and rates of arterial hypertension and dyslipidemia also appeared

Table 2 Concentration of the phenolic compounds in the selected Galician EVOO

Phenolic compounds	Acronym	Concentration (mg kg^{-1} of EVOO)
Oleuropein derivatives		
Decarboxymethyl oleuropein aglycone (oleacein)	DOA	196.02 ± 26.54
Dehydro oleuropein aglycone	DH-OlAgl	2.58 ± 0.50
Hydroxy oleacein	Hy-DOA	13.83 ± 0.62
Hydroxy oleuropein aglycone	Hy-OlAgl	1.45 ± 0.49
Oleuropein aglycone (isomer I)	OlAgl (Is I)	<LD
Oleuropein aglycone (isomer II)	OlAgl (Is II)	1.00 ± 1.05
Oleuropein aglycone (isomer III, main peak)	OlAgl (main peak)	102.73 ± 3.95
Oleuropein aglycone (isomer IV)	OlAgl (Is IV)	2.35 ± 0.86
Total		**319.96**
Ligstroside derivatives		
Decarboxymethyl ligstroside aglycone (oleocanthal)	DLA	201.35 ± 16.27
Ligstroside aglycone (isomer I)	LigAgl (Is I)	<LD
Ligstroside algycone (isomer II)	LigAgl (Is II)	39.34 ± 4.24
Ligstroside aglycone (isomer III, main peak)	LigAgl (main peak)	400.81 ± 31.68
Total		**641.50**
Simple phenols		
Hydroxytyrosol	HTy	20.38 ± 1.20
Hydroxytyrosol acetate	HTy-Ac	<LD
Oxidized hydroxytyrosol	O-HTy	1.41 ± 0.10
Tyrosol	Ty	11.59 ± 0.30
Total		**33.38**
Phenolic acids		
p-Coumaric acid	*p*-Cou	0.30 ± 0.03
Vanillic acid	Van	0.03 ± 0.002
Total		**0.33**
Flavonoids		
Apigenin	Api	0.57 ± 0.04
Diosmetin	Dios	0.30 ± 0.01
Luteolin	Lut	2.94 ± 0.30
Total		**3.81**
Lignans		
Acetoxy pinoresinol	Ac-Pin	0.13 ± 0.02
Pinoresinol	Pin	1.11 ± 0.04
Total		**1.24**

LD: limit of detection.

balanced, as indicated by p-values $\geq$ 0.05. With regard to comorbidities, including heart-related diseases and nephropathy, no significant differences were observed between both groups. Medication use patterns for glucose regulation, lipid control, blood pressure and heart failure were also consistent between groups (Table S3 of ESI†). Most participants in both groups were treated with metformin to lower glucose levels (91.8% in the Control group and 86.4% in the Interventional group, p = 0.37).

Baseline values for primary and secondary outcomes in both Control and Interventional groups are presented in Table 3.

With respect to the indicators of diabetes control (primary outcomes)—fasting glucose, estimated average glucose, HbA1c, and fasting insulin levels—, although the profile of the Control group exhibited a marginally superior trend, this did not reach statistical significance. Insulin resistance (HOMA IR) values did not differ significantly. A comprehensive review of the data revealed elevated fasting glucose levels (>115 mg dL^{-1}) and HbA1c levels (>6.0%), which is consistent with the diagnosis of diabetes in all subjects.

Lipid parameters, including HDL-c, LDL-c, VLDL-c, total cholesterol, and triglycerides, showed no significant differences between groups. No significant differences were observed in anthropometric indices, including weight and BMI, between the groups. Systolic and diastolic blood pressure and heart rate were found to be similar across groups, with no statistical discrepancies identified.

Biomarkers of adherence

The adherence to the OILDIABET trial was evaluated by measuring the sulfHTy and sulfHTyAc levels in the 24-hour urine samples collected at the baseline (T0) and the endpoint (T24) for all volunteers. These metabolites were proposed by Rubió *et al.*, (2014)[19] as compliance biomarkers following sustained consumption of a phenol-enriched virgin olive oil. A comparison of their concentration levels at baseline and at the end of intervention confirmed that both sulfHTy and

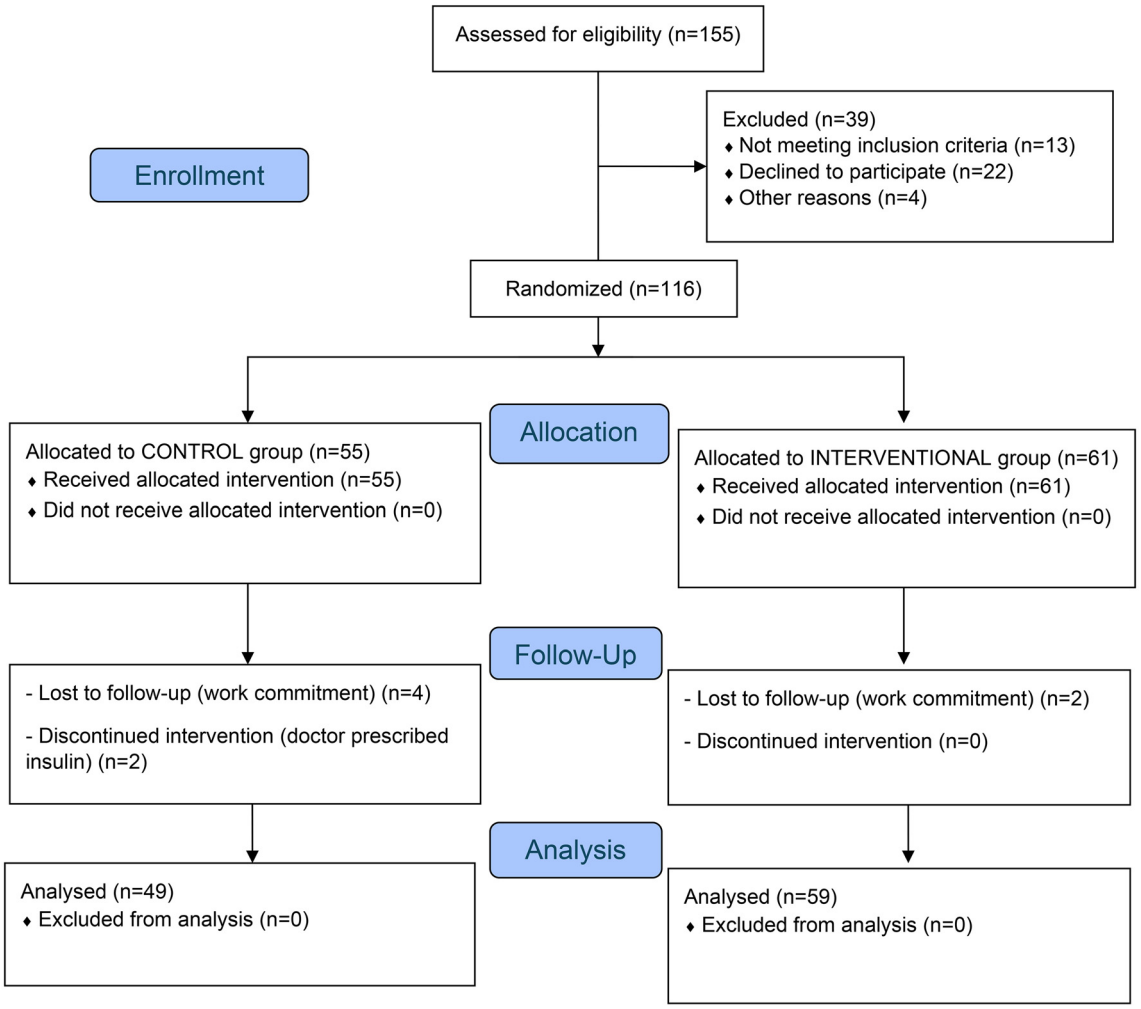

Fig. 1 Consort flow diagram of selection and allocation of the participants included in the OILDIABET study.

sulfHTyAc levels increased 310% (p = 0.00) and 367% (p = 0.00) respectively, in the Interventional group with respect to the Control group (Fig. 2).

Effect of EVOO consumption on primary and secondary outcome variables

Between-group comparisons over the follow-up period

Total sample. Table S4† presents the mean differences (Control group – Interventional group) for every outcome variable (primary and secondary), along with the corresponding p-values derived from independent-samples Student's t-tests conducted at each evaluation time (baseline, 12 weeks and 24 weeks). No statistically significant differences were observed between both groups for any variable ($p \geq 0.05$).

Obesity and insulin-resistant subgroups. In order to account for inter-individual variability, it is necessary to establish a cut-off point in the response, to ascertain whether this divides subjects into two distinct categories: those who are responsive and those who are non-responsive.[24] In this sense, the total sample was dichotomized according to health status, and two subgroups were identified. The first subgroup comprised individuals with obesity (BMI $\geq$ 30 kg m^{-2}) (Table S5†) and the second subgroup comprised subjects with high HOMA IR ($\geq$3.8) (Table S6†). This cut-off point for insulin resistance was selected based on previous evidence in Spanish populations,[25] corresponding to the 90th percentile of the HOMA IR distribution. In both cases, as well as for the entire sample, no statistically significant differences were observed between the groups at any time point for any variable ($p \geq 0.05$).

Within-group comparisons over the follow-up period

Total sample. Table 4 shows the evolution of the primary and secondary outcomes in the total sample (n = 108) for Control and Interventional groups, respectively.

The data presented in Table 4 revealed significant insights into the biochemical, anthropometric and blood pressure changes of the **Control group** over time.

In terms of diabetes control, there was a gradual improvement in fasting and estimated average glucose levels, along with HbA1c, with the last two reaching statistical significance (p = 0.005). Fasting insulin and HOMA IR values exhibited minor changes without statistical significance.

Table 3 Baseline characteristics of primary and secondary outcomes in the Control and Interventional groups of the OILDIABET study

Parameter	Control group (n = 49)	Interventional group (n = 59)	p-Value
Primary Outcomes			
Diabetes control			
Fasting glucose (mg dL^{-1})	139.00 (115.00–156.50)	128.00 (114.00–144.00)	0.061
Estimated average glucose (mg dL^{-1})	152.00 (138.50–170.50)	146.00 (131.00–158.00)	0.051
HbA1c (%)	6.90 (6.45–7.55)	6.70 (6.20–7.10)	0.051
Fasting insulin (μU mL^{-1})	7.10 (4.60–11.90)	9.00 (6.30–14.00)	0.051
HOMA IR	2.20 (1.45–4.10)	2.70 (2.00–4.20)	0.19
Secondary outcomes			
Lipid profile control			
HDL cholesterol (mg dL^{-1})	50.00 (43.00–55.50)	49.00 (42.00–56.00)	0.72
LDL cholesterol (mg dL^{-1})	85.00 (54.00–106.00)	82.50 (62.00–106.00)	0.75
VLDL cholesterol (mg dL^{-1})	25.00 (20.00–31.00)	26.00 (20.00–33.25)	0.73
Total cholesterol (mg dL^{-1})	163.00 (104.00–192.00)	167.00 (135.00–185.00)	0.72
Triglycerides (mg dL^{-1})	123.00 (100.50–163.00)	131.00 (98.00–172.00)	0.90
Anthropometric control			
Weight (kg)	84.00 (75.00–91.00)	82.00 (73.00–90.00)	0.70
BMI (kg m^{-2})	29.74 (26.80–33.25)	30.60 (27.70–35.30)	0.37
Hypertension control			
SBP (mm Hg)	140.00 (132.00–152.00)	140.00 (128.00–151.00)	0.69
DBP (mm Hg)	82.00 (79.50–90.00)	84.00 (79.00–90.00)	0.88
Heart rate (bpm)	74.00 (65.00–84.50)	77.00 (69.00–85.00)	0.38

Data expressed as median (IQR). p-Value was derived from Mann–Whitney test. HbA1c: glycated hemoglobin; HDL: high-density lipoproteins, LDL: low-density lipoproteins, VDL: very low-density lipoproteins; BMI: body mass index; SBP: systolic blood pressure, DBP: diastolic blood pressure.

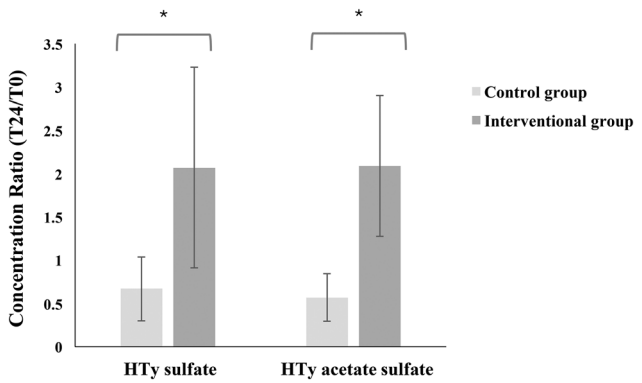

Fig. 2 Concentration ratio (T24/T0) of the selected compliance biomarkers of EVOO intake in 24 h urine collected samples for both Control and Interventional groups. * Indicates significant differences (p < 0.05); p-value was derived from the Mann–Whitney test.

Changes in key parameters of lipid profile and hypertension control were non-significant ($p \geq 0.05$). Weight decreased after 24 weeks, indicating a statistically significant reduction in body weight ($p < 0.05$). Furthermore, BMI showed a slight decrease, which aligns with the weight reduction.

The results of Table 4 also disclosed substantial information on the biochemical, anthropometric and blood pressure modifications in the **Interventional group** throughout time. In terms of glycemic control, the Interventional group showed a marked reduction in estimated average glucose and HbA1c (p = 0.009 and p = 0.019, respectively), and in contrast to the Control group, HOMA IR decreased significantly after 24 weeks (p = 0.015 adjusted by the Bonferroni correction for T0 and T24 comparison). Stratified analyses of study participants by gender concluded that women were responsible for this improvement in insulin resistance, the median values (IQR) for 0, 12 and 24 weeks were 3.30 (2.10–5.45), 2.90 (1.55–4.37), 2.90 (1.60–3.77), respectively. Using the Bonferroni correction *post-hoc* test for multiple pairwise comparisons between group medians, significant differences were identified between 0 and 24 weeks (p = 0.013).

No statistically significant changes were observed in the remaining parameters related to lipid profile and hypertension. Nevertheless, as in the Control group, there was a significant reduction in weight among participants of the Interventional group (p = 0.028). BMI also showed a corresponding decrease.

Importantly, there were no statistically significant differences between the Control and Interventional groups in the proportion of individuals whose medication for the treatment of T2D, dyslipidemia and hypertension was modified as shown in Table S7 of ESI.†

Obesity and insulin-resistant subgroups. In the **obesity subgroup** (n = 57), significant changes were observed in parameters associated with improved glycemic control exclusively within the Interventional group (n = 34). Specifically, there were notable reductions in fasting glucose, estimated average glucose and HbA1c levels at the end of the intervention with EVOO (Table 5). Stratifying this sub-sample by gender, fasting glucose levels improved significantly in women with obesity (n = 19), and the median values (IQR) for 0, 12 and 24 weeks were 117 mg dL^{-1} (103–136), 114 (98–141), 109 (94–129), respectively. Using the Bonferroni correction *post-hoc* test for multiple

Table 4 Evolution of the primary and secondary outcomes for the Control group (n = 49) and the Interventional group (n = 59) during the follow-up period of the OILDIABET trial

	Control group				Interventional group			
Parameter	T0 (0 weeks)	T12 (12 weeks)	T24 (24 weeks)	p-Value	T0 (0 weeks)	T12 (12 weeks)	T24 (24 weeks)	p-Value
Primary outcomes								
Diabetes control								
Fasting glucose (mg dL^{-1})	139.00 (115.00–156.50) a	121.00 (107.50–146.00) a	123.00 (108.50–146.00) a	0.030	127.75 ± 22.33	125.80 ± 23.93	123.69 ± 22.39	0.33
Estimated average glucose (mg dL^{-1})	**154.12 ± 22.21 b**	**150.00 ± 20.34 ab**	**146.76 ± 17.41 a**	**0.005**	**145.66 ± 19.14 b**	**144.17 ± 16.15 b**	**140.73 ± 15.20 a**	**0.009**
HbA1c (%)	**6.99 ± 0.76 b**	**6.85 ± 0.70 ab**	**6.74 ± 0.61 a**	**0.005**	**6.70 (6.20–7.10) ab**	**6.70 (6.20–7.10) b**	**6.70 (6.10–6.90) a**	**0.019**
Fasting insulin (μU mL^{-1})	7.10 (4.60–11.90)	8.50 (4.50–12.70)	7.40 (4.65–11.70)	0.61	9.00 (6.30–14.00)	10.70 (5.70–13.90)	8.40 (6.00–11.70)	0.41
HOMA IR	2.20 (1.45–4.10)	2.50 (1.42–4.10)	2.20 (1.35–4.10)	0.96	**2.70 (2.00–4.20) b**	**2.80 (1.60–4.20) ab**	**2.60 (1.60–3.70) a**	**0.015**
Secondary outcomes								
Lipid profile control								
HDL cholesterol (mg dL^{-1})	50.14 ± 10.22	50.43 ± 9.61	51.08 ± 10.05	0.40	49.00 (42.00–56.00)	50.00 (43.00–57.00)	49.00 (42.00–58.00)	0.69
LDL cholesterol (mg dL^{-1})	88.51 ± 35.61	82.63 ± 28.55	76.77 ± 26.86	0.087	85.55 ± 33.59	83.19 ± 26.36	82.91 ± 25.93	0.56
VLDL cholesterol (mg dL^{-1})	25.00 (20.00–31.00)	25.00 (18.00–33.25)	23.00 (18.00–33.00)	0.31	26.00 (20.00–33.25)	24.00 (18.50–34.50)	24.50 (18.00–30.25)	0.23
Total cholesterol (mg dL^{-1})	163.00 (140.00–192.00)	162.00 (139.00–181.00)	158.00 (134.00–177.50)	0.72	164.03 ± 38.95	161.41 ± 31.65	160.19 ± 30.27	0.51
Triglycerides (mg dL^{-1})	123.00 (100.50–163.00)	125.00 (90.00–174.00)	119.00 (92.50–174.50)	0.23	131.00 (98.00–172.00)	122.00 (93.00–184.00)	126.00 (92.00–155.00)	0.48
Anthropometric control								
Weight (kg)	**84.00 (75.00–91.00) b**	**82.50 (73.75–90.00) ab**	**81.00 (72.00–89.00) a**	**0.000**	**82.00 (73.00–90.00) b**	**80.00 (71.00–92.00) ab**	**82.00 (71.00–91.00) a**	**0.028**
BMI (kg m^{-2})	**29.74 (26.80–33.25) b**	**29.84 (26.68–33.92) ab**	**29.40 (26.30–33.15) a**	**0.000**	**30.60 (27.70–35.30) a**	**30.41 (28.11–34.70) a**	**30.09 (28.00–34.37) a**	**0.039**
Hypertension control								
Systolic arterial pressure (mm Hg)	141.50 ± 16.49	137.02 ± 16.91	139.02 ± 15.37	0.094	136.15 ± 12.86	132.44 ± 17.52	133.12 ± 12.83	0.15
Diastolic arterial pressure (mm Hg)	83.71 ± 8.53	81.23 ± 8.91	82.02 ± 8.59	0.077	83.88 ± 8.35	83.35 ± 8.57	84.32 ± 9.14	0.79
Heart rate (bpm)	75.16 ± 12.09	75.73 ± 11.24	74.00 ± 10.08	0.52	76.78 ± 11.19	76.33 ± 12.09	75.86 ± 10.85	0.90

Values are expressed as mean ± SD for normal variables or as median (IQR) for non-normal variables. Repeated measures one-way ANOVA for variables with normal distribution and Friedman one-way repeated measure analysis of variance by ranks for variables without normal distribution were used to compare the three related groups (0, 12, 24 weeks) of paired data. In the statistically significant results, a *post-hoc* analysis adjusted by the Bonferroni correction was performed. Different letters in the same line mean a significant difference at 5% probability level. BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HbA1c: glycated hemoglobin, HDL: high-density lipoproteins, LDL: low-density lipoproteins, VDL: very low-density lipoproteins.

Table 5 Evolution of the primary and secondary outcomes for subjects with obesity (BMI ≥ 30 kg m^{-2}) of the Control group (n = 23) and the Interventional group (n = 34) during the follow-up period of the OILDIABET trial

	Control group				Interventional group			
Parameter	T0 (0 weeks)	T12 (12 weeks)	T24 (24 weeks)	p-Value	T0 (0 weeks)	T12 (12 weeks)	T24 (24 weeks)	p-Value
Primary outcomes								
Diabetes control								
Fasting glucose (mg dL^{-1})	134.30 ± 32.80	127.96 ± 35.14	129.61 ± 31.81	0.58	**120.50 (109.75–145.25) b**	**120.50 (103.00–141.00) ab**	**117.00 (103.75–135.50) a**	**0.049**
Estimated average glucose (mg dL^{-1})	152.00 (141.00–166.00)	146.00 (141.00–151.00)	144.00 (134.00–160.00)	0.47	**143.32 ± 19.32 b**	**141.21 ± 16.70 ab**	**138.21 ± 15.86 a**	**0.031**
HbA1c (%)	6.90 (6.50–7.40)	6.70 (6.50–6.90)	6.60 (6.30–7.20)	0.37	**6.63 ± 0.67 b**	**6.54 ± 0.58 ab**	**6.44 ± 0.56 a**	**0.031**
Fasting insulin (μU mL^{-1})	12.40 ± 5.75	12.60 ± 4.88	12.16 ± 5.97	0.91	11.05 (7.32–14.67)	10.90 (6.17–14.67)	9.25 (6.72–13.87)	0.77
HOMA IR	4.15 ± 2.25	3.91 ± 1.66	3.99 ± 2.32	0.86	2.75 (2.17–4.42)	3.00 (1.67–3.97)	3.00 (1.67–3.70)	0.11
Secondary outcomes								
Lipid profile control								
HDL cholesterol (mg dL^{-1})	48.26 ± 8.63	48.61 ± 8.15	48.48 ± 8.30	0.92	49.00 (42.00–55.25)	50.00 (43.50–55.50)	48.50 (43.00–55.00)	0.54
LDL cholesterol (mg dL^{-1})	84.32 ± 31.89	83.95 ± 25.61	75.57 ± 27.12	0.43	86.56 ± 31.20	85.15 ± 23.33	86.21 ± 23.81	0.96
VLDL cholesterol (mg dL^{-1})	27.00 (19.75–33.00)	24.50 (18.00–32.25)	24.00 (19.00–37.00)	0.95	25.50 (21.00–34.50)	24.00 (18.50–32.00)	24.50 (17.25–31.50)	0.11
Total cholesterol (mg dL^{-1})	161.57 ± 36.51	159.52 ± 28.56	152.87 ± 32.70	0.34	164.88 ± 36.74	162.56 ± 28.50	162.56 ± 27.30	0.80
Triglycerides (mg dL^{-1})	138.00 (99.00–163.00)	126.00 (92.00–178.00)	121.00 (96.00–185.00)	0.88	126.00 (103.75–173.50)	124.00 (92.25–170.75)	123.00 (88.25–158.00)	0.27
Anthropometric control								
Weight (kg)	**91.00 (84.00–100.00) b**	**89.00 (82.00–97.00) ab**	**89.00 (81.00–97.00) a**	**0.005**	**89.63 ± 11.19 b**	**88.16 ± 11.40 a**	**88.05 ± 11.79 a**	**0.007**
BMI (kg m^{-2})	**33.40 (32.00–37.89) b**	**33.70 (30.80–36.50) ab**	**32.70 (30.40–36.90) a**	**0.003**	**33.71 (31.42–37.40) b**	**32.84 (30.96–37.17) ab**	**32.61 (30.72–36.57) a**	**0.010**
Hypertension control								
Systolic arterial pressure (mm Hg)	135.70 ± 14.97	132.26 ± 18.45	133.74 ± 13.86	0.51	136.15 ± 12.86	132.44 ± 17.52	133.12 ± 12.83	0.15
Diastolic arterial pressure (mm Hg)	83.70 ± 8.42	80.70 ± 9.35	84.30 ± 7.33	0.067	83.88 ± 8.35	83.35 ± 8.57	84.32 ± 9.14	0.79
Heart rate (bpm)	75.35 ± 12.85	76.00 ± 11.10	72.57 ± 8.53	0.29	77.12 ± 11.48	77.18 ± 12.63	76.53 ± 10.91	0.90

Values are expressed as mean ± SD for normal variables or as median (IQR) for non-normal variables. Repeated measures one-way ANOVA for variables with normal distribution and Friedman one-way repeated measure analysis of variance by ranks for variables without normal distribution were used to compare the three related groups (0, 12, 24 weeks) of paired data. In the statistically significant results, a *post-hoc* analysis adjusted by the Bonferroni correction was performed. Different letters in the same line mean a significant difference at 5% probability level. BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HbA1c: glycated hemoglobin, HDL: high-density lipoproteins, LDL: low-density lipoproteins, VDL: very low-density lipoproteins.

pairwise comparisons between group medians, significant differences were identified between 0 and 24 weeks ($p = 0.006$).

In the **insulin-resistant sub-sample** ($n = 33$), clinical markers related to diabetes control (*viz.* fasting insulin and HOMA IR) exhibited significant enhancement at the conclusion of the intervention period for the Interventional group (Table 6). Stratifying this sub-sample by gender, in the Interventional group, the percentage of males with HOMA IR ≥ 3.8 was significantly greater at the outset of the study than at the 24-week follow-up (100% *vs.* 37.5%, respectively; $p = 0.037$, adjusted by the Bonferroni correction).

Furthermore, it was observed that in the Interventional group of the obesity subgroup, the number of subjects with HOMA IR > 3.8 exhibited a significant decline after 24-weeks of intervention period (20.6% in the Interventional group *vs.* 52.2% in the Control group, respectively, $p = 0.013$), in contrast to observations made at 0 and 12 weeks.

Discussion

OILDIABET trial characteristics

Dietary interventions have successfully been expanded over the past three decades to supply robust evidence for the development of dietary guidelines. A number of short-term intervention studies have been conducted to analyze the effects of olive oil consumption on individuals with T2D and/or overweight status. Nevertheless, the results of these studies have not provided definitive evidence regarding the advantages of this dietary strategy.[26,27] One of the primary sources of variability observed across these studies is the varying amounts and types of olive oil consumed by the participants, as well as the number of patients included and the duration of the study period. It is well known that for a comprehensive assessment of the health-related properties of a functional food, intervention studies should be replicated in several populations to mitigate potential biases linked to the study design and participant characteristics, which are inherent to any study. For this reason, in the OILDIABET trial, adults diagnosed with T2D—including individuals with and without obesity—were subjected to a dietary supplementation comprising 30 mL of phenolic-rich EVOO per day and were subsequently monitored for a period of six months to evaluate the effect on glycemic, lipid, anthropometric and blood pressure measurements.

One of the key strengths of this study is that, for the first time, a dietary intervention has been carried out with Galician EVOO, elaborated with autochthonous varieties recently recovered. Of particular interest is the high concentration of phenolic compounds present in this olive oil, recognized as bioactive compounds (Table 2).

A parallel rather than a cross-over design was chosen for this dietary intervention study. Although a parallel-group design typically requires a larger sample size, it offers a shorter overall duration compared to cross-over trials, thereby reducing the likelihood of participant dropout without compromising statistical power. Nevertheless, it should be acknowledged that a cross-over design generally provides more robust results, since each participant serves as their own control, thereby reducing inter-individual variability. Despite this limitation, the sample size for the OILDIABET trial was appropriately calculated, and the required number of participants was successfully recruited, ensuring adequate statistical power.

Randomization is the principal component of a well-designed dietary intervention. As evidenced in Table 1 and Table S3 of ESI† the baseline characteristics of the Control and Interventional groups were well matched, thereby supporting the robustness of the randomization process. This equivalence at baseline provides a reliable basis for assessing the impact of the intervention on the metabolic profile of subjects with T2D.

Evolution of primary and secondary outcome variables over the follow-up period

Analyses comparing outcomes at baseline, 12, and 24 weeks (T0, T12, and T24, respectively) between the Control and Interventional groups for the total sample (Table S4†) revealed no statistically significant results. A similar pattern was observed for obesity (Table S5†) and insulin-resistant (Table S6†) sub-samples. In addition, to verify whether group allocation influenced the metabolic biomarker results over the follow-up period, a repeated measures linear regression model was performed, using the study group as the between-subjects factor; once again, no statistically significant results were identified. Although our randomized controlled trial (RCT) was adequately powered to detect moderate between-group changes, the final sample size may have been insufficient to uncover the relatively small mean differences that are typical of dietary interventions. Therefore, our strategy focused on comparing the values of the main clinical markers related with diabetes (primary outcomes), lipid profile, anthropometric and hypertension individually for each group during the follow-up period (within-group changes) for the entire sample (Table 4) and for obesity (Table 5) and insulin-resistant (Table 6) sub-samples.

Diabetes control. The primary outcomes of the OILDIABET trial were related to glycemic control parameters in T2D participants, specifically fasting glucose, estimated average glucose, HbA1c, fasting insulin and insulin resistance (HOMA IR). Overall, the intervention resulted in significant improvement in HOMA IR after 24 weeks. The novelty of our study lies in the specific evaluation of Galician EVOO, characterized by a distinctive high phenolic profile, in a T2D cohort over a 24-week period. Unlike shorter intervention studies, this trial provides evidence supporting sustained glycemic benefits potentially linked to monounsaturated fatty acids (MUFAs) and EVOO-derived phenolic compounds. The observed significant reduction in insulin resistance (HOMA IR) in the Interventional group highlights the potential clinical relevance of phenolic compounds such as oleacein and oleocanthal. These secoiridoids are known to modulate key pathways involved in glucose metabolism. These compounds have been shown to enhance insulin receptor activity, likely through their

Table 6 Evolution of the primary and secondary outcomes for insulin resistant subjects (HOMA IR ≥ 3.8) of the Control group (n = 14) and the Interventional group (n = 19) during the follow-up period of the OILDIABET trial

	Control group				Interventional group			
Parameter	T0 (0 weeks)	T12 (12 weeks)	T24 (24 weeks)	p-Value	T0 (0 weeks)	T12 (12 weeks)	T24 (24 weeks)	p-Value
Primary outcomes								
Diabetes control								
Fasting glucose (mg dL^{-1})	**160.57 ± 31.84 b**	**141.57 ± 30.63 ab**	**132.57 ± 25.99 a**	**0.012**	136.00 (120.00–156.00)	131.00 (114.00–146.00)	132.00 (113.00–142.00)	0.18
Estimated average glucose (mg dL^{-1})	163.50 (148.25–188.50)	149.50 (145.75–174.25)	149.50 (139.75–166.25)	0.098	145.58 ± 17.44	147.84 ± 14.74	141.95 ± 14.87	0.098
HbA1c (%)	7.30 (6.77–8.15)	6.85 (6.70–7.70)	6.85 (6.50–7.42)	0.11	6.70 (6.20–6.90) a	6.90 (6.40–7.10) a	6.70 (6.00–7.00) a	0.047
Fasting insulin (μU mL^{-1})	15.25 (11.47–18.77)	13.05 (9.37–18.65)	10.50 (7.25–17.57)	0.14	**16.70 (13.80–21.20) b**	**14.10 (11.30–16.30) a**	**12.50 (11.00–17.10) a**	**0.019**
HOMA IR	5.90 (4.42–7.55)	5.05 (3.10–5.97)	4.10 (2.25–5.42)	0.13	**5.300 (4.20–6.90) b**	**4.20 (3.60–6.30) ab**	**3.70 (3.10–5.50) a**	**0.029**
Secondary outcomes								
Lipid profile control								
HDL cholesterol (mg dL^{-1})	40.93 ± 6.31	42.71 ± 7.43	43.00 ± 7.48	0.17	45.89 ± 11.77	48.22 ± 13.33	47.63 ± 9.91	0.37
LDL cholesterol (mg dL^{-1})	72.54 ± 35.31	72.69 ± 29.05	73.62 ± 31.96	0.95	79.50 ± 40.54	78.41 ± 26.87	77.83 ± 24.64	0.73
VLDL cholesterol (mg dL^{-1})	30.00 (23.00–65.50)	31.00 (20.00–46.50)	35.00 (23.75–47.00)	0.61	29.00 (25.75–39.50)	28.00 (25.00–39.00)	28.50 (25.75–33.00)	0.61
Total cholesterol (mg dL^{-1})	153.00 ± 37.08	150.07 ± 30.30	150.36 ± 39.06	0.92	159.68 ± 47.02	159.63 ± 31.34	156.05 ± 27.40	0.71
Triglycerides (mg dL^{-1})	155.50 (118.00–338.75)	166.50 (104.75–246.00)	175.50 (119.25–234.25)	0.49	144.00 (129.00–205.00)	143.00 (127.00–204.00)	145.00 (128.00–167.00)	0.89
Anthropometric control								
Weight (kg)	96.91 ± 17.81	94.27 ± 18.25	92.07 ± 17.95	0.20	85.09 ± 12.88	84.78 ± 12.64	84.79 ± 13.34	0.91
BMI (kg m^{-2})	34.00 ± 4.83	32.84 ± 4.70	32.43 ± 5.59	0.17	32.41 ± 4.38	32.25 ± 4.08	32.81 ± 4.67	0.57
Hypertension control								
Systolic arterial pressure (mm Hg)	142.50 (130.25–156.75)	143.00 (126.00–146.50)	137.50 (125.50–144.25)	0.21	138.00 (131.00–145.00)	137.00 (125.00–140.00)	139.00 (129.00–143.00)	0.22
Diastolic arterial pressure (mm Hg)	83.00 (80.00–87.50)	79.00 (74.00–87.00)	81.00 (73.50–86.00)	0.16	85.00 (78.00–90.00)	84.00 (80.00–88.00)	87.00 (84.00–92.00)	0.42
Heart rate (bpm)	74.36 ± 12.31	77.23 ± 15.44	72.29 ± 10.16	0.46	80.95 ± 10.71	81.06 ± 12.54	78.37 ± 9.66	0.40

Values are expressed as mean ± SD for normal variables or as median (IQR) for non-normal variables. Repeated measures one-way ANOVA for variables with normal distribution and Friedman one-way repeated measure analysis of variance by ranks for variables without normal distribution were used to compare the three related groups (0, 12, 24 weeks) of paired data. In the statistically significant results, a *post-hoc* analysis adjusted by the Bonferroni correction was performed. Different letters in the same line mean a significant difference at 5% probability level. BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HbA1c: glycated hemoglobin, HDL: high-density lipoproteins, LDL: low-density lipoproteins, VDL: very low-density lipoproteins.

anti-inflammatory and antioxidant effects. Specifically, they inhibit pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which are strongly associated with insulin resistance. TNF-α, in particular, inhibits the secretion of adiponectin, a cytokine known to improve glucose metabolism by enhancing insulin sensitivity. Furthermore, Silveira *et al.*, (2022)[28] demonstrated that oleic acid in combination with HTy could counteract TNF-α-induced suppression of adiponectin in adipocytes, further emphasizing the anti-inflammatory potential of EVOO. Additionally, HTy and Ty have been reported to reduce oxidative stress markers and improve mitochondrial function, further supporting their role in glucose homeostasis.[27,29]

Moreover, the ability of these phenolic compounds to improve endothelial function and increase nitric oxide bioavailability may also contribute to better glucose uptake in peripheral tissues. These findings align with prior *in vitro* and *in vivo* studies suggesting that EVOO phenolics positively influence glucose transporter activity and hepatic glucose metabolism.

When evaluating specific subgroups, significant improvements were observed among participants with obesity (BMI $\geq$ 30 kg m^{-2}), who demonstrated notable reductions in fasting glucose, estimated average glucose, and HbA1c levels after EVOO intervention. Similarly, insulin-resistant participants (HOMA IR $\geq$ 3.8) displayed significant improvements in fasting insulin and HOMA IR. Nonetheless, these subgroup analyses should be cautiously interpreted due to the limited sample sizes, potentially affecting statistical power. These findings are consistent with previous research, notably the meta-analysis by Schwingshackl *et al.* (2017),[13] which demonstrated that olive oil supplementation significantly reduced HbA1c and fasting plasma glucose compared to control groups. Similarly, the recent umbrella review conducted by Chiavarini *et al.* (2024)[26] reinforced these conclusions, highlighting the beneficial impact of EVOO consumption on glucose homeostasis and insulin sensitivity, thus supporting the potential of EVOO consumption for the prevention and control of T2D.

Focusing specifically on interventions in individuals with overweight/obesity and prediabetes or T2D, previous shorter-duration trials have shown mixed results. Santangelo *et al.* (2016)[30] observed that, after 4 weeks of consuming high-phenolic EVOO (25 mL day^{-1}), overweight non-insulin-treated T2D patients showed reductions in fasting plasma glucose and HbA1c compared to consuming refined olive oil (25 mL day^{-1}, without phenolic compounds), despite following the same diet throughout the intervention. Likewise, Ruíz-García *et al.* (2023)[10] reported improved fasting glucose levels after a one-month intervention with EVOO *versus* common olive oil in individuals with obesity (30–40 kg m^{-2}) with prediabetes (HbA1c 5.7–6.4%), although no significant changes in HbA1c, insulinemia, insulin resistance, or HOMA B. Additionally, Silveira *et al.* (2022)[28] found that adherence to a traditional Brazilian diet supplemented with EVOO significantly reduced fasting insulin levels in adults with obesity and T2D, although other glycemic parameters remained unchanged. These contrasting results underline the complexity and variability in dietary responses among diverse diabetic populations.

Lipid profile control. Olive oil has been extensively studied for its beneficial effects on plasma concentrations of LDL-c, HDL-c, and total cholesterol.[31–33] However, its role in the management of dyslipidemia remains inconclusive.[34]

In our study, no significant changes were observed in lipid profile parameters, which may be attributed to the duration of the intervention being insufficient to induce substantial alterations. These align with a recent systematic review and dose–response meta-analysis of RCTs on the effects of olive oil consumption on blood lipids in adults. Based on existing evidence, olive oil has trivial effects on levels of serum lipids.[34] Furthermore, Santangelo *et al.* (2016),[30] reported no significant changes in lipid profile following high-phenolic EVOO intake in overweight individuals with T2D.

Some studies have suggested that the maintenance of HDL-c concentrations observed with olive oil intake may be explained by the competition between olive oil chylomicron remnants and HDL particles for hepatic lipase activity.[35] This mechanism could help prevent the postprandial decline in HDL-c levels, potentially contributing to a more favorable lipid profile and cardiovascular health.

Anthropometric control. Regarding anthropometric parameters, both groups exhibited a statistically significant reduction in body weight and BMI after 24 weeks. Although volunteers were encouraged to maintain their daily habits, the participation in this trial as well as a more rigorous medical follow-up may have encouraged them to become more mindful of their dietary habits decreasing for example, the amount of solid fats, which would translate into a greater weight loss and BMI for the Control group in the total sample and the obesity subgroup (Tables 4 and 5, respectively). It is plausible to hypothesize that the reduction in anthropometric parameters within the Control group could be attributed to adherence to a balanced diet, rather than voluntary increase in EVOO consumption, given that the levels of HTy metabolites in urine were lower compared to those in the Interventional group (Fig. 1).

Our findings verified that consumption of Galician phenolic-rich EVOO by T2D adults beneficially affected the anthropometric parameters with a reduction in body weight and BMI for the total sample and the obesity subgroup (Tables 4 and 5, respectively) suggesting that the intervention not only facilitated weight loss but also contributed to overall improvements in body composition.

This behavior is in accordance with several studies documented in the literature, such as the randomized cross-over trials conducted by Santangelo *et al.*, (2016)[30] in non-insulin-treated T2D patients and Ruíz-García *et al.*, (2023)[10] in adults diagnosed with prediabetes and obesity. Additional research indicated that subjects with T2D and obesity, randomized into a group adhering to a traditional Brazilian diet supplemented with EVOO, had a reduction in BMI and weight at the end of the intervention.[28]

The hypothesis that decreasing oxidative stress through antioxidant intake could improve obese phenotypes is supported by a body of research indicating that dietary interventions, particularly those involving the MedDiet enriched with VOO (rich in monounsaturated fatty acids and polyphenols with high antioxidant activity), can have beneficial effects on obesity.[36] It was demonstrated that a MedDiet enriched with EVOO may be an effective alternative to low-fat diets aimed at maintaining weight in adults with overweight or obesity. This is due to the increase in postprandial fat oxidation, as observed after following a meal rich in olive oil.[37,38] Silveira *et al.* (2022)[28] emphasized that a high percentage of body fat is linked to decreased adiponectin production; this, in turn, contributes to insulin resistance and chronic inflammation, which can have catabolic effects on muscle mass.

The hypothesis that EVOO could improve body composition is primarily based on the effect of oleic acid (C18:1) on stearoyl-CoA desaturase 1 (SCD1). SCD1 is an enzyme that catalyzes the conversion of saturated fatty acids (SFAs) into monounsaturated fatty acids (MUFAs), such as oleic acid (C18:1). The consumption of saturated fatty acids has been shown to stimulate SCD1 activity, which may promote obesity by favoring the accumulation of fat. Conversely, oleic acid, derived from EVOO, has been associated with the downregulation of SCD1 activity, which could potentially support weight loss by positively influencing the expression of genes related to adiposity.[39] Furthermore, the possibility that bioactive compounds also contribute to the observed anthropometric changes cannot be ruled out; HTy and Ty (released metabolites of oleacein and oleocanthal after gastrointestinal digestion process) have been found to reduce body weight in people with overweight and obesity.[10]

Hypertension control. EVOO has been studied for its beneficial effects on blood pressure, contributing to hypertension control.[39,40] This protective effect is primarily attributed to the incorporation of oleic acid into cell membranes, which modulates their lipid structure and regulates G protein-mediated signaling, ultimately leading to a reduction in blood pressure.[41] Additionally, the polyphenols in EVOO enhance nitric oxide production and suppress the expression of endothelin-1, a vasoconstrictive peptide implicated in hypertension. Moreover, certain phenolic compounds, such as those in EVOO, have demonstrated the ability to inhibit the angiotensin-converting enzyme, further contributing to antihypertensive effects.[42]

In our study, blood pressure values remained within normal and safe ranges throughout the intervention period for both Control and Interventional groups with no statistically significant variations. This fact may be partially attributed to the administration of angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors to a substantial proportion of the participants, with 53.1% in the Control group and 59.3% in the Interventional group receiving these medications (Table S3†). Moreover, subgroup analyses in participants with obesity and insulin-resistant similarly revealed no significant changes in blood pressure parameters, although caution is advised when interpreting these findings due to limited sample sizes and consequent statistical power.

Similarly, other studies have reported no significant changes in blood pressure following EVOO intake in individuals with T2D and obesity.[28] Furthermore, a single-dose ingestion of high-polyphenolic EVOO, compared to refined olive oil, in adults at risk for T2D did not result in improvements in either systolic or diastolic blood pressure.[32]

Strengths and limitations of the study

The OILDIABET trial has several strengths, including: the rigorous selection and characterization of Galician phenolic-rich EVOOs; the high homogeneity in baseline demographic and clinical characteristics among participants of both groups; the high adherence and compliance rates to the trial; and, finally, the comparatively extended intervention period relative to other analogous studies, featuring three measurement points (baseline, midway, and end of the follow-up period), which allowed for continuous monitoring and analysis of outcome variables, enhancing the robustness of the results.

Nevertheless, this study has several limitations that should be acknowledged. First, no systematic dietary evaluations, such as food frequency questionnaires or 24-hour dietary recalls, were performed either before or during the intervention period. Therefore, baseline EVOO consumption habits of participants were not formally quantified, and actual adherence to dietary recommendations during the trial could not be precisely verified. Additionally, participants in the Control group were advised to minimize the consumption of EVOO, favoring refined olive oil blends instead; however, adherence to this advice was not quantitatively monitored.

Another limitation was the absence of a placebo in the Control group, inherently precluding blinding. This lack of blinding could introduce potential biases related to participant and investigator expectations, possibly affecting subjective outcomes or adherence behavior.

In future interventions with these Galician EVOOs, it is necessary to focus the recruitment process on those diabetic subjects who are in a more vulnerable situation, with a BMI $\geq$ 30 kg m^{-2} and a high insulin resistance. Although the intervention period can be extended, it is essential to include cytokine determination, given that inflammatory processes are intertwined processes which contribute to the etiology and physiopathology of obesity and T2D.

Conclusion

The rising prevalence of diabetes in recent decades underscores the urgent need for effective strategies to delay or prevent its onset. One potential approach is the sustained consumption of EVOO. In this sense, the OILDIABET trial provides new evidence on the effects of daily intake of high-phenolic EVOOs in the treatment of T2D.

Although no statistically significant differences were detected between the Interventional and Control groups, an exploratory within-group analysis revealed a time-dependent benefit of EVOO in participants at higher metabolic risk.

Specifically, subgroup analyses stratified by baseline health status indicated that individuals with obesity (BMI ≥ 30 kg m^{-2}) and insulin resistance (HOMA IR ≥ 3.8) showed improvements in key diabetes-control parameters, identifying them as potential responder subgroups to high-phenolic EVOO intake.

These findings suggest that regular consumption of high-phenolic EVOO could potentially offer beneficial effects as part of dietary strategies for T2D management. Nevertheless, further larger-scale and longer-term studies are required before definitive recommendations can be included in dietary guidelines. Future dietary advice might consider emphasizing the phenolic content of olive oils, exploring the potential therapeutic advantages of regional varieties such as Galician EVOOs.

Abbreviations

Api	Apigenin
BMI	Body mass index
DH-OlAgly	Dehydro oleuropein aglycone
Dios	Diosmetin
DLA	Decarboxymethyl ligstroside aglycone, oleocanthal
DOA	Decarboxymethyl oleuropein aglycone, oleacein
DPP-4	Dipeptidyl peptidase-4
EVOO	Extra virgin olive oil
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide-1
HbA1c	Glycosylated hemoglobin
HDL-c	High-density lipoprotein cholesterol
HIV	Human immunodeficiency virus
HLB	Hydrophilic-lipophilic balance
HOMA IR	Homeostatic model assessment of insulin resistance
HTy	Hydroxytyrosol
HTy-Ac	Hydroxytyrosol acetate
Hy-OlAgl	Hydroxy oleuropein aglycone
IC_{50}	Half maximal inhibitory concentration
IDF	International Diabetes Federation
IL-6	Interleukin-6
IQR	Interquartile range
LD	Limit of detection
LDL-c	Low-density lipoprotein cholesterol
LigAgl	Ligstroside aglycone
Lut	Luteolin
MedDiet	Mediterranean diet
MUFAs	Monounsaturated fatty acids
O-HTy	Oxidized hydroxytyrosol
OlAgl	Oleuropein aglycone
p-Cou	*p*-Coumaric acid
Pin	Pinoresinol
RCT	Randomized controlled trial
SCD1	Stearoyl-CoA desaturase 1
SD	Standard deviation
SGLT2	Sodium-glucose co-transporter 2
sulfHTy	Hydroxytyrosol sulfate
sulfHTyAc	Hydroxytyrosol acetate sulfate
T2D	Type 2 diabetes
TNF-α	Tumor necrosis factor-alpha
Ty	Tyrosol
Van	Vanillic acid
VLDL-c	Very low-density lipoprotein cholesterol
μSPE	Microelution solid phase extraction

Author contributions

María Figueiredo-González: conceptualization, investigation, methodology, validation, writing – original draft, writing – review & editing. Inés Seoane-Cruz: conceptualization, investigation, methodology, supervision, validation. Patricia Reboredo-Rodríguez: data curation, investigation, methodology, validation, writing – original draft. Eva Fernández-Rodríguez: conceptualization, investigation, methodology, validation, writing – review & editing. Manuel Marcos-García: investigation, methodology. María José Menor-Rodríguez: investigation. Beatriz Calderón-Cruz: formal analysis, writing – review & editing. Carmen González-Barreiro: conceptualization, data curation, investigation, project administration, validation, visualization, writing – original draft, writing – review & editing. José Antonio Mato-Mato: resources, funding acquisition. Beatriz Cancho-Grande: conceptualization, funding acquisition, investigation, project administration, supervision, visualization, writing – original draft, writing – review & editing.

Ethical statement

This study was conducted in accordance with the principles of the Declaration of Helsinki, the CONSORT reporting guidelines and the Good Clinical Practice Guidelines of the International Council for Harmonization. Ethical approval for the study was obtained from the Regional Ethics Committee for Clinical Research of the Galician Health Service (Registration identifier: 2019/309). The study protocol was also registered in ClinicalTrials.gov with the Identifier: NCT06757751. The research required the acquisition of written informed consent forms from all subjects before participation, ensuring informed and voluntary engagement in the study.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

The data supporting this article have been included as part of the ESI.†

Acknowledgements

This work is part of the Grant RTI2018–098633-B-I00 funded by MCIN/AEI/10.13039/501100011033 and, as appropriate, by "ERDF A way of making Europe", by the "European Union". María Figueiredo-González is financed as Distinguished Researcher by the Universidad de Vigo (Programa de Retención de Talento 2021). Patricia Reboredo-Rodríguez would like to thank the Ramón y Cajal contract RYC2022-037075-I, funded by MCIN/AEI/10.13039/501100011033 and FSE+. Open access funding was provided by Universidade de Vigo/Consorcio Interuniversitario do Sistema Universitario de Galicia (CISUG).

The authors express their profound gratitude to the clinical staff of the Primary Care Center of A Ponte (Ourense), particularly María Luisa González Barreiro and Pilar Alonso Álvarez, as well as to Carmen Tellado González from the Primary Care Center of Castrelo de Miño (Ourense), for their indispensable assistance in patient recruitment. The authors also sincerely thank all the participants who voluntarily contributed to this study.

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