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MELI-POP Study: MEditerranean LIfestyle in Pediatric Obesity Prevention. Study protocol for a randomized controlled trial

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Abstract

Background Childhood obesity is a significant public health challenge, with Mediterranean countries showing high prevalence rates. While genetic factors play a role, diet and physical activity (PA) are critical modifiable influences. Emphasizing healthy dietary patterns, like the Mediterranean diet, and promoting regular PA can help mitigate obesity risk.

Methods MELI-POP is a randomized controlled multi-center clinical trial in a cohort of children aged 3 to 6 years at baseline and being at obesity risk. The main objective consists on assessing the efficacy of an intervention during early childhood, considering a healthy lifestyle based on the promotion of a Mediterranean dietary pattern and regular PA, compared with a control group, on decreasing obesity incidence 5 and 10 years after the beginning of the intervention. It is expected to include 310 children, aged 3 to 6 years, having at baseline a normal weight or overweight according to the International Obesity Task Force (IOTF) criteria, and at least one parent having a body mass index > 25 kg/m^2. The clinical trial has two arms and is performed in Spanish Primary Health Care centers. The control group receive usual care by healthcare professionals. The intervention group receive education on Mediterranean diet and PA, combined with the provision of extra-virgin olive oil and fish, in order to be consumed at least 2 times per week. Free PA sessions with a physical education teacher are also offered for the children (3 sessions of 60 min of moderate-vigorous PA per week). The participants' adherence to the intervention is periodically monitored. The study primarily focuses on adiposity as the main outcome, with secondary outcomes encompassing dietary intake and eating habits, physical activity and lifestyle behaviors (including extracurricular sports, screen time,

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and sleep duration), physical fitness, biochemical parameters (such as inflammation and cardiometabolic biomarkers, fatty acids, and oxidation), as well as microbiota, genetic, omic and metabolomic analyses.

Discussion Beneficial results are expected by preventing obesity during childhood, and associated comorbidities.

Trial registration ClinicalTrials.gov, ID: NCT04597281.

Keywords Obesity prevention, Mediterranean Diet, Physical Activity, Pediatric Obesity

Background

Overweight and obesity are one of the major public health challenges of the 21 st century [1]. In Europe, the WHO's COSI project revealed that Mediterranean countries have the highest prevalence of overweight and obesity among children aged 6 to 9 years [2]. According to recent Spanish data on children, 40.6% had excess weight (23.3% and 17.3% suffering from overweight or obesity, respectively) [3].

Childhood obesity is linked to a broad spectrum of short-term comorbidities, including diabetes, dyslipidemia, and psychological disorders, which contribute to an increased risk and earlier onset of chronic conditions later in life [4].

The development of obesity in children is influenced by genetic susceptibility. However, certain risk factors related to diet and physical activity (PA) contribute to its development and have driven the increase in its prevalence over recent decades [5, 6]. Regarding diet, it is suggested that dietary patterns, rather than specific foods, are associated with a higher or lower risk of obesity development. Therefore, obesity prevention strategies have recently focused on promoting healthy dietary patterns instead of targeting individual foods [7]. Among these dietary models, the Mediterranean diet stands out for its nutritional value and well-known beneficial effects in preventing chronic diseases [8]. However, in children, there are not many studies assessing adherence to the Mediterranean diet and its relationship with weight gain, obesity, or metabolic syndrome. In a study involving European children aged 2 to 9 years, an inverse association between the degree of adherence to this dietary pattern and the prevalence of overweight/obesity was described in a cross-sectional analysis, as well as the incidence of this condition in the longitudinal analysis after two years of follow-up [9]. It has also been observed in European adolescents that there is an association between adherence to the Mediterranean diet and a lower inflammatory state [10].

Moreover, regular PA contributes to weight management by promoting muscle development, reducing body fat percentage, decreasing energy intake, and improving metabolism [11–13]. By the other hand, a sedentary lifestyle contributes to the development of obesity over time. Therefore, incorporating regular PA into daily routines is essential for maintaining a healthy weight and overall well-being [14].

Moreover, predicting cardiometabolic outcomes in children with obesity is complex due to limited biomarkers and unclear contributing factors. Advances in omics technologies and Artificial Intelligence (AI), particularly Machine Learning (ML), have improved predictive models [15, 16]. These are key for developing clinical systems that aid early diagnosis of complications.

Based on the incidence and prevalence of childhood obesity, since early stages of life, the implementation of preventive strategies is required. Therefore, the MELI-POP (Mediterranean Lifestyle and Physical Activity Intervention for the Pediatric Obesity Prevention) Study is designed to promote healthy dietary and PA patterns while reducing sedentary behavior through a comprehensive approach. We aim for this intervention to play a pivotal role in preventing obesity and advancing our understanding of its development and comorbidities from early childhood through adolescence.

Objectives

The primary aim of MELI-POP (https://melipop.es/) is to evaluate the effectiveness of a lifestyle intervention targeting preschool children at high risk of developing obesity. In addition to this overarching goal, the study has six specific objectives: i) to assess the reduction in the incidence of obesity at 5 and 10 years, following the initiation of the intervention; ii) to improve body composition, evaluated annually through measures such as the z-score of body mass index, z-score of fat mass index, and waist circumference; iii) to enhance health-related physical fitness, evaluated annually; ix) to improve cardiovascular risk factors associated with obesity, assessed at 1, 3, 5, and 10 years from the start of the intervention, using both traditional and multiomics approaches; v) to increase adherence to a Mediterranean dietary pattern at 1, 3, 5, and 10 years since the beginning of the intervention; vi) to increase adherence to age-specific PA recommendations at 1, 3, 5, and 10 years after the initiation of the intervention.

Material and methods

Study design

MELI-POP is a multicenter, parallel, randomized and controlled clinical trial (RCT) involving children aged 3

STUDY PERIOD									
TIMEPOINT	Enrolment	Allocation	Post-allocation					Close-out	Follow-up (5 years)
	-V00	0	V00	V12	V24	V36	V48	V60	Anually
Study Procedures									
Informed consent	X								
Eligibility screen	X								
Medical history	X								
Physical examination	X		X	X	X	X	X	X	X
Allocation									
Randomization		X							
Intervention									
Control group			◆	—	—	—	—	◆	
Intervention group			◆	—	—	—	—	◆	
Assessments									
Biochemistry			X	X	X	X	X	X	X*
Hematology			X	X	X	X	X	X	X*
Blood pressure			X	X	X	X	X	X	X
Urine sampling			X	X	X	X	X	X	
Fecal sampling			X	X	X	X	X	X	
Anthropometry/body composition			X	X	X	X	X	X	X
Physical fitness			X	X	X	X	X	X	
Dietary intake and eating habits			X	X	X	X	X	X	X
Lifestyle behaviors, extracurricular sports, screen time and sleeping patterns			X	X	X	X	X	X	X

Fig. 1 Schedule of enrollment, interventions, and assessments of the MELI-POP Study. *These measurements are optional during the first 3 years of follow-up and are performed only with parental consent for blood collection. In the final 2 years, all evaluations are fully conducted, consistent with visits V00 to V60

to 6 years (preschool stage), identified as being at risk of developing obesity. The participant's allocation among groups is detailed in Fig. 1. The intervention aims to promote a healthy lifestyle based on the Mediterranean dietary pattern and regular PA to reduce the incidence of obesity, compared to a control group. The SPIRIT (Standard Protocol Items: Recommendations for Intervention Trials) statement (supplementary material) was used as guideline to draft the current study protocol (Fig. 1). Moreover, the CONSORT (Consolidated Standards of Reporting Trials) statement has been considered in the study design report.

Study setting

MELI-POP is conducted in three Spanish cities: Córdoba, Santiago de Compostela, and Zaragoza. The intervention is conducted by a multidisciplinary team involving pediatricians, dieticians-nutritionists, nurses, and PA experts. Research centers that participate in the MELI-POP study belongs to the CIBEROBN-ISCIII Spanish network. Moreover, this RCT was registered in the Clinical Trials Database on October 22, 2020 (ClinicalTrials.gov, ID: NCT04597281). This RCT has two arms: 1) the control group that receive general education (usual care) from primary care professionals, and 2) the intervention group receive detailed education on the Mediterranean lifestyle (Mediterranean diet and PA promotion), in addition to providing families with extra virgin olive oil and fish, which are consumed at least three times per week throughout the study duration. Additionally, two free weekly sessions for children, from September to June, excluding school holidays, with 60 min of moderate or vigorous PA, are thought. Due to the COVID-19 pandemic, sessions transitioned to online format.

Initially, a pilot study was conducted, following the same recruitment pattern and inclusion/exclusion criteria for participants. The sample size involved 98 participants, distributed among recruiting centers, undergoing a 3-month intervention (from February 2019 to April 2019). Following its completion, data were analyzed, and necessary protocol adjustments were made.

Participants

Children were recruited by pediatricians in health care centers or at schools located nearby. In schools, with prior permission from educational institutions, families received an invitation to participate. Participants who

completed the family history questionnaire and met the eligibility criteria were contacted.

To be eligible for participation, the following criteria were considered: i) be between 3 to 6 years old; ii) to have normal weight or overweight according to the International Obesity Task Force (IOTF) criteria [17]; iii) with at least one parent having a body mass index (BMI) equal to or greater than 25 kg/m^2, without no disease responsible for overweight or obesity. Exclusion criteria were: i) children under 3 years old or over 7 years old; ii) children whose parents had a BMI lower than 25 kg/m^2; iii) children with chronic diseases, including obesity, or following a therapeutic diet; iv) children from families with dietary habits that did not align with the characteristics of the dietary intervention; v) children from families facing difficulties in following the protocol.

The initial contact with the participating families took place during the screening visit at the beginning of the run-in period. At that time, the inclusion criteria for the project were assessed (Fig. 1). Exclusive data were collected from the child (sex/gender, date of birth, weight, height, and potential allergies and food intolerances) and the parents/caregivers (body weight, height).

Randomization

Between 1 and 3 weeks after the run-in period concluded, participants at each center underwent random assignment to either the control or intervention group. The randomization was conducted via a centralized computer system, Sealed Envelope (www.sealedenvelope.com), using block randomization with a fixed block size of 8. The investigator was not aware of the block size, and no stratification was used. Once assigned, group allocation could not be altered. To facilitate the study procedure for the families, siblings were randomized to the same group.

Ethical and legal aspects

The study protocol was approved by the Ethics Committee of each recruitment center (references: 3669—Institutional Hospital Ethics Committee (Córdoba), 2017/501—Santiago-Lugo Committee of Ethics in Clinical Research (Santiago de Compostela), PI17/0338—Aragon Committe of Ethics in Clinical Research (Zaragoza), and it was conducted following the standards of the Declaration of Helsinki and the Good Clinical Practice standards recommended for intervention trials involving humans (RD 1090/2015), and in compliance with current legislation and Spanish legal regulations governing clinical research in humans (Royal Decree 561/1993 on clinical trials). Informed consent was collected from the parents or legal representatives. Any contact information obtained was securely stored under clinical custody in health centers by pediatricians and the research team. All data and samples were codified according to each center and subsequently centralized at the Instituto de Investigación Sanitaria de Aragón (Zaragoza, Spain). Furthermore, general results after evaluations were sent to parents/caregivers by letter annually. If pathological values were detected, the pediatricians provided parents with appropriate health recommendations. Moreover, a civil liability insurance policy has been secured to cover any potential damage or harm that participants may suffer as a result of the study.

Procedures

Training for researchers

A training session was organized for the investigators tasked with fieldwork to ensure consistency and standardization of procedures outlined in the operations manual. This session encompassed various aspects including questionnaire administration, body composition and impedance measurements according to ISAK protocol [18], blood pressure assessment, physical fitness testing, and processing of biological samples. During the training session, an intra- and inter-observer reliability study was conducted for anthropometric and body composition assessments.

Physical examination

At baseline, pediatricians conducted a complete physical examination by organs and systems. Blood pressure was measured in the non-dominant arm three times, with a 5-min gap between each measurement, using an automatic oscillometer (Omron M3 Intellisense HEM-75051-EV; IOMRON Healthcare Europe) equipped with a child-specific cuff. The average of the three measurements was recorded for both systolic and diastolic blood pressure. The mean blood pressure values are expressed in mmHg, and the percentiles are determined and adjusted for sex and age according to international guidelines [19].

Blood, urine and fecal sampling

After a 12-h overnight fast, blood samples are obtained for biochemical and hematologic screening tests between 08:00 am and 09:00 am. A maximum of 3 ml is drawn via the antecubital vein. General biochemical analyses (glucose, uric acid, total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc), transaminases, and urea) are performed at the participating hospitals following internationally standardized accepted protocols. Furthermore, plasma and serum fractions are extracted by centrifugation and distributed in aliquots (100-300μL) and immediately frozen at −80ºC until analyzed. Peripheral white blood cells (buffy coat) are taken for deoxyribonucleic acid (DNA) extraction.

Additionally, urine samples are obtained and transported in cold conditions (4ºC) until being divided into three aliquots of 1 ml each and immediately frozen at −80ºC until analysis. Stool samples (100–200 g) are also collected in a sterile container by parents or caregivers and immediately frozen at −20ºC until being transported to the corresponding hospitals, where they are stored at −80ºC until analysis.

All biological samples are collected at the beginning and during the 1st, 2nd, 5th, and 10th years of follow-up. In the pilot study, these samples were collected at the beginning and the end of the 3-months period.

Interventions

The main objective of the intervention is to promote an overall adequate quality of the diet, fitting with the Mediterranean dietary pattern, aiming to promote the consumption of extra virgin olive oil, crushed nuts (age-appropriate), fruits and vegetables, legumes, whole grains, whole wheat bread and pasta, fermented foods, especially yogurt, and low-aged cheeses. It was also aiming to avoid (not prohibit) low-nutrient-density foods such as sugary beverages, pre-packaged meals, refined products (white bread, white rice, etc.), snacks, fried foods, trans fats (especially in industrial pastries), sweets, sugar, and processed meats.

The promotion of an active lifestyle is also considered, as developing a scheduled and directed exercise, and ensuring compliance with recommendations for moderate and vigorous PA. This takes place after school hours to avoid interfering with academic activities and consists of two one-hour sessions per week. Exercises, activities, or games are adapted to the psychomotor development of the participants. During the sessions, adherence to an active lifestyle is encouraged, such as walking to school or engaging in outdoor activities with family members.

For the control group, educational activities focusing on topics unrelated to diet and PA or exercise are implemented annually. Among these activities, workshops on road safety are included, addressing crucial aspects of accident prevention and the promotion of safe behaviors in road environments. Moreover, hygiene workshops are conducted to foster healthy practices and cleanliness habits that contribute to the overall well-being of the participants.

Healthy eating behaviors (nutritional education)

Participants from the intervention group, including their parents or caregivers, receive guidance to gradually improve adherence to the Mediterranean diet. During the first two years of the intervention, dieticians conduct monthly group sessions focused on various topics related to Mediterranean habits (e.g., using extra virgin olive oil as the main fat, or the importance of eating five portions of vegetables and fruits daily). Moreover, dieticians contact with these families individually every four months to implement reachable changes in their food habits. Following the first two years, individual follow-ups occurred every six months, while the monthly group sessions continued.

A healthy eating pattern distributed across five daily meals (breakfast, mid-morning snack, lunch, afternoon snack, and dinner) is promoted. No specific quantitative diet is prescribed. Nevertheless, families are oriented on appropriate portion sizes for children's meals, and the importance of portion control is emphasized during group sessions. The inclusion of foods characteristic of the Mediterranean diet is encouraged while minimizing the consumption of unnecessary foods and snacks at other times. Specifically, extra virgin olive oil and nuts consumption is promoted as preferred sources of fat. Proteins should be obtained preferably from plant sources, fish, lean animal sources, and fermented dairy products. Carbohydrate intake preferably should come from solid foods rich in fiber such as vegetables, fruits, and whole grains. Dietary recommendations are also tailored to accommodate the unique characteristics of the family, including their food preferences and socioeconomic status.

To achieve healthy food habits based on the Mediterranean diet, several materials (in paper and digital formats) were developed to help the implementation of a healthy lifestyle pattern among children and their families. These materials encompass guidelines, presented as booklets and posters. Through these resources, the Mediterranean diet and an active lifestyle are promoted. Some booklets include advice to encourage the regular consumption of Mediterranean foods (including recommended frequencies for fish, extra virgin olive oil, fruits, and vegetables) and dynamic tasks to promote lifestyle recommendations (e.g., "Have you consumed your daily five portions of fruits and vegetables?" or "Build your healthy sandwich!"). MELI-POP characters were also designed (Melipop, Creta, Olimpia, and Oliver; https://melipop.es/personajes-melipop/) to help children see themselves reflected in the stories. These materials were registered as intellectual property (identification code: Safe Creative 2,406,208,329,598).

Exercise and daily physical activity

Group sessions are designed around activities tailored to psychomotor development, maintaining an instructor/student ratio of 1:20. These sessions incorporate vigorous activities to strengthen muscles and bones, with an underlying focus on promoting health across all activities. Ensuring a gradual progression of workload in each session and throughout the intervention period is prioritized. Moreover, the varying physical conditions

and developmental stages of each child are considered. Each session, lasting 60 min, includes warm-up and cool-down periods, with hydration and breaks adjusted according to weather conditions and the season. Annually, the intervention period lasts 9 months, from September to June. Weekly sessions are planned as: the first one, with emphasizing games and exercises designed for high-intensity engagement, alongside osteogenic exercises to foster the accumulation of bone minerals. The second one is dedicated to sport-specific activities, rotating through disciplines such as football, basketball, athletics, and handball. In the final 10 min of each session, reinforcement messages regarding the Mediterranean diet and the adoption of active, healthy lifestyles are emphasized. Throughout all physical exercise sessions, heart rate monitors are used to measure the achieved intensities.

In addition to the two weekly sessions during the school year, participants are encouraged to engage in at least 60 min of moderate-to-vigorous PA daily, especially through extracurricular sports activities. To facilitate it involvement, researchers establish partnerships with local sports clubs, providing MELI-POP boys and girls the opportunity to participate free of charge.

For maintaining and promoting an active lifestyle, supporting materials were also developed (e.g. daily: walk, bicycle, go upstairs; various days per week: sport activities; punctually: watch television, videogames). For the COVID-19 pandemic, posters were created to promote PA at home, encouraging individuals to engage in 45 min of movement daily (e.g. treasure play, yoga exercises or dancing). During the lockdown, a PA instructor devised virtual sessions to be held twice a week.

Outcomes

Primary outcome (adiposity)

Body weight (kg) is measured using a precision balance incorporated in a Tanita MC780SMA (Tanita Europe, B.V.) including an octopolar multifrequency bioelectrical impedance system. Height (cm) is determined using a portable stadiometer (SECA 213, Scale 20–205 cm; SECA). These assessments are conducted with the child wearing light clothing, without shoes, and following standard procedures. Waist circumference is measured with a measuring tape (Cescorf 1 mm accuracy), midway between the lowest rib and the iliac crest. BMI is calculated as weight (kg) divided by the square of height (m) [17]. The BMI z-score is obtained from standardized residuals conducted with linear regression models within the studied sample. Body fat mass (kg) is directly estimated by bioelectrical impedance using the algorithms provided by the company and the fat mass index is estimated by dividing the body fat mass (kg) by the square of the height (m) [20].

The determination of skinfold thickness is conducted using high-precision calipers (Holtain or Harpenden, Holtain Ltd.) applied to the right side of the body. The measurements are taken according to the International Society for the Advancement of Kinanthropometry ISAK protocol [18]. The average of three measurements, taken over a period of 3 s, was considered. Measurements of the tricipital fold are taken at the midpoint of the posterior aspect of the relaxed arm, while the subscapular fold was measured immediately below the tip of the scapula, at a 45-degree angle with respect to the spine. Suprailia fold is measured above the iliac crest at the axillary midline level and at an angle of 45 degrees with respect to the latter.

Secondary outcomes

During the baseline visit, the parents'weight and height, and children data about gestational age, birth weight and height, duration and type of breastfeeding, introduction of complementary feeding, and allergies or food intolerances, among other factors, were assessed. Socioeconomic status, family size and structure, and living conditions were also evaluated. Information regarding medical history, family background, and medication use is gathered at the baseline visit and subsequently, on an annual basis.

Dietary intake and eating habits A recently validated semi-quantitative food and beverage frequency questionnaire tailored for the childhood stage [21] is used to assess food and nutrient intakes. At each individual visit, parents or caregivers also complete an 18-item questionnaire assessing the adherence to the Mediterranean diet of their children. Moreover, the adherence of the parents or caregivers to the Mediterranean diet is assessed annually using a 14-item questionnaire designed for adults.

Physical activity PA is evaluated objectively using ActiGraph GT3X + accelerometers. Summary data is used to generate the results shown in ActiLife version 6.13.3 (ActiGraph Software Department: Pensacola, FL, USA) at 15 epochs. To evaluate PA and sedentary time, a minimum of eight hours of monitoring per day for at least three days, including one weekend day, is considered acceptable. Two rules are applied to exclude low-quality records: (a) all negative counts are replaced with missing data, and (b) periods of 20 min or more of consecutive zero counts are replaced with missing data before downstream analysis [22]. Exclusion criteria for this analysis include invalid data availability, non-compliance with the minimum number of monitoring hours, or insufficient time on valid days during the week or weekend. The ActiLife data scoring program is used to determine daily minutes spent in sedentary time and PA for each

epoch length dataset, using the Butte et al. activity cut-off points accelerometry protocol [23], where sedentary time is defined as ≤ 819 counts per minute (CPM), light PA as 820–3907 CPM, moderate PA as 3908–6111 CPM, and vigorous PA as ≥ 6112 CPM.

Lifestyle behaviors, extracurricular sports, screen time and sleep duration A set of self-administered questionnaires about lifestyle behaviors is completed at home by parents or caregivers. An active lifestyle is assessed using the Outdoor Playtime Checklist and the Outdoor Playtime Recall Questions [24] and an ad hoc questionnaire based on leisure-time PA that included sedentary behaviors is used to complement objective measurements for sedentary time and PA. Details concerning participation in extracurricular sports activities, daily life commutes, and time spent in outdoor play involving PA on both school days and weekends are collected. An additional ad hoc questionnaire measures screen time (on school days and weekends) and the child's sleep duration based on night-time sleep and nap duration during weekdays, weekend days, or holidays. The total sleep duration (hours per day) includes the nighttime sleep hours and nap time on weekdays plus weekend days, or holidays, divided by seven (representing the total days of the week).

Physical fitness Physical fitness evaluation is scheduled on a different day from the anthropometric measurements and blood extraction to mitigate any potential impact as mild discomfort, potential changes in hydration status, or psychological stress associated with the procedures [25].

Lower body explosive strength (standing long jump test)

Lower body muscular strength is evaluated through the standing long jump test [26]. This assessment required participants to jump forward as far as possible with their feet together while maintaining an upright position. Preschoolers are instructed to perform three jumps recording the greatest distance achieved. Initially, a mark is placed at the take-off line to assist participants in positioning their feet correctly (referred to as the standing long jump test). This practice continues throughout subsequent assessments to prevent participants from crossing the starting line, thereby invalidating their attempts and necessitating repetition. Participants should be urged to make the maximum possible effort.

Upper body isometric strength (handgrip strength test)

Upper body muscular strength is assessed by the handgrip strength test using an analogic dynamometer (TKK 5001, Grip-A, Takei, Tokyo) [27]. Pre-schoolers are instructed to gradually and continuously squeeze the dynamometer for a minimum of 2 or 3 s, conducting the test twice (alternating between hands). To ensure accuracy, participants are required to fully extend their elbows and refrain from any contact between the dynamometer and other parts of the body, aside from the hand being measured. The optimal grip span is standardized at 4.0 cm [28]. The highest recorded value from the two trials for each hand, and the average strength from both hands are recorded. Participants are urged to make the maximum possible effort.

Cardiorespiratory fitness (adaptation of 20-m shuttle run test)

Cardiorespiratory fitness is evaluated using a modified version of the initial 20-m shuttle run assessment (SRT) [29]. The PREFIT 20-m SRT [30]. Participants shuttle between two lines 20 m apart upon hearing an audio cue. The assessment concludes either when the child fails to reach the lines in sync with the audio signal on two consecutive occasions or when exhaustion prompts them to stop. Given the young age of the children, adjustments have been made to the original test, including a reduction in the starting speed (6.5 km/h instead of the original 8.5 km/h) and the presence of two evaluators running alongside a smaller group of children (e.g., 4–6 with similar ages) to maintain an appropriate space. An audio track reproduces acoustic signals related to the adapted starting speed of 6.5 km/h, with 0.5 km/h increments every minute. The feasibility, reliability, and maximality of this test in preschool children have been previously reported [31]. The participants receive verbal support throughout every phase, with common expressions like"you're doing great"and"keep it up"delivered in a clear, loud voice to ensure complete understanding. A heart rate monitor Polar H10 (Polar Electro Oy, Kempele, Finland) is also used to ensure that children are achieving maximum effort throughout the test [32].

The data about time and stadiums reached are recorded, and subsequently converted into total laps and speed (km/h) [30], then further transformed into an estimation of maximum oxygen intake (VO_2max) [32].

Biochemical parameters Biochemistry analysis includes general parameters as a lipid profile with plasma total cholesterol, LDLc, HDLc, apolipoprotein A1 and B, triacylglycerols, transaminases, an iron profile (iron, ferritin, transferrin). The carbohydrate metabolism markers used are glucose and insulin. Analyses are performed by standardized laboratory methods using Architect c16000 and i2000SR autoanalyzers (Abbott Diagnostics®, Abbot Laboratories, Madrid, Spain). External and internal quality controls are performed according to hospital protocols. Insulin resistance will be calculated by the Homeostatic Model Assessment for Insulin Resistance index (HOMA-IR) calculated as insulin (mU/L) × glucose (mmol/L)/22.5.

Inflammation and cardiometabolic biomarkers Specific inflammatory and cardiovascular risk biomarkers will be measured in plasma. C-reactive protein (CRP) levels are quantified using the autoanalyzer Architect c16000 (Abbott Diagnostics®, Abbott Laboratories, Madrid, Spain) by turbidimetric immunoassay with latex particles. Furthermore, adipokines (adiponectin, leptin, resistin), plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor-alpha (TNF-α), monocyte chemo- attractant protein-1 (MCP-1), interleukin-6 (IL-6), interleukin-8 (IL-8) will be analyzed in duplicate on a Luminex 200 system with the XMap technology (Luminex Corporation, Austin, TX, USA) and using human monoclonal antibodies (Milliplex Map Kit, Millipore, Billerica, MA, USA) at the"José Mataix Verdú"Institute of Nutrition and Food Technology (INYTA, Granada, Spain).

Fatty acids An analysis of the total and the plasma and erythrocyte fatty acid profile will be performed. First, plasma lipids will be extracted and then the fatty acid composition will be determined in a Hewlett Packard gas chromatograph, model 5890 A (Philadelphia, PA, USA). To determine plasma fatty acids, lipid extraction and transesterification of fatty acids will be carried out in a single step. The quantification of fatty acid methyl esters will be carried out by gas–liquid chromatography at the INYTA (Granada, Spain).

Oxidation Blood analysis will be completed by measuring oxidized low-density lipoprotein (oxLDL) levels and determining the activity of enzymes involved in the antioxidant defense system at the INYTA (Granada, Spain). These enzymes include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Levels of total antioxidant capacity (TAC) and malondialdehyde (MDA) will be assessed to evaluate oxidative stress. OxLDL levels will be measured using the ELISA kit from Mercodia (Uppsala, Sweden). SOD activity will be determined using the SOD Assay Kit-WST (Sigma-Aldrich, St. Louis, MO, USA), CAT activity using the Catalase Assay Kit (Cayman Chemical, Ann Arbor, MI, USA), and GPx activity using the Glutathione Peroxidase Assay Kit (Abcam, Cambridge, UK). TAC will be assessed with the TAC Assay Kit (Sigma-Aldrich, St. Louis, MO, USA), and MDA will be measured using the TBARS Assay Kit (Cayman Chemical, Ann Arbor, MI, USA).

Microbiota Stool samples will be homogenized in a Stomacher-400 blender. Subsequently, a QIAamp DNA Stool Mini Kit (QIAGEN, Barcelona, Spain) will be used for the DNA extraction as indicated by the manufacturer with the exception of the incubation at 70 °C. Instead, the samples will be mixed with the lysis buffer and incubated at a temperature of 95 °C to ensure that both Gram-positive and Gram-negative bacteria will be lysed. The DNA concentration and purity will be conducted at the INYTA (Granada, Spain), with a NanoDrop ND1000 spectrophotometer (Thermo Fisher Scientific, DE, USA). The Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) will be used for metagenomics library construction. Amplified libraries will be purified using AMPureXP (Agencourt, Brea, CA, USA) and the quality will be assessed using an Agilent High Sensitivity DNA Kit on an Agilent 2100Bioanalyzer (Santa Clara, CA, USA). The sequencing libraries will be quantified using the KAPA Library Quantification Kit. The raw data samples will be analyzed using MetaPhlAn version 3.0 [33].

Genetic and omic analyses Genomic DNA will be extracted from peripheral white blood cells (buffy coat) using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), with concentration and purity measured by a NanoDrop ND1000 spectrophotometer, and integrity verified by agarose gel electrophoresis. All extractions were purified using a DNA Clean and Concentrator kit from Zymo Research (Zymo Research, Irvine, CA, USA). Genotyping will be performed using the Infinium Global Screening Array-24 BeadChip (Illumina, Madrid, Spain) Using the iScan™ System, integrated analysis software, and the Infinium high-throughput screening (HTS) assay. This technology allowed us to measure 651,563 single nucleotide polymorphisms (SNPs) with a small percentage of missing values. Quality control analysis will be performed: low allelic frequency (less than 0.2); low call rate (less than 0.95); and repeated variants or without information will be excluded. A second quality control analysis will be performed with PLINK 1.9 software with the exclusion criteria for low imputation quality ($R2 < 0.9$); variants that did not meet the Hardy–Weinberg equilibrium ($HWE - P > 10 - 6$); and low minor allele frequency ($MAF < 0.01$).

Metabolomic analyses An untargeted metabolomic assay will be conducted. Medium to non-polar compounds will be detected by an Agilent Series 1290 HPLC (Agilent Technologies, Santa Clara, CA, USA) using a Waters Atlantis T3 HPLC column (Waters Corporation, Milford, MA, USA) and coupled to AB SCIEX TripleTOF 5600 quadrupole time-of-flight quadrupole mass spectrometer (QTOF-MS). Low molecular weight and polar metabolites will be analyzed using gas chromatography coupled to mass spectrophotometry. Metabolites candidates'biomarkers identified by untargeted metabolomics strategy will be quantified and validated by a targeted approach. Statistical analyses and pathway analysis will be conducted using MetaboAnalyst to interpret the biological relevance of the findings. The i2b2-tranSMART open-source framework will be used for the integration

of large amounts of data, along with the application of machine learning models.

All genetic and omics data will be integrated and analyzed to identify potential biomarkers and molecular pathways associated with the study outcomes.

Sample size and power

The sample size was estimated considering the main objective and the effect size on the primary variable (BMI z-score). Few international studies have evaluated lifestyle interventions for preventing childhood obesity from preschool age [34], and no data exist for this age group in our country. Among the limited intervention programs conducted in the last decade, the Home Styles study implemented a web-based lifestyle program for preschoolers with normal weight or overweight over 6–8 months [35]. It reported a BMI z-score reduction of 0.14 in the control group and 0.27 in the intervention group, with a between-group difference of 0.13 [35]. Given that MELIPOP is a personalized lifestyle intervention with a longer duration (1 to 5 years), a larger effect size was anticipated. Specifically, the estimated difference in BMI z-score reduction between groups is 0.20. Based on a power of 0.95, an α-error of 0.05, and an expected follow-up loss of 15%, the required minimum sample size was calculated to be 300 participants. For the pilot study, 5–10% of the total sample has been considered appropriate to assess the adequacy of instruments and intervention protocols.

Statistical analysis

All data are integrated into the database in duplicate to detect possible errors during this process, using SPSS V.29.0 for Windows (SPSS Inc., Chicago, IL, USA). Variables with a normal distribution will be analyzed using parametric tests, and those with a skewed distribution will be logarithmically transformed or through another mathematical expression to normalize their distribution. The chi-square test will be used to compare qualitative variables, and the t-test will be employed to compare quantitative variables based on a qualitative variable with 2 categories. If the distribution is not normal, the U-Mann–Whitney test will be used. Parametric data will be presented as means with standard deviation (SD), and non-parametric distributed variables as median with interquartile ranges (IQRs). When the qualitative variable has three categories, ANOVA and Kruskal–Wallis tests will be used, respectively. To assess the effect of the intervention, generalized estimating equation (GEE) models will be established, based on the intention-to-treat approach. The center will be considered a factor to adjust the statistical models. The fixed effects will include *time* × *treatment* interaction. Puberty stage will be considered in the adjustments for analysis. A Bonferroni test will be used to assess the specific differences between the interventions. The accuracy of the estimation of variables and statistical indices will be assessed using 95% confidence intervals. A p-value < 0.05 will be considered statistically significant.

Several specific analyses will be performed on various outcomes: To examine the relationship between lifestyle habits and microbiota profiles across control and intervention groups, clusters will be generated using principal component analysis (PCA).

For the genetic data, multiple linear regression models will be implemented to assess the effect of each genetic variant on the response to the lifestyle intervention. Furthermore, PCA will be applied to assess the quality of metabolomic data against quality controls to detect potential outliers and identify clustering patterns. Different extensions of partial least squares discriminant analysis (PLS-DA), such as sPLS-DA or OPLS-DA models will be built to identify metabolite patterns and specific features that discriminate between study groups.

Discussion

Childhood obesity has become a significant public health concern, drawing considerable attention in recent decades because of its short- and long-term adverse effects just from an early stage of life [36]. This research protocol emphasizes the evaluation of specific variables related to body composition, metabolic status, diet, and PA. This approach enables to gather information about preschoolers at a higher risk for developing obesity, particularly in those whose parents have obesity. Therefore, the intervention aims to be a preventive strategy against the development of obesity and its associated comorbidities.

The present work is notable for the design and multidisciplinary approach. The methods used are designed to validate findings from both quantitative and qualitative data for a personalized intervention over time. Some studies examining diet and PA habits in children have included PA measurements using accelerometry, dietary assessments through questionnaires, and anthropometry [37, 38]. In this study, the effectiveness assessment is conducted using reliable measures whenever feasible (e.g., accelerometer data, anthropometric and body composition measurements by standardized and validated methods), valid assessment of dietary intake [23] and validated questionnaires (related to practices). This evaluation allows to assess PA, nutrition, and other habits as well as health status over time and to inform children and families about their progress.

Qualitative measurements can provide valuable insights to explain and interpret quantitative results; for example, caregivers'and children's perceptions of their experience with the intervention can provide deeper

insights into the"how"and"why"of its effectiveness [39]. Moreover, it is crucial to explore the expectations and experiences of both patients and healthcare professionals with the intervention before implementing it in clinical practice or developing similar interventions in other settings. Secondly, the systematic and personalized approach of this intervention enhances its applicability. Literature indicates that interventions are more successful when tailored to the specific needs, interests, and environments of the participants [40]. By employing a personalized approach, the integration of the intervention into daily life becomes more feasible, thereby increasing the likelihood of successful implementation upon the study's completion. Besides the strengths of this study, there are some limitations. Given the vulnerability of children receiving this intensive intervention, it may be overly demanding for both, children and parents, potentially leading to high dropout rates.

In the MELI-POP study, the intervention focuses primarily on diet and PA, as these are deemed the most critical factors in preventing obesity [41]. The main objective is to reduce sedentary behavior and foster a shift towards healthy lifestyle habits based on dietary and exercise recommendations, ensuring that participants adopt or sustain these habits. The sedentary behavior most significantly associated with obesity is the time spent watching TV, with a positive association found in most of studies conducted in children when the time exceeds 2 h per day [42]. Both, for PA [43] and sedentary behavior [44], systematic reviews have shown that intervention studies generally succeed modifying both positively [45]. Furthermore, studies using objective PA measures support the idea that a high level of PA, especially through intense activities, is associated with lower total and abdominal fat [46]. Muscle strength and aerobic capacity are also associated with lower adiposity and lower levels of some cardiovascular risk factors and obesity [47].

Due to the early age at which participants were selected, and the expected 10-year follow-up period, changes during childhood and adolescence can be observed to evaluate obesity risk of development, also considering pubertal stage. Young children are generally more active, engaging in physical play and movement [48]. However, during adolescence, there is a tendency to increase sedentary behavior. Activities such as sitting, using electronic devices, and less physical play become more common. This shift can contribute to weight gain and the development of obesity during these critical years [49]. Therefore, starting this intervention at a young age can ensure the monitoring of the behavioral and physiological changes over time to plan modifications if needed. Understanding the transition from active childhood to more sedentary adolescence provides valuable insights into “when” and “how” to intervene effectively [50] and it also helps in identifying critical periods where targeted strategies can prevent the onset of obesity and its related health issues.

The participants included in this study had one or both parents having overweight or obesity, as this is considered a significant risk factor for the children developing obesity in the future [51]. Research has shown that parental obesity greatly increases the likelihood of obesity in their children due to a combination of genetic, environmental, and behavioral factors [52]. Children with parents suffering from overweight or obesity are more likely to adopt similar dietary and PA patterns, which can contribute to weight gain [53]. Genetic factors can also condition changes in metabolism, appetite, and fat storage, further increasing the risk of obesity [54]. By focusing on children with this high-risk background, this study aims to implement early interventions that can help prevent the onset of obesity during childhood. In MELI-POP, the quality of the intervention is emphasized. Hence, individualized educational interventions are implemented, and follow-up is conducted to verify their compliance with dietary and PA habits.

Interventions focused solely on increasing PA have also shown positive effects, such as increasing exercise practice, improving physical fitness, and reducing waist circumference [55]. However, some studies did not find improvements in physical fitness or anthropometric measures [56]. The positive effects of PA interventions appear to be sustained long-term, with studies finding decreases in body fat and lower obesity risk, 2 years after the intervention [56]. However, several studies have found that interventions combining changes in diet, increased PA, and behavioral modifications in children with overweight or obesity have shown short-term and long-term beneficial effects [57]. These interventions have associated significant weight loss, reduced body fat, and improvements in lipid profile [58]. In a Cochrane review [59] about the effectiveness of programs to prevent obesity in children and adolescents, the effects are observed especially in the short term, although there is solid evidence of the positive impact of obesity prevention programs on BMI. In the present study, changes could be observed from the start of the intervention through several years also considering the puberty influence. Since our participants begin with a good state of health, the intervention aims to sustain or further enhance their well-being over time.

Moreover, an intervention providing olive oil and fish in the context of a Mediterranean diet has planned to increase adherence to the general Mediterranean diet advice. In adults at high risk for cardiovascular disease, those who received guidance on the Mediterranean diet with olive oil supplementation experienced a notable decrease in central obesity and fasting glucose levels

compared to those who received general dietary advice [60].

Recent advances in the biomedical field have initiated a big data revolution, particularly through omics technologies. These technologies enable comprehensive molecular analyses, enhancing our understanding of cell biology and disease mechanisms. Key advancements include genome-wide association studies, RNA sequencing and epigenome-wide association studies. Omics research is also improving clinical treatments by facilitating the development of predictive biomarker panels for personalized disease risk assessments and stratified clinical guidelines. Moreover, the integration of ML, a branch of artificial intelligence, has significantly enhanced predictive modeling through sophisticated algorithms, including ensemble modeling and deep learning [15, 61].

Finally, it seems that some integral strategies are particularly effective, especially, when education on healthy eating habits in schools, providing food at school, increasing PA sessions, and developing movement skills are included [59]. Therefore, it is essential to promote healthy habits encouraging a balanced diet and regular PA from an early age. This not only helps to prevent obesity and other chronic diseases but also fosters a lifelong commitment to a healthy lifestyle [62]. Moreover, instilling these habits in children can have a positive impact on their families and communities, creating an environment that supports overall well-being [63].

In conclusion, as MELI-POP is a multicenter clinical trial in children aged 3 to 6 years at risk of developing obesity and based on a methodology designed for a comprehensive and personalized intervention, beneficial results are expected by preventing obesity during childhood, and associated comorbidities.

Abbreviations

AI	Artificial Intelligence
BMI	Body mass index
CAT	Catalase
CONSORT	Consolidated Standards of Reporting Trials
CPM	Counts per minute
CRP	C-reactive protein
COSI	Childhood obesity surveillance initiative
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
GPx	Glutathione peroxidase
GEE	Generalized estimating equation
HDLc	High-density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment for insulin resistance
HPLC	High-performance liquid chromatography
i2b2	Informatics for integrating biology and the bedside
IL-6	Interleukin-6
IL-8	Interleukin-8
IQR	Interquartile Range
IOTF	International Obesity Task Force
LDLc	Low-density lipoprotein cholesterol
MELI-POP	Mediterranean Lifestyle and Physical Activity Intervention for the Pediatric Obesity Prevention
MCP-1	Monocyte chemoattractant protein-1
ML	Machine learning
MDA	Malondialdehyde
PA	Physical activity
PAI-1	Plasminogen activator inhibitor-1
PCA	Principal component analysis
PLS-DA	Partial least squares discriminant analysis
QTOF-MS	Quadrupole time-of-flight mass spectrometer
RCT	Randomized controlled trial
SD	Standard deviation
SOD	Superoxide dismutase
SPIRIT	Standard Protocol Items: Recommendations for Intervention Trials
SPSS	Statistical package for the social sciences
SRT	Shuttle run test
TAC	Total antioxidant capacity
TNF-α	Tumor necrosis factor alpha
VO2max	Maximum oxygen uptake
WHO	World Health Organization
oxLDL	Oxidized low-density lipoprotein

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

PME, KFR, RVC, JMJC, ALG, RPL, CCC, FJLC, and BPV are responsible for data and sample collection. JMJC, CCC, and BPV contributed to writing the manuscript. MGC, RL, and LM designed the study and secured funding. PME, KFR, and RVC managed child recruitment and oversaw the RCT. CMA developed the sampling protocols and will conduct the sample analysis. MGC and LM were coordinators of the study. All authors assume full responsibility for the manuscript's content and have read, reviewed, and approved the final version.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
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Competing interests
The authors declare no competing interests.

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