Trials

STUDY PROTOCOL

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The impact of the timing of pasta intake on sleep quality and health outcomes: a protocol for a randomized controlled trial

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Abstract

Background Pasta is a fundamental component of the Mediterranean diet and a key source of carbohydrates. Despite its nutritional benefits, misconceptions persist regarding its potential to promote weight gain, particularly when consumed at dinner. While no evidence supports this concern, emerging chrononutritional research suggests that evening carbohydrate intake may positively influence sleep quality by promoting serotonin production. This study aims to assess, for the first time, whether pasta consumption (lunch vs. dinner) affects sleep quality, circadian rhythms, cardiometabolic health, and gut microbiota composition in healthy, normal-weight adults.

Methods A 7-month randomized, open-label, cross-over trial will enroll 70 participants, assigned to two isocaloric, Mediterranean-style diets differing only in pasta consumption timing. Each phase will last 3 months, separated by a 1-month wash-out period. At the beginning and end of each phase, participants will wear an actigraph for 7 days and provide saliva, blood, and stool samples. Additional assessments include body composition analysis, indirect calorimetry, and food and lifestyle diaries. The primary outcome will be changes in sleep quality from baseline. Secondary outcomes include anthropometric measurements, body composition, metabolic rate, biochemical and hormonal markers, inflammatory and oxidative stress markers, gut microbiota composition, and short-chain fatty acid production. The study has been approved by the Tuscany Regional Ethics Committee of the Azienda Ospedaliera Universitaria (AOU)—Careggi, Florence.

Discussion This study will provide experimental data on how the timing of pasta consumption affects sleep quality and a range of health outcomes, contributing to the debate on the optimal timing of carbohydrate intake.

Trial registration ClinicalTrials.gov NCT06185634. Registered on 07/01/2024.

Keywords Carbohydrate timing, Circadian rhythms, Sleep, Cardiometabolic health, Gut microbiota

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Administrative information

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Name and contact information for the trial sponsor {5b}	Trial sponsor: Azienda Ospedaliero-Universitaria Careggi (AOU-Careggi) Contact name: Clinical Trial Center Address: Largo Giovanni Alessandro Brambilla, 3, 50,134, Florence, Italy Telephone: + 39 055 7,947,807 Email: ctc.smo@aou-careggi.toscana.it
Role of sponsor {5c}	The sponsor is responsible for managing trial coverage and overseeing the study's initiation, management, reporting, and indemnity arrangements. However, it does not participate in study design, data collection, management, analysis, interpretation, report writing, or the decision to submit the report for publication

Introduction

Background and rationale {6a}

Pasta, rich in complex carbohydrates, is a staple food and a significant source of energy globally [1]. It plays a central role in the Mediterranean diet (MD), which is universally recognized as a healthy and balanced eating pattern [2]. Despite the well-documented health benefits of moderate pasta consumption, particularly regarding cardiovascular health and weight management [3–5], there has been a noticeable decline in its consumption. This trend is particularly evident in Mediterranean countries, including Italy [6, 7], driven in part by the increasing popularity of low-carbohydrate, high-protein diets, often promoted for weight loss.

In addition to concerns about carbohydrates in general, many individuals specifically avoid consuming pasta in the evening, based on the belief that eating carbohydrates later in the day may lead to weight gain. Research suggests that glucose tolerance—the body's ability to regulate blood sugar levels after consuming glucose—decreases as the day progresses, supporting the hypothesis that evening carbohydrate intake might have negative metabolic effects [8–10]. However, this hypothesis stems mainly from physiological and chronobiological theories—which examine how the timing of biological processes affects health—rather than robust experimental evidence, and it remains unclear whether carbohydrate consumption at dinner as part of a balanced diet results in adverse outcomes. Contrarily, some studies suggest that consuming carbohydrates in the evening may improve sleep latency and duration through the modulation circadian rhythms and sleep architecture [11–14]. Carbohydrates are known to enhance tryptophan availability, an amino acid crucial for serotonin production. Serotonin in turn plays a key role in regulating sleep by promoting relaxation and modulating the sleep–wake cycle [15]. This mechanism suggests that carbohydrate intake in the evening may positively influence sleep parameters, offering potential health benefits rather than risks.

Another emerging area of interest is how the timing of carbohydrate consumption affects gut microbiota. Animal studies suggest that the timing of carbohydrate intake may influence the composition and activity of gut microbes, with short-chain fatty acid (SCFA) production fluctuating according to circadian rhythms [16, 17]. Since SCFAs play a significant role in metabolic regulation and are produced from carbohydrates, understanding how meal timing affects microbial activity could offer key insights into metabolic health.

Given that previous research has mainly focused on the quantity and quality of pasta consumption [18], little is known about how the timing of consumption may influence health outcomes. This study aims to fill this knowledge gap by investigating how the timing of pasta consumption affects sleep quality, metabolic health, and gut microbiota. The hypothesis is that consuming pasta in the evening, as part of a balanced diet, may have beneficial effects on sleep quality and metabolic health, possibly through modulation of circadian rhythms and microbial activity. The findings of this study could provide valuable insights into personalized dietary strategies that maximize the health benefits of pasta and inform more tailored nutritional recommendations.

Objectives {7}

The present study aims to investigate, for the first time, whether the timing of pasta consumption—at lunch versus dinner—has differential effects on sleep quality, circadian rhythms, anthropometric measurements, body composition, cardiovascular risk factors, and the composition and functionality of the gut microbiota in a sample of healthy, normal-weight individuals.

Trial design {8}

A 7-month randomized, cross-over, two-arm, open-label, superiority trial with a 1:1 allocation ratio to either pasta at lunch or pasta at dinner arms will be conducted, adhering to the SPIRIT reporting guidelines (see Fig. 1 and Supplementary file #1). A cross-over study design was chosen because it allows each participant to act as his or her own control, thus minimizing interindividual variability and improving the internal validity of the study. In the development of the study protocol, there

	STUDY PERIOD				
	Enrolment	Allocation	Post-allocation		Close-out
PROTOCOL ACTIVITY	*day-14 to 1*	day 0	*mo 3*	*mo 4*	*mo 7*
TIMEPOINT	$-t_1$	t_0	t_1	t_2	t_3
ENROLMENT:					
Eligibility screen	X				
Informed consent	X				
Demographic details	X				
Medical history	X				
Medi-Lite questionnaire		X	X	X	X
3-day dietary records		X	X	X	X
IPAQ		X	X	X	X
MEQ		X	X	X	X
Allocation		X			
INTERVENTIONS:					
Pasta at lunch		◆		◆	
Pasta at dinner		◆		◆	
ASSESSMENTS:					
Actigraphy		X	X	X	X
DMLO		X	X	X	X
Anthropometric measurements		X	X	X	X
Body composition		X	X	X	X
Indirect calorimetry		X	X	X	X
Blood samples		X	X	X	X
Stool samples		X	X	X	X
Adherence questionnaire			X		X
24-hour diet recall			X		X

Fig. 1 SPIRIT figure reporting the phases of the trial and data collection time points

was no direct involvement from the public or patients. Despite the acknowledged significance of patient and public engagement in research, the protocol for this study was formulated within the framework of scientific and clinical expertise.

Methods: participants, interventions, and outcomes

Study setting {9}

The study will take place at the Clinical Nutrition Unit, Careggi University Hospital, Florence, Italy.

Eligibility criteria {10}

Adults aged 18 to 65 years of both sexes, with a body mass index (BMI) between 18.5 and 24.9 kg/m^2, will be eligible to participate. Exclusion criteria include individuals with night shifts, long-distance travel plans, or irregular sleep schedules. Additionally, participants on medication affecting sleep or metabolism, or with ongoing illnesses requiring dietary supervision (e.g., recent myocardial infarction, chronic liver disease, diabetes) will be excluded. Pregnancy, intention to become pregnant in the next 12 months, and breastfeeding, as well as current or recent (within the last 3 months) use of supplements or antibiotics, will also be reasons for exclusion.

Who will take informed consent? {26a}

Trained research personnel will obtain informed consent. These individuals will be responsible for clearly communicating the study's objectives, procedures, potential risks, and benefits to potential participants. Before enrollment, participants will have the opportunity to ask questions and receive comprehensive information about the trial, ensuring they fully understand what their participation entails.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Study participants will also be asked for written informed consent to keep the biological samples for future research and to publish the data anonymously.

Interventions

Explanation for the choice of comparators {6b}

Our comparator approach involves the implementation of two normo-caloric Mediterranean-type diets with identical food group composition, differing only in the timing of pasta consumption. The diets will be customized based on indirect calorimetry and the habits reported by participants in a 3-day food diary. By controlling for caloric intake and food composition, we aim to minimize confounding factors, allowing us to evaluate the specific effects of pasta consumption timing on sleep and other health parameters.

Intervention description {11a}

Upon recruitment, participants will be randomized into two groups. Group 1 will begin the study by consuming pasta exclusively at dinner for a duration of 3 months, while group 2 will consume it solely at lunch during the same period. After this initial phase, both groups will undergo a 1-month wash-out period, followed by a crossover to the alternate intervention: group 1 will then consume pasta only at lunch, and group 2 will switch to dinner consumption.

Participants will be required to avoid cereals other than pasta (e.g., rice, spelt, barley, couscous). The nutritional value of the pasta administered during the study is shown in Supplementary file #2. The dietary intervention will be a normo-caloric diet tailored to individual energy needs, determined by measuring the resting metabolic rate (RMR) through indirect calorimetry and analyzing participants' calorie intake based on a 3-day food diary. This approach will ensure isocaloric equivalence between the two diets. Each diet will adhere to a Mediterranean dietary pattern, comprising approximately 50–55% of total energy from carbohydrates (primarily complex), less than 30% from total fat, and 15–20% from protein. The MD was selected as the traditional dietary pattern of Italy, the country in which the study will be conducted.

For both dietary interventions, daily caloric distribution will be structured as follows: 20% of daily energy intake will be allocated to breakfast, 5% to a morning snack, 40% to lunch, 5% to an afternoon snack, and 30% to dinner. The sole distinction between the diets will be the timing of pasta consumption—restricted to either lunch or dinner, depending on the assigned group. Participants will receive a comprehensive 1-week menu plan along with a handout detailing the assigned diet and possible substitutions. They will have the flexibility to prepare their own meals or dine at restaurants. Alcohol will be limited to one drink per day for women and two for men. Additionally, participants will be instructed to maintain their regular exercise habits throughout the study.

Criteria for discontinuing or modifying allocated interventions {11b}

Participants may discontinue the intervention or withdraw from the study for the following reasons: (1) at the request of the participant; (2) if the investigator considers that a participant's health will be compromised due to adverse events or concomitant illness that develop after entering the study.

Strategies to improve adherence to interventions {11c}

To improve adherence to the intervention protocols, participants will receive detailed 1-week menu plans from qualified nutritionists, specifying the quantities of food ingredients by weight and/or volume. Additionally, a comprehensive handout detailing the assigned diet, including possible substitutions, will be provided. We will implement recurring telephonic follow-ups to ensure ongoing support and monitor adherence to the intervention. During these follow-up sessions, participants will have the opportunity to discuss their adherence to the intervention, address any challenges encountered, and provide feedback on their dietary choices and preferences.

Relevant concomitant care permitted or prohibited during the trial {11d}

During the trial, participants will be requested to advise if they require initiation of cortisone therapies, antidiabetic drugs, or vitamin/mineral supplements, and their continuation in the study will be evaluated accordingly. If a participant requires the initiation of any of these products based on a doctor's recommendation during the intervention period, they will be excluded from the study.

Provisions for post-trial care {30}

Due to the nature of the study, no post-trial arrangements are required.

Outcomes {12}

The primary endpoint will be the change in sleep quality from baseline expressed as sleep efficiency (SE) measured through actigraphy. Key secondary endpoints will include changes in anthropometric parameters, body composition, glycemic profile, lipid profile, minerals, liver marker enzymes, homocysteine, circulating levels of adipokines, inflammatory markers, oxidative stress, hormonal parameters, dim light melatonin onset (DLMO), gut microbiota composition, and SCFA production. Sleep quality will be measured as a unique and established quantitative primary outcome, while the assessment of anthropometric parameters, body composition, biochemical profile, DLMO, gut microbiota composition, and SCFA production will provide information on the cardiometabolic status and the circadian rhythm of participants. The metric used for analysis will be the change in mean values from the beginning to the end of each dietary intervention.

Participant timeline {13}

The study timeline is depicted in Fig. 2. Before starting the intervention, a 2-week run-in period will be implemented during which participants will be asked to fill in informed consent and a medical history form. Subsequently, participants will be randomly allocated into two groups: one assigned to the pasta at lunch arm and the other to the pasta at dinner arm. There will be four

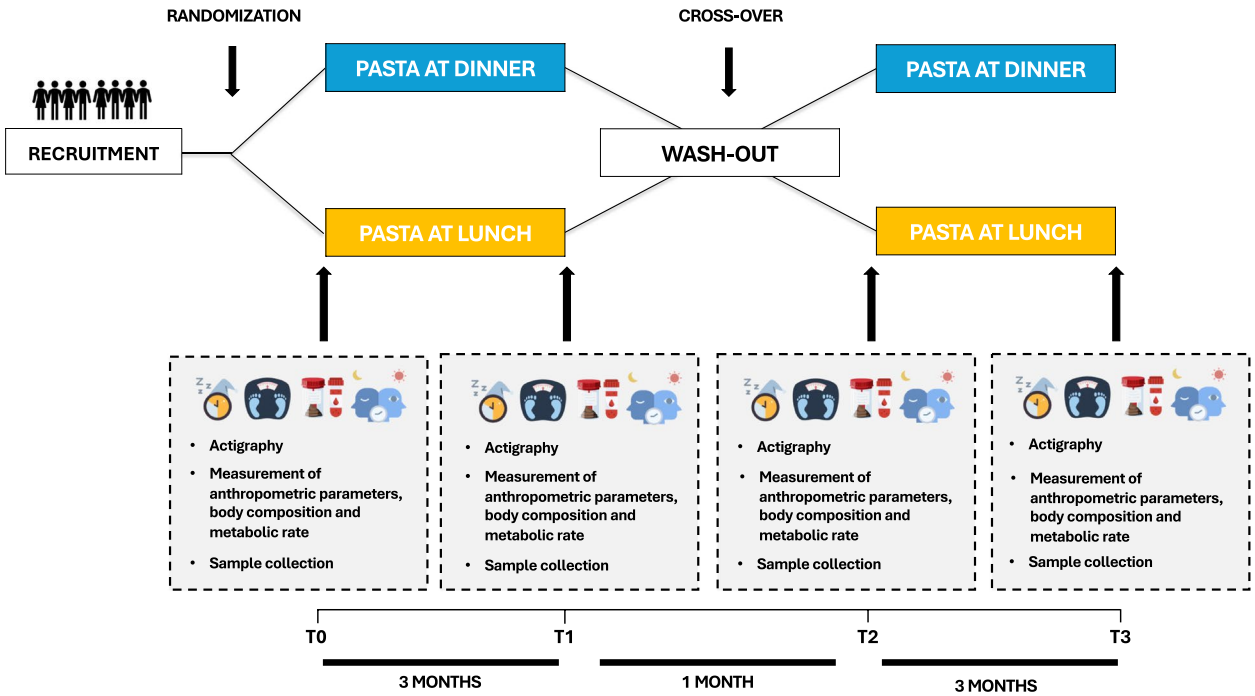

Fig. 2 Organization of the intervention study

clinical evaluations of the study population: at the beginning (time 0) and end (T1) of the first dietary intervention and at the beginning (T2) and end (T3) of the second dietary intervention. At each time point, assessments will include evaluations of sleep quality, anthropometric measurements, body composition, individual RMR, and DLMO, as well as the collection of blood and stool samples.

Sample size {14}

Sample size was calculated using Cohen's "*d*" effect size estimation. Assuming an average effect size of $d=0.5$, a power of 80% (beta), and an error probability of 5% (alfa), the required sample size is 64 participants, with 32 assigned to each group in this cross-over study design. To account for a 10% dropout rate, 6 additional participants were added, totaling 70 participants. Dropouts will be included in the intention-to-treat analysis, ensuring that all participants who were initially randomized are accounted for in the results. Additionally, per-protocol analyses will be conducted, focusing on participants who complete the trial without any major deviations from the study protocol.

Recruitment {15}

Female and male participants will be recruited using advertisements on local media, newspapers, social media, official papers, and websites. We will also recruit from our existing database of participants and friends or relatives of the hospital and university staff.

Assignment of interventions: allocation

Sequence generation {16a}

A web-based online randomization procedure, free from adaptive randomization procedures, will be employed to generate the allocation sequence. The random assignment sequence will be conducted and overseen by an investigator who will not be involved in participant recruitment, ensuring an impartial and unbiased sequence generation.

Concealment mechanism {16b}

The allocation sequence for participant assignments will be concealed from the experimenters involved in both participant enrollment and intervention assignment. Specifically, group assignments will be reported on folded sheets of paper and placed within sealed envelopes. This method ensures that the integrity of the allocation sequence remains protected until the moment of intervention assignment, thereby minimizing any potential bias in the assignment process and maintaining the study's rigor.

Implementation {16c}

The allocation sequence will be generated by an external researcher. Participant enrollment will be conducted by trial staff, while the assignment of participants to their respective interventions will be carried out by personnel who are directly involved in visiting the participants.

Assignment of interventions: blinding

Who will be blinded {17a}

Blinding of study participants and nutritionists will not be feasible due to the apparent differences between intervention diets. However, outcome measures, being less susceptible to observer influence, will be objectively assessed. Study personnel involved in participant enrollment, data collection, outcome assessment, and data analysis will be blinded to the treatment assignments. To ensure data integrity, an external researcher, independent of the research team, will enter information into the database.

Procedure for unblinding if needed {17b}

N/A.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Assessment and data collection will take place at the Unit of Clinical Nutrition of the Careggi University Hospital in Florence, Italy, with trained study staff conducting all procedures. Each participant will undergo evaluations between 7:30 AM and 10:30 AM after a 12-h fasting period. Prior to enrollment, participants will receive comprehensive instructions regarding the trial's objectives and methodologies to ensure clarity and understanding.

One week before each scheduled visit, from T0 to T4, participants will be provided with an actigraph, which they will wear continuously for 7 days. Additionally, they will receive a kit containing five salivettes for collecting saliva samples to be used in melatonin assays. Participants will be instructed on how to properly collect these samples the evening before their scheduled visits.

During each visit, participants' eating habits will be assessed using the MD adherence questionnaire (MediLite) [19] and by completing a 3-day food diary (two weekdays and one weekend day), enhancing the accuracy of energy and nutrient intake estimation. A dietitian, utilizing specific nutritional software linked to a country-specific food-nutrient database, will retrieve these data. Physical activity and individual chronotype will be investigated using the International Physical Activity Questionnaire (IPAQ) [20] and the Morningness-Eveningness Questionnaire (MEQ) [21], respectively. In addition, each

participant will undergo anthropometric measurements, including height, weight, and BMI, using a stadiometer and a professional weighing scale. Body composition will be assessed using a bioelectrical impedance analyzer, while indirect calorimetry will be applied to each participant to define individual RMR. Blood and fecal samples will be collected at baseline and follow-up visits for subsequent analyses of biochemical, inflammatory, oxidative stress markers, hormonal parameters, and microbiota-associated markers.

Within 1 week after the visit, the study staff will distribute the intervention diets, allowing time for the development of customized diet plans based on indirect calorimetry and the 3-day food diary. All participant sessions will maintain consistent duration and content.

Plans to promote participant retention and complete follow-up {18b}

To enhance participant retention and ensure completion of follow-up assessments, the study will implement various strategies based on behavior change methodologies. This includes encouraging self-monitoring and providing continuous access to study staff for dietary counseling. Throughout both phases of the study, investigators will conduct 24-h check-in calls to assess adherence to the dietary guidelines. At the conclusion of each study phase, participants will be asked to complete an adherence questionnaire. This will gather insights into their compliance with the assigned diets, any modifications made, and the challenges faced during the intervention.

If participants miss scheduled appointments, the study team will initiate up to three phone calls and send a follow-up email before considering withdrawal from the study. Participants who choose to discontinue their involvement will not undergo further clinical and laboratory evaluations. The reasons for their withdrawal will be documented to facilitate analysis during the interpretation of results. Notably, the study will be discontinued if the observed results indicate a need for premature termination.

Data management {19}

All data will be systematically recorded in an electronic database, ensuring participants are identified solely by a unique study identification number to maintain pseudonymization. No personally identifiable information will be stored within the database. To uphold data quality, several strategies will be implemented, including careful participant recruitment, adherence to a structured and time-limited protocol, a run-in period, and efforts to minimize participant burden. Establishing a trusting relationship between research units and participants will also be prioritized. Additionally, a double data entry process will be employed to enhance accuracy. Biological samples will be stored under optimal conditions according to standard procedures. Blood samples will be aliquoted and preserved at − 20 °C for up to 5 years, with detailed documentation maintained regarding their usage or destruction. These preserved samples will only be used for research purposes, contingent upon the donor's consent, and any destruction of samples will be documented. The data will be made available upon request following publication.

Confidentiality {27}

Identifiable information will be protected by assigning unique study identification numbers to each participant, ensuring their anonymity throughout data storage and analysis. Hard copies that link participant identification numbers to contact details will be securely stored in a locked file cabinet within a restricted-access office. Access to this sensitive information will be limited to essential members of the research team. Furthermore, participant files, source data, and associated study documents will be retained for 5 years, adhering to the maximum retention period permitted by the institution.

Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

Blood, fecal (four or five scoops totaling 4 g), and saliva samples will be collected at the beginning and end of each intervention phase.

Actigraphy

Each participant will undergo a 7-day monitoring period using an actigraph (Fitbit Alta HR) to evaluate their sleep–wake cycle. Participants will be instructed to wear the actigraph on their non-dominant hand and not to remove it until the visit with the research team. Data collected from the actigraph will be downloaded and analyzed using the DORMI—Sleep Analysis Evolution software, developed by Sleepacta. DORMI is a certified software, included in the register of class I medical devices. It analyzes movement data and specific physiological parameters recorded by the actigraph to compute a set of sleep metrics. The following key variables will be derived from the actigraphy data: Total Sleep Time (TST), total sleep duration (in hours and minutes); Waking After Sleep Onset (WASO), time spent in a waking state between the beginning and end of sleep (in minutes); sleep efficiency (SE), time spent sleeping between the beginning and end of sleep (in %); Number of Awakenings (Naw), number of awakenings detected; Duration of Awakenings (Daw), duration of awakenings detected (in min); Movement Index (MI), amount of movement

detected during sleep (in %); and Activity Index (AI), amount of movement detected during sleep (in minutes).

Dim light melatonin onset (DLMO)

The circadian rhythm of each participant will be assessed using the DLMO test, employing the NovoLytiX Direct Saliva Melatonin ELISA (EK-DSM). This competitive immunoassay, utilizing the capture antibody technique, is recognized as the gold standard for evaluating circadian rhythms governed by the central hypothalamic biological clock [22]. To perform this test, each participant will be instructed to collect five saliva samples using a saliva kit provided by the study team the evening prior each scheduled visit. Sample collection will begin 3 h before the participant's usual bedtime, with subsequent samples collected at 1-h intervals until the final sample is obtained 1 h after the usual bedtime. Participants will use a cotton swab, which they will chew gently for 2 min to collect saliva. Comprehensive instructions will be provided by the study staff to ensure optimal sample collection. Once collected, the saliva samples will be stored in a refrigerator at 2–8 °C until they are delivered for analysis, which should occur within a maximum of 1 day. To recover saliva from the cotton swab, the salivettes will be centrifuged for 2 min at 1000 × g and then the saliva samples will be stored and preserved at − 20 °C until analysis.

Anthropometric parameters, body composition, and indirect calorimetry

Height will be measured using a stadiometer, while weight will be assessed with a professional weighing scale (TANITA, model TBF-410) that has an accuracy of 0.1 kg. BMI will be calculated as weight (kg)/height (m^2). Body composition will be determined with a bioelectrical impedance analyzer (Akern, model SE 101) at baseline and at follow-up visits. RMR will be measured under fasting conditions using the Fitmate GS indirect calorimetry device (COSMED). The Fitmate GS is a portable desktop metabolic monitoring device that calculates oxygen consumption (VO2), ventilatory power (VP), and expired fraction of oxygen (FEO2). RMR is estimated using the abbreviated Weir equation: $(3.9 \times (VO2) + 1.1 \times (RQ \times VO2)) \times 1.44$, with a fixed Respiratory Quotient (RQ) of 0.85. Participants will be instructed to fast for 12 h prior to testing and to avoid intense physical activity the day before the test. During the measurement, participants will lie supine for 15 min, breathing normally within a ventilated hood.

Analysis of biochemical parameters, hormones, adipokines, inflammatory, and oxidative stress markers

Blood samples will be centrifuged at 3000 rpm for 15 min, aliquoted to obtain serum, and stored at − 20 °C until analysis. The following biochemical measures will be evaluated in all participants according to standardized routine laboratory protocols: complete blood count, glycemic profile [fasting glucose, insulin, HOMA Index, glycated hemoglobin], homocysteine, lipid profile [total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides], liver function tests [aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (γGT)], renal function tests (serum creatinine, urea, uric acid), mineral profile (sodium, potassium, magnesium, calcium), iron metabolism (iron, ferritin), and vitamin profile (vitamin B12, folic acid). Fasting plasma concentrations of ghrelin, leptin, glucagon-like peptide 1 (GLP-1), peptide YY, C-peptide, glucagon, and pancreatic polypeptide will be measured using commercial enzyme-linked immunosorbent assay kits, according to the manufacturer's instructions. Pro-(anti)-inflammatory cytokines (e.g., interleukin (IL)−6, interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNFα), monocyte chemoattractant protein-1 (CCL2/MCP-1)) will be analyzed in plasma according to manufacturers' instructions. Markers of oxidative stress will also be studied, in terms of total antioxidant profile and lipid peroxidation.

Analysis of fecal microbiota profiles and SCFAs

Fecal sample collection kits, including containers, will be provided to each participant. Fecal microbiota profiles and SCFAs (acetic, butyric, isobutyric, propionic, valeric, and isovaleric acids) will be evaluated. Total microbial DNA will be extracted from the faces by repeated vortexing. The V3 and V4 hypervariable regions of the 16S rRNA gene for bacteria and ITS1-4 for fungi will be sequenced on the Illumina MiSeq platform, following the Illumina protocol for preparing 16S metagenomic sequencing libraries. SCFAs will be extracted using aqueous sodium hydroxide (NaOH) containing an internal standard. After extraction, an aliquot of supernatant fecal water will be derivatized with a propanol/pyridine mixture. The organic extract will be analyzed by gas chromatography-mass spectrometry (GC–MS) using deuterated internal standards and an appropriate GC Wax column.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

Statistical analyses will be performed using SPSS software for Macintosh (SPSS Inc., Chicago, IL, USA). A P value < 0.05 will be considered statistically significant. Data will be analyzed using intention-to-treat and on-treat procedures. The primary outcome (change in sleep efficiency) will be analyzed within each group using Student's t-test for paired data. The difference in absolute change (mean value at baseline subtracted from the

mean value after the intervention for each subject) will be estimated using Student's *t*-test for independent data. Evaluation of distributions and checking for outliers will be performed using histograms and box plots. If appropriate, variables will be logarithmized to normalize the distributions of data. Continuous variables that follow a normal distribution will be summarized using the mean and standard deviations. Categorical variables will be presented in terms of frequencies and percentages. After testing the regression assumption, a general linear model for repeated measurements with adjustments for possible confounding factors will be run to compare the effects of different interventions. Data for the general linear model will be reported as geometric means and 95% confidence intervals. The same analyses will be performed for secondary outcomes. Microbial alpha-diversity (richness, Simpson's, Gini-Simpson's, inverse Simpson and Shannon's indices, evenness, and dominance) and beta (weighted UniFrac and Bray–Curtis dissimilarity) diversity measures will be assessed using QIIME (v. 1.9).

Interim analyses {21b}

No interim analysis will be conducted.

Methods for additional analyses (e.g., subgroup analyses) {20b}

Subgroup analyses will be performed to analyze possible differences in the changes, according to specific characteristics of the study population (age, sex, and chronotype).

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

Before starting data analysis, the extent and potential causes of missing data will be systematically evaluated using appropriate tables and descriptive statistics. This assessment will help determine whether the level and type of missing data could introduce bias into the analysis results or significantly reduce the precision of the estimates derived from the proposed statistical methods. Sensitivity analyses will be conducted under the assumption that the missing outcomes represent the worst or best-case scenarios within the different randomization groups. Should these analyses indicate that the study conclusions could vary based on the missing values, further multiple imputation methods will be applied to address the missing data. These imputation analyses will consider follow-up losses, operating under the assumption of random missingness, and will be guided by the relationships observed in the measured variables.

Plans to give access to the full protocol, participant-level data, and statistical code {31c}

Access to data will be limited to qualified personnel with unique password-protected accounts.

Oversight and monitoring

Composition of the coordinating center and trial steering committee {5d}

Regular monitoring of the study will be conducted by both the research team and the local Institutional Review Board (IRB). Due to the study's limited objectives, short duration, and low-risk nature of the intervention, the establishment of a Data Monitoring Committee and a Trial Steering Committee was deemed unnecessary. Instead, a management board, chaired by the principal investigator and two co-investigators, will be responsible for overseeing the progress of activities and ensuring alignment with the study targets. This board will define deadlines and ensure timely submission of related reports. Additionally, annual reports detailing both study activities and financial matters will be prepared to maintain transparency and accountability throughout the study duration.

Composition of the data monitoring committee, its role and reporting structure {21a}

The research group, appointed by the principal investigator, comprises three other nutritionists, two dieticians, one physiologist, one biostatistician, and one gastroenterologist.

Adverse event reporting and harms {22}

Adverse events, including any unfavorable or undesirable signs, symptoms, abnormal laboratory results, or illnesses temporally associated with the intervention diet, will be systematically collected from the time of randomization until the final 7-month follow-up visit for each participant. This collection will occur regardless of whether the events are considered related to the intervention. All reported adverse events will be monitored until their resolution, ensuring participant safety and well-being throughout the study.

Frequency and plans for auditing trial conduct {23}

The research team will convene regularly, typically monthly, to comprehensively evaluate the ongoing progress of the trial. These meetings will involve a thorough review of various aspects, including recruitment rates (actual versus projected), data quality, return rates, protocol amendments, and other relevant research matters. The principal investigator will lead these meetings, setting the agenda and acting as the

primary contact for the study. At both the halfway point and the conclusion of the study, the protocol team will prepare and submit a comprehensive monitoring report to the local Institutional Review Board. This report will detail activities undertaken, progress made, challenges encountered, and any significant issues of concern that may arise during the study.

Plans for communicating important protocol amendments to relevant parties (e.g., trial participants, ethical committees) {25}

Any changes to the protocol and information provided to participants will be submitted to the Ethics Committee for approval prior to implementation.

Dissemination plans {31a}

The findings of the study will be disseminated through multiple channels to reach a wide audience. Investigators will present the results at both national and international scientific conferences, facilitating discussions and feedback from the scientific community. Additionally, the study's outcomes will be published in peer-reviewed journals to ensure accessibility to researchers and practitioners in the field.

Discussion

This study represents a pioneering exploration of how the timing of pasta consumption—whether eaten at lunch or dinner—can influence a range of health outcomes, including sleep quality, circadian rhythm, body composition, cardiovascular risk factors, and gut microbiota in normal-weight individuals. Given the high prevalence of sleep disturbances worldwide and their well-established link to metabolic and cardiovascular diseases [23], understanding how meal composition influences sleep could lead to more effective dietary strategies. With over a third of adults in industrialized nations experiencing insufficient or poor-quality sleep, any dietary modification that alleviates sleep disturbances could have substantial health benefits.

If the timing of carbohydrate-rich meals, such as pasta, is found to positively impact sleep and metabolic outcomes, it could provide a novel, non-pharmacological support for addressing sleep disorders through dietary adjustments. These findings may also advance personalized nutrition by highlighting not only *what* to eat, but also *when* to eat, aligning with the emerging field of chrononutrition. Additionally, this study comes at a time when low-carbohydrate diets have gained widespread popularity, often based on the misconception that all carbohydrates are harmful [24, 25]. Our findings could challenge this "carbophobia" by fostering a more holistic view of diet, one that shifts the focus away from rigid calorie counting or macronutrient modulation to the overall quality and timing of the diet. Such insights could lead to more flexible, personalized diets that promote long-term adherence, an especially important factor given that rigid diets frequently fail in real-world settings. The results may also stimulate further research into the broader implications of meal composition and timing in populations with diverse body compositions and health statuses, potentially informing public health strategies aimed at improving both sleep hygiene and dietary habits.

Several limitations of this study must be acknowledged. The relatively short duration and modest sample size may limit the generalizability of the findings to larger or more diverse populations. Although the sample is sufficient to detect moderate effects, it may not capture more subtle variations or account for the range of dietary habits and health profiles seen in the general population. Additionally, the reliance on self-reported data introduces the risk of recall bias, which may affect the accuracy of reported dietary intake. Nevertheless, the study has several strengths. The randomized controlled trial design minimizes confounding factors, while the cross-over design allows participants to serve as their own controls, reducing interindividual variability and improving internal validity. The use of DLMO to assess circadian rhythms is another strength, enabling a detailed exploration of the interactions between meal composition and circadian biology, and providing deeper insights into the mechanisms behind observed health effects. Finally, the comprehensive range of outcomes evaluated offers a holistic view of how the timing of carbohydrate consumption, especially pasta, affects sleep quality and cardiometabolic health. This multidimensional approach allows for a more thorough understanding of the complex relationships between diet, sleep, and metabolic function.

In conclusion, this study has the potential to significantly advance our understanding of how carbohydrate intake at different times of the day influences sleep, metabolic health, and circadian rhythms. By addressing a critical gap in the literature, these findings could inform future research and provide actionable insights for public health strategies. This is especially relevant in light of the global shift toward personalized nutrition, which must account for individual variability in circadian biology, dietary preferences, and metabolic responses. Ultimately, by providing more flexible and individualized dietary recommendations, this research could contribute to improving long-term adherence to

healthy eating patterns, benefiting billions of people worldwide.

Trial status

The study has received all necessary regulatory approvals. The currently approved version of the protocol is 2.0 (version date 26/09/2023). Recruitment started in November 2023 and the completion date is scheduled for November 2025.

Abbreviations

ALT	Alanine transaminase
AI	Activity Index
AST	Aspartate aminotransferase
BMI	Body mass index
CCL2/MCP-1	Monocyte chemoattractant protein-1
DAW	Duration of Awakenings
DLMO	Dim light melatonin onset
γGT	Gamma-glutamyl transferase
GLP-1	Glucagon-like peptide 1
HDL	High-density lipoprotein
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
IFN-γ	Interferon-gamma
IL	Interleukin
IPAQ	International Physical Activity Questionnaire
LDL	Low-density lipoprotein
MD	Mediterranean diet
MEQ	Morningness-Eveningness Questionnaire
MI	Movement Index
NAW	Number of Awakenings
RCT	Randomized controlled trial
RMR	Resting metabolic rate
SCFAs	Short-chain fatty acids
SE	Sleep efficiency
TNFα	Tumor necrosis factor-alpha
TST	Total Sleep Time
WASO	Waking After Sleep Onset

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13063-025-08859-x.

Supplementary Material 1.

Supplementary Material 2.

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N/A.

Authors' contributions {31b}
SL has made substantial contributions to the conception and design of the work, wrote the study protocol, and drafted the article. She is currently involved in participant enrollment, analysis of food diaries, analysis of laboratory parameters, and data acquisition and interpretation. She is part of the protocol team. MD has made substantial contributions to the conception and design of the work, wrote the study protocol, and drafted the article. She is currently involved in participant enrollment and data acquisition and interpretation. She is part of the protocol team. AN is currently involved in participant enrollment, analysis of food diaries, analysis of laboratory parameters, and data acquisition and interpretation. She is part of the protocol team. IG is currently involved in clinical evaluations of the participants and data acquisition. She is part of the protocol team. MTA is currently involved in participant enrollment and data acquisition and interpretation. She is part of the protocol team. GP is currently involved in participant enrollment and data acquisition and interpretation. She is part of the protocol team. BC has made substantial contributions to the conception and design of the work. She is currently involved in participant enrollment and data acquisition and interpretation and has provided critical intellectual revisions to the manuscript. FS has made substantial contributions to the conception and design of the work. He is responsible for clinical evaluations and the analysis of laboratory parameters and has provided critical intellectual revisions to the manuscript. He is part of the protocol team. All authors read and approved the submitted manuscript.

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Data availability {29}
Access to the final trial dataset will be restricted to the research team to ensure confidentiality and protect participants' privacy. The principal investigator will be responsible for safeguarding participant privacy and ensuring that data, along with source documents, are stored securely for potential monitoring or inspection by the Ethics Committee. Upon study completion, participants may request a copy of the study results from the principal investigator, thereby promoting transparency and engagement. The final report will comply with CONSORT 2010 guidelines to ensure comprehensive reporting standards. Study results will be submitted for publication in a peer-reviewed journal. Additionally, plans for dissemination at national and international conferences will be discussed among researchers prior to implementation.

Declarations

Ethics approval and consent to participate {24}
The study protocol was reviewed and approved by the Ethics Committee of the University of Florence (n spe123.23, version 2.0, date 26/09/2023). The study will be conducted in accordance with the Declaration of Helsinki and the Data Protection Act. Ethics Committee approval included the study protocol, information sheet and consent form, questionnaires, interviews, any other written information that will be provided to participants, and any advertising that will be used during the study. The study is registered at the Clinical Trials Registry (clinicaltrials.gov: NCT06185634, registered on 07/01/2024) in accordance with the requirements of the International Committee of Medical Journal editors (ICMJE).

Consent for publication {32}
Not applicable—no identifying images or other personal or clinical details of participants are presented here or will be presented in reports of the trial results. The participant information materials and informed consent form are available from the corresponding author on request.

Competing interests {28}
The authors declare that they have no competing interests, and the research is not being supported by any commercial organization.

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