Trials

STUDY PROTOCOL

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The Ex-Timing trial: evaluating morning, afternoon, and evening exercise on the circadian clock in individuals with type 2 diabetes and overweight/obesity—a randomized crossover study protocol

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Abstract

Background Exercise is known to provide multiple metabolic benefits such as improved insulin sensitivity and glucose control in individuals with type 2 diabetes mellitus (T2DM) and those at risk. Beyond the traditional exercise dose, exercise timing is perceived as a contemporary hot topic, especially in the field of T2DM; however, the number of intervention studies assessing exercise timing and glucose metabolism is scarce. Our aim is to test the effect of exercise timing (i.e., morning, afternoon, or evening) on the inter-individual response variability in glycemic control and related metabolic health parameters in individuals with T2DM and those at risk during a 12-week intervention.

Methods A randomized crossover exercise intervention will be conducted involving two groups: group 1, individuals with T2DM; group 2, age-matched older adults with overweight/obesity. The intervention will consist of three 2-week blocks of supervised post-prandial exercise using high-intensity interval training (HIIT). Between each training block, a 2-week washout period, where participants avoid structured exercise, will take place. Assessments will be conducted in both groups before and after each exercise block. The primary outcomes include the 24-h area under the curve continuous glucose monitoring-based glucose. The secondary outcomes include body composition, resting energy expenditure, insulin response to a meal tolerance test, maximal aerobic capacity, peak power output, physical activity, sleep quality, and insulin and glucose levels. All primary and secondary outcomes will be measured at each assessment point.

Discussion Outcomes from this trial will provide us additional insight into the role of exercise timing on the inter-individual response variability in glycemic control and other related metabolic parameters in two distinct populations, thus contributing to the development of more effective exercise prescription guidelines for individuals with T2DM and those at risk.

Trial registration ClinicalTrials.gov NCT06136013. Registered on November 18, 2023.

Keywords Glycemic control, Inter-individual variability, Aging, Obesity, Crossover randomized controlled trial

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Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

Title {1}	Ex-Timing study protocol: another look to exercise prescription: exercise timing and the circadian clock in individuals with type 2 diabetes and those at risk
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Introduction

Background and rationale {6a}

The prevalence of type 2 diabetes mellitus (T2DM) continues to increase globally, with implications for cardiovascular diseases and all-cause mortality [1]. Exercise is known to provide multiple metabolic benefits and is one of the main components included in the treatment plan for improving glycemic control and reducing the development of further comorbidities in individuals with T2DM [2–5].

Despite the benefits, not all individuals with T2DM obtain improvement in glycemic control to a given exercise program. Several sources of inter-individual variability in the glycemic response to exercise have been identified and investigated, with the major one being that of exercise dose [6]. Many investigations have been done to determine the exercise that can elicit the greatest benefits on glycemic control and metabolic health, with findings suggesting that the incorporation of exercise of higher intensity and longer duration may eliminate much of the "non-response" observed following exercise training [7]. Nevertheless, even with the incorporation of higher-intensity exercise into interventions aimed at improving glycemic control in individuals with T2DM, we [8] and others [9] continue to have mixed results, with not all individuals with T2DM obtaining benefits in insulin sensitivity and glycemic control to a given exercise training program.

Exercise timing has recently been explored as another potential source of inter-individual variability, given that glucose/insulin metabolism is controlled by cellular clock genes with many of these genes being downstream of exercise-stimulated pathways [10–12]. In healthy humans, insulin sensitivity and beta-cell function tend to decrease in the evening hours, whereas the diurnal patterns in glucose tolerance and insulin sensitivity appear to be reversed in individuals with T2DM [12]. Despite the growing interest in exercise timing, most of the experimental evidence originates from animal models [13, 14], with only three studies being performed in humans with T2DM, which displayed conflicting results [15–17]. Moreover, two of these studies included a mixed population of both individuals with T2DM, who were on either metformin or a combination of antihyperglycemic drugs, and sedentary overweight/obese individuals [15, 17], all of which increase inter-individual variability and limits the ability to assess the group-dependent metabolic adaptations to exercise timing specific to individuals with T2DM and overweight/obese individuals with metabolic risk.

Objectives {7}

This paper describes the protocol for the Ex-Timing trial, which aims to test the effect of exercise timing (i.e., morning, afternoon, or evening) on the inter-individual response variability in glycemic control and related metabolic health parameters in two distinct populations: (1) individuals with T2DM on the most common monohyperglycemic drug therapy (i.e., metformin) [2] and (2) age-matched sedentary overweight/obese individuals, where glycemic control is known to deteriorate [16], hence increasing their risk of developing insulin resistance and T2DM, using a randomized crossover design.

Trial design {8}

This project will consist of a randomized crossover exercise intervention involving two groups: group 1, individuals with T2DM; group 2, age-matched

older adults with overweight/obesity. Each study will consist of three 2-week blocks of 3 days per week of supervised post-prandial (30 min after the last meal) exercise using high-intensity interval training (HIIT). Exercising post-prandially has consistently been shown to be effective at improving glucose control in individuals with T2DM [18]. Between each training block, a 2-week washout period, where participants avoid structured exercise, will take place. This has been shown to be enough time to eliminate any exercise effects on insulin sensitivity and glycemic control (Fig. 1) [5].

The crossover groups for the randomized crossover trial are the following:

1) Morning HIIT 30 min following breakfast between 9:00 and 10:00 am
2) Afternoon HIIT 30 min following lunch between 2:00 and 3:00 pm
3) Evening HIIT 30 min following dinner between 7:00 and 8:00 pm

Our crossover design will allow each participant to serve as their own control, helping to account for the

	STUDY PERIOD						
	Enrolment	Allocation	Post-allocation				Close-out
TIMEPOINT	*Pre-Baseline*	*Baseline 1*	*Week 2 (End 1)*	*Baseline 2*	*Week 2 (End 2)*	*Baseline 3*	*Week 2 (End 3)*
ENROLMENT:							
Eligibility screen	X						
Informed consent	X						
Allocation		X					
INTERVENTIONS:							
Morning		♦—	—♦				
Afternoon				♦—	—♦		
Evening						♦—	—♦
ASSESSMENTS:							
24-hour AUC CGM		X	X	X	X	X	X
Body composition		X	X	X	X	X	X
Body mass index (BMI)		X	X	X	X	X	X
Waist and hip circumference		X	X	X	X	X	X
Resting energy expenditure		X	X	X	X	X	X
Metabolic flexibility		X	X	X	X	X	X
Maximal aerobic capacity,		X		X		X	
Peak power output (PPO)		X		X		X	
Dietary records		X	X	X	X	X	X
Physical activity volume and intensity		X	X	X	X	X	X
Time spent in sedentary behavior (SB)		X	X	X	X	X	X
PA and SB patterns		X	X	X	X	X	X
Sleep quality		X	X	X	X	X	X
Blood Analysis		X	X	X	X	X	X
Questionnaires		X					

Fig. 1 SPIRIT figure

biological variability within the individual, as well as their individual chronotype, such as sleep/wake cycles, which are known to slightly alter their circadian rhythm [19]. This study protocol was approved by the Faculty of Human Kinetics Institutional Review Board and has been registered at ClinicalTrials.gov (Trial ID #NCT06136013).

This protocol follows the SPIRIT checklist and figure guidelines (see Fig. 1).

Methods: participants, interventions, and outcomes

Study setting {9}

Individuals with T2DM and age-matched older adults with overweight/obesity will be recruited via Associação Protectora dos Diabéticos de Portugal (APDP) clinic and by using other referral sources, media advertisements, brochures/posters, and community events within the Lisbon Metropolitan Area. Following an initial telephone screening to determine eligibility, all eligible participants will be scheduled for an orientation session where detailed information about the study will be provided. Written informed consent will be obtained from all participants, and they will be informed that their participation is solely voluntary and that they may withdraw their consent at any time.

Eligibility criteria {10}

The inclusion criteria for this study comprises the following: previous diagnosis of T2DM in accordance with American Diabetes Association criteria and currently taking metformin (group 1 only); BMI $\geq$ 25 without diagnosis of T2DM (group 2 only); adults aged 55 to 75; and able to participate in exercise sessions. Exclusion criteria includes taking any other antihyperglycemic medication; major micro- or macro-vascular complications from T2DM; and the inability to provide informed consent. No direct or indirect incentives will be offered to the participants.

Who will take informed consent? {26a}

The principal investigator of the study will contact all potential participants for a detailed explanation of the trial (the number and type of assessments, the length and nature of the intervention, and the time commitment required to complete the study) so that all doubts can be solved prior to the baseline evaluation moment. On the baseline assessment, one trained evaluator (i.e., Ph.D. candidate with a Master in Exercise and Health and a certified Exercise Physiologist by the Portuguese Government) will answer any questions prior to the potential participants signing the written informed consent.

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

On the consent form, participants will be asked if they agree to the use of their data should they choose to withdraw from the trial. Participants will also be asked for permission for the research team to share relevant data with people from the university taking part in the research or from regulatory authorities, where relevant.

Interventions

Explanation for the choice of comparators {6b}

Ex-Timing is designed for individuals with T2DM and for age-matched overweight/obese individuals that have an increased risk of developing insulin resistance and T2DM. Both groups will have access to the exercise intervention. Our crossover design will allow each participant to serve as their own control, helping to account for the biological variability within the individual, as well as their individual chronotype, such as sleep/wake cycles.

Intervention description {11a}

Ex-Timing will be delivered over 3 months per participant. Given that most of the participants will not be accustomed to regular exercise, especially at the high-intensity levels of HIIT, in the first week before the beginning of the intervention, we will gradually phase all participants up to the full HIIT protocol. In phase 1 of the exercise phasing (1st session), participants will perform continuous exercise of moderate intensity (40–60% of their peak power output (PPO) determined by their cardiopulmonary exercise test). During phase 2 (2nd session), HIIT will be introduced progressively, starting with bouts of 2 min at 70% of their PPO, followed by 1 min at 40–60% of their PPO. Finally, on phase 3 (3rd sessions), the intensity will be increased to bouts of 80% of their PPO, followed by 1 min at 40–60% of their PPO. Following this preparatory week, participants will begin their first training block (i.e., morning, afternoon, or evening) at the full HIIT protocol (i.e., 1 min of exercise at 90% of their PPO, followed by 1-min resting at 40–60% of their PPO).

The HIIT sessions will consist of participants performing 1 min of exercise at 90% of their PPO, followed by a 1-min rest at 40–60% of their PPO using a cycle ergometer. In addition to the prescribed power output, all exercise sessions will be closely monitored with heart rate sensors (polar H-10, USA) to ensure compliance with the target prescription and to measure each exercise training energy expenditure. The exercise prescription will be standardized according to body weight and based on physical activity guidelines to achieve a weekly target of 10 kcal/kg. The duration of the exercise sessions will be based on the weekly target for energy expenditure,

considering weight and individual VO2peak, and will be updated at the beginning of each intervention block.

Following each intervention block, there will be a 2-week washout period where the participants will be asked to not partake in any structured exercise training. In addition, each participant will be provided with a step counter during this 2-week washout period to control physical activity levels. The range of steps per day in which they will be instructed to maintain will be defined based on their physical activity recorded through accelerometry prior to the start of the intervention. This will help to eliminate any compensatory physical activity that may be performed by the participants during the washout period.

Criteria for discontinuing or modifying allocated interventions {11b}

After the randomization procedure that will place all participants to one of the defined exercise block orders, modifying the allocated order of exercise timing will not be allowed.

Strategies to improve adherence to interventions {11c}

To increase exercise compliance, we intend to establish a community outreach program to increase motivation and provide the conditions for the participants to continue exercising after the cessation of the intervention. Moreover, we will use the already established collaborative support of the Associação Protectora dos Diabéticos de Portugal (APDP) and their qualified health professionals, who will assure close proximity with participants throughout the intervention, helping with compliance.

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

Participants should not increase their physical activity patterns, and this will be controlled by having the participants wear an accelerometer during the intervention period. Other than that, no control will exist regarding any extra behavior that participants may develop through the intervention.

Provisions for post-trial care {30}

We do not expect any harm from this intervention; nevertheless, to limit the potential for injury and dropout, there will be a phasing period to the HIIT exercise intervention to gradually integrate the HIIT regimen.

Outcomes {12}

Primary

The primary outcome of Ex-Timing is the 24-h AUC continuous glucose monitoring (CGM)-based glucose, which will be objectively assessed with a CGM monitor (Freestyle Libre, Abbott). The "Data collection and management" section provides more details regarding the assessments that generate these outcomes. This outcome will be measured at the beginning and at the end of each 2-week block of exercise.

Secondary

The secondary outcomes of Ex-Timing are body composition (i.e., total and regional fat mass and lean body mass, abdominal and gynoid body fat mass, and bone mineral content and density, all estimated based on dual-energy X-ray absorptiometry), body mass index (BMI); waist and hip circumference; resting energy expenditure, and metabolic flexibility (measured through insulin response to a meal tolerance test) by a gas analyzer; and maximal aerobic capacity, PPO, and pre-existing heart conditions assessed through a cardiopulmonary exercise test (CPET). Alongside the CGM evaluation, participants will fill out a 24-h dietary record the day before and the day immediately after the CGM assessment in order to account for the effect of diet and time of the meal on glycemic control and other metabolic outcomes. ActiGraph GT9X accelerometers (ActiGraph, Pensacola, FL) will be used to measure physical activity volume and intensity (total minutes at light, moderate, and vigorous intensity, total counts, and counts/minute), time spent in sedentary behavior, as well as patterns on how each of these behaviors are accumulated (i.e., bouts of time spent in sedentary behavior or physical activity). Furthermore, to objectively evaluate sleep quality, participants will use the accelerometer during the night to evaluate sleep latency (minutes), sleep efficiency (%), total time in bed (minutes), total time sleeping (minutes), wake before sleep onset (minutes), number of awakenings, and average awakening length (minutes). Insulin and glucose will also be analyzed as secondary outcomes through blood samples collected by APDP-certified staff. Lastly, questionnaires will be performed at baseline and will gather information on several domains: sociodemographic, health, physical activity, sedentary behavior and sedentary behavior correlates, and residence domain and physical activity correlates. More details regarding the assessment that generates these outcomes can be found in the "Data collection and management" section. All secondary outcomes will also be measured at the beginning and at the end of each 2-week exercise block.

Participant timeline {13}

The time schedule of enrolment, interventions, assessments, and visits for participants are presented in the intervention guide (see Fig. 2). All participants will enter the study and be assessed at the same time points.

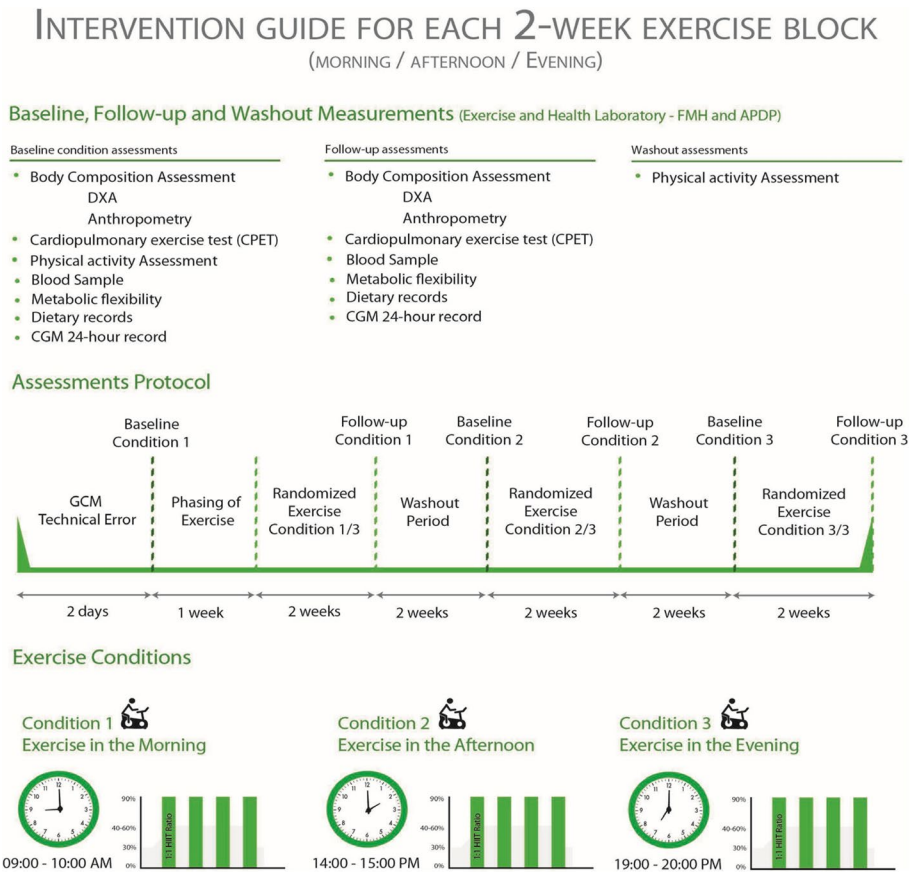

Fig. 2 Study design for the intervention

Sample size {14}

To detect a predicted mean difference in 24-h AUC CGM-based glucose of 1.0 mmol/L with a within-patient SD of 0.85 mmol/L [20] considering a type I error of 5%, a power of 80%, and a 30% dropout rate, we calculated a required sample size of 17 individuals for each group, making a total sample of 34 individuals (group 1: T2DM; group 2: age-matched overweight/obese).

Recruitment {15}

Through our longstanding collaboration with the Associação Protectora dos Diabéticos de Portugal (APDP), we will be able to recruit individuals with T2DM using referral sources, media advertisement, brochures/posters, and community events within the Lisbon Metropolitan Area.

Following an initial telephone screening to determine eligibility, all eligible participants will be scheduled for an orientation session where detailed information about the study will be provided. The detailed information will include the number and type of assessments, the length and nature of the intervention, and the time commitment required to complete the study. All participants will be informed about any potential risks of partaking in the intervention prior to giving their written informed consent. Following consent, individuals will complete the baseline assessments and then the order of the 2-week blocks of exercise intervention (i.e., morning HIIT intervention, afternoon HIIT, and evening HIIT session) will be randomly allocated for each participant. Experimental order will be randomized by a third-party investigator using a computer-generated list of random numbers (block randomization by group with balanced block sizes). Ethics approval will be obtained from all the institutions involved prior to study implementation.

Assignment of interventions: allocation

Sequence generation {16a}

After baseline data collection, block randomization by group with balanced block sizes using REDCap (https://imm.medicina.ulisboa.pt/group/redcap/redcap_v7.6.7/) will be performed by a researcher not involved in recruitment or data collection. The different possible exercise timing orders will be calculated and planned, and

participants will be randomly allocated to one of these block orders.

Concealment mechanism {16b}

Utilizing a REDCap feature, participants will be randomized to an exercise timing order. After all baseline measurements have been completed, the allocation concealment technique will be implemented so that the participants are not informed of their exercise timing order prior to the beginning of the intervention. Experimental order will be randomized by a third-party investigator using a computer-generated list of random numbers (block randomization by group with balanced block sizes).

Implementation {16c}

The allocation sequence will be created by an independent researcher. Once participants are registered, the research team will assign them to interventions. The groups that the participants were assigned to (i.e., based on a randomly generated selection made by a separate researcher) will be communicated to them in the last sessions of exercise phasing.

Assignment of interventions: blinding

Who will be blinded {17a}

Prior to randomization, baseline assessment will be conducted; as a result, neither participants nor fieldwork staff will be aware of the group assignment at this time. After baseline, it will not be possible to blind participants or staff due to the intervention's nature.

Procedure for unblinding if needed {17b}

Unblinding will be necessary after randomization has been completed and before telling the participants their group assignment. The trial manager, data coordinator, facilitators of the implementation, and participants will then have access to group allocations.

Data collection and management

Plans for assessment and collection of outcomes {18a}

All study measurements will be collected at the Faculty of Human Kinetics, University of Lisbon. Body composition, CPET, blood collection, metabolic flexibility, dietary records, and 24-h GCM recordings will be done at baseline as well as at the end of each 2-week intervention block. Physical activity assessment will be performed at baseline and during each 2-week washout period. The evaluations inherent to the project at baseline and at the end of each 2-week intervention block will take place on two different days.

Primary data collection

Continuous glucose monitoring Twenty-four-hour blood glucose levels will be measured with a CGM monitor (Freestyle Libre, Abbott).

The sensor will be placed subcutaneously on the right side of the arm prior to eating any of the standardized pre-made meals with macronutrient distribution based on the recommended dietary guidelines (either breakfast, lunch, snack, or dinner). The monitor will provide interstitial glucose values every 10 min and will be removed when the battery life ends. During the first 24-h period, all meals will be standardized and provided to the participants. Participants will be asked to keep all non-eaten food and bring it to the after the 24-h period. This will allow the research team to determine the participants' exact dietary intake during the 24-h CGM period. In addition, the time of all medications taken will be recorded.

This procedure will be performed as follows: To obtain the technical error of the CGM (needed for secondary objective b to quantify the number of responders exercising at different times of the day on glycemic control), participants will be assessed on two non-consecutive days at baseline before the implementation of the intervention. Furthermore, for each exercise block (i.e., 2 weeks of morning HIIT, 2 weeks of afternoon HIIT, and 2 weeks of evening HIIT), participants will be assessed prior to the start of the exercise sessions and at the end of week 2.

Secondary data collection

Demographic data Questionnaires will be performed at baseline and will measure several domains: sociodemographic, health, physical activity, sedentary behavior domain and sedentary behavior correlates, and residence domain and physical activity correlates.

The selected questionnaires are EHIS-PAQ, S/R, Bone Density Questionnaire, WHOQOL Questionnaire, PSQI Questionnaire, FRAQ, CPF Scale, IPAQ, Eurobarometer Wave 88.4, GPAQ, SBQ, and NEWs.

Body composition Participants will be weighed to the nearest 0.01 kg wearing minimal clothes and without shoes, and their height will be measured to the nearest 0.1 cm on a digital scale with an integrated stadiometer (Seca, Hamburg, Germany). From weight and height, BMI will be calculated as weight (kg) divided by the square of height (m).

Dual-energy X-ray absorptiometry (DXA) (Hologic Explorer-W, fan-beam densitometer, software QDR for Windows version 12.4, Waltham, USA) will be used to estimate total and regional fat mass and lean body mass. Moreover, bone mineral content and density will also be estimated. A whole-body scan will be performed, and the attenuation of X-rays pulsed between 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image that will be measured. Abdominal and gynoid body fat mass will be measured through partial analyses of the DXA scan, based on regions of interest set by default in the DXA settings. Following the protocol for DXA described by the manufacturer, a phantom with six fields of acrylic and aluminum of varying thickness and known absorptive properties will be scanned alongside each participant to serve as an external standard for the analysis of different tissue components. The same laboratory technician will position the participants, perform the scans, and execute the analyses according to the operator's manual using the standard analysis protocol. Based on ten participants, the coefficient of variation in our laboratory for fat mass and abdominal fat mass was 1.7% and 0.01%, respectively.

Resting energy expenditure/metabolic flexibility Oxygen consumption, carbon dioxide production, and respiratory quotient variations will be measured in a resting condition via indirect calorimetry (Quark RMR w/CPET, version 9.1) for 2 h and 30 min. The first 30 min will be conducted in a fasting condition. After that, a blood sample will be taken to measure insulin concentration, and the participants will consume a standardized meal (2 bottles of Boost Complete Nutritional Drink). After that, indirect calorimetry will be performed for 2 h, with a blood collection every 60 min. This procedure will take place after an overnight fast of 10 h and refraining from exercise and alcohol ingestion the previous day.

Energy intake/diet Participants will fill out a 24-h dietary record the day before and the day immediately after the CGM assessment in order to account for the effect of diet and time of the meal on glycemic control and other metabolic outcomes. Prior to the beginning of the intervention, participants will be instructed on portion sizes, aspects of food preparation, and other relevant information in order for them to record their dietary intake as accurately as possible. Records will be analyzed to obtain macro- and micronutrient content using two software packages: Food Processor SQL, which carries the USDA (USA) recently updated food database.

Participants will be instructed to maintain the timing of their meals and diet throughout the study. In the event that any participant is losing or gaining substantial weight during each intervention block, a consultation will be set with the nutritionist in order to achieve a neutral energy balance and avoid any potential bias on glycemic control outcomes.

Free-living physical activity and energy expenditure Daily physical activity will be measured using ActiGraph GT9X accelerometers (ActiGraph, Pensacola, FL). Participants will be asked to maintain their physical activity patterns unchanged during the intervention period and to wear the accelerometer on the non-dominant wrist, through the full duration of the study, in order to control physical activity levels. Device activation and download will be performed using Actilife® software (v.6.9.1; Fort Walton Beach, FL), whereas processing will be done using an open-source R package GGIR version 2.6. The accelerometer data will provide a measure of physical activity volume and intensity (total minutes at light, moderate, and vigorous intensity, total counts, and counts/minute), time spent in sedentary behavior, and how each of these behaviors are accumulated (i.e., bouts of time spent in sedentary behavior or physical activity). Furthermore, sleep quality will be sassed through sleep latency (minutes), sleep efficiency (%), total time in bed (minutes), total time sleeping (minutes), wake before sleep onset (minutes), number of awakenings, and average awakening length (minutes).

Blood samples Fasting blood samples will be collected at baseline and at the end of each of the 2-week exercise intervention blocks by APDP-certified staff. Blood collection will be performed in a seated position from the antecubital vein at rest after an overnight fast into dry tubes and into tubes containing ethylenediaminetetraacetic acid, an anticoagulant. Biological samples will be centrifuged at 500 g at 4 °C for 15 min, and plasma samples will be frozen at − 80 °C for posterior analysis. Blood samples will be delivered on ice to the APDP for glucose and insulin analysis. All samples will be used once for analysis and disposed of after following the APDP clinical protocols.

From serum blood samples, glucose will be analyzed using colored enzymatic tests in an automated analyzer (auto analyzer, Olympus AU640, Beckman Coulter). Serum insulin will be analyzed using an electrochemiluminescence immunoassay (Liaison, Diasorin).

Physical fitness The CPET will be used to assess each participant's maximal aerobic capacity, PPO, and to check for pre-existing heart conditions. To determine maximal aerobic capacity and PPO, a ramp incremental protocol to exhaustion will be performed on a cycle

ergometer (Monark 839E, Kroons Vag, Sweden). Participants will start the assessment at 20 W/min, and then the workloads will increase in increments of 5–20 W/min (according to personal cardiopulmonary response to exercise during the first minute), while on a constant pedal frequency of 60 rotations/min. All the tests will be monitored using a 12-lead electrocardiogram PC-based acquisition module, and all data, including heart rate, will be monitored and recorded using Omnia software (Cosmed, Rome, Italy). During this test, expired and inspired gasses are continuously analyzed, breath-by-breath, through a portable gas analyzer (Quark RMR w/ CPET, version 9.1, Cosmed, Rome, Italy). VO2peak will be defined as the highest 20-s value for peak oxygen consumption (ml/kg/min) attained in the last minute of the exercise test, provided that participants attained volitional fatigue and met at least one of the following objective test criteria: (1) respiratory exchange ratio reached 1.1 or higher; (2) participants reached predicted maximal heart rate; and (3) oxygen uptake did not increase in spite of increasing workload. PPO will be defined as the last ramp stage achieved by the participants during the incremental output.

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

At the conclusion of each block of the intervention period, assessments will be carried out. To increase retention at all assessments, we will:

- Schedule all future assessments and training sessions in advance on a calendar hand-delivered to all the participants
- Text participants on the previous day leading up to the next day's assessment/training session to remind them about the time, day and any important requirements

The importance, specificity, and depth of the data obtained through these assessments will be emphasized from the first moment of the research, for instance in the dissemination contacts and trial explanation. Additionally, in this first session, we will emphasize the value of trial participation.

Data management {19}

Data entry, processing, and management will be done by two researchers supervised by the PI as follows:

(1) We will compile all measurements received during data collection into a database. To ensure each participant's privacy, an identity code will be generated for them.
(2) All data collected through surveys will be exported and preserved in a data set.
(3) To collect and securely store anonymized data from all project domains, a new database will be created.
(4) Documentation checks, keeping an eye out for potential punching mistakes, and systematic controls will be used to ensure data quality.

Confidentiality {27}

All data collected from the participants will be kept strictly confidential. The data will be computerized and encrypted in a database and will not contain any identifying elements of the participants. All participants will have a unique ID number and their name will not be used, apart from the consent form. A second database containing the decryption key, that only the principal investigator of the study will have access to, will retain full identification and personal data of the participants. Both databases will be maintained by those responsible for the investigation, on a secure server at FMH for 10 years and used for research purposes only. The second database containing personal information of the participants will be stored for 5 years after the end of the study, after which it will be destroyed. During and after the completion of the study, all biological samples collected throughout the research, as well as all original/copied documents filled out by the assessors and participants, will be archived at the Exercise and Health Laboratory of FMH. All archived documents and samples will be anonymized to ensure data confidentiality.

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

Fasting blood samples will be collected at baseline and at the end of each of the 2-week exercise intervention blocks by APDP-certified staff. Blood collection will be performed in a seated position from the antecubital vein at rest after an overnight fast into dry tubes and into tubes containing ethylenediaminetetraacetic acid, an anticoagulant. Biological samples will be centrifuged at 500 g at 4 °C for 15 min and plasma samples will be frozen at − 80 °C for posterior analysis. From serum blood samples, glucose will be analyzed using colored enzymatic tests in an automated analyzer (auto analyzer Olympus AU640, Beckman Coulter). Serum insulin will be analyzed using an electrochemiluminescence immunoassay (Liaison, Diasorin). Blood samples will be used once for analysis and disposed after following the APDP clinical protocols.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

Statistical analysis will be performed using STATA version 13.1 (StataCorp LLC, College Station, TX, USA) and the Statistical Package for the Social Sciences (SPSS) 24.0 (IBM Corp., Armonk, New York). Significance will be set at $p \leq 0.05$.

For both groups (i.e., individuals with T2DM and age-matched individuals with overweight or obesity), normality plots and Kolmogorov–Smirnov tests will be used to test the normality of outcome variables. For normally distributed outcomes, mean and standard deviation will be used as measurements of central tendency and variability, whereas median and interquartile range will be used for non-normally distributed data. Baseline differences between study groups will be assessed using ANOVA and non-parametric tests (e.g., Kruskal–Wallis) for any non-normally distributed and categorical variables.

For assessing between-group differences (T2DM vs. overweight/obesity) in the primary outcome (24-h AUC provided by the CGM in the morning, afternoon, and evening), a 3×2-way ANCOVA will be used, controlling for baseline AUC, changes in physical activity/sedentary behavior, changes in weight, sex, and age. To assess differences in AUC between the morning, afternoon, and evening as well as to assess if there is a time effect, separately for both the individuals with T2DM and the age-matched overweight/obese group, generalized estimating equations will be used, while controlling for physical activity/sedentary behavior, weight, baseline AUC, sex, and age. All these analyses will be performed using an intention-to-treat (i.e., inclusion of all participants that were initially randomized) and per-protocol approach (i.e., inclusion of only participants who completed the study and had at least 80% adherence to the total number of trainings). Statistical significance will be set at $p < 0.05$.

To quantify responder variability to exercise at different times of the day, we will first calculate the technical error of the 24-h AUC measurement provided by the CGM for each participant. This value will be calculated by taking the difference between the repeated baseline measures at each exercise block. The observed changes in AUC for each participant will be adjusted by the technical error for that participant. Individuals will be classified as having a true response to exercise at the given time of day if their adjusted change score is beyond the calculated minimal clinically important difference (i.e., effect size of 0.2×baseline between-subject SD). This technique for establishing responder thresholds has previously been suggested and used by others as a viable approach for assessing individual response variability [21].

Interim analyses {21b}

The research team did not feel that it will be necessary to conduct interim analysis and stopping guidelines.

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

Not applicable. The sample size in this trial will not allow subgroup analysis.

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

Other than the intention-to-treat (i.e., inclusion of all participants that were initially randomized) and per-protocol approach (i.e., inclusion of only participants who completed the study and had at least 80% adherence to the total number of trainings), we will not use any other statistical methods to handle missing data.

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

Public access to the full protocol is granted by the registration in a public online platform https://clinicaltrials.gov/study/NCT06136013.

Oversight and monitoring

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

At the start of the investigation, a steering committee will be established to oversee the trial. This committee will be conducted by the principal investigator (PI) in a small group to provide efficient oversight of the trial's operations. Every week, the steering committee will meet with the research team to discuss the study's progress and to recommend counter measures when deviations from the predetermined trial plan take place.

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

A data monitoring committee will not be needed for the Ex-Timing trial, since the intervention has minimal risk of harm. Participants' safety and intervention progress will be constantly monitored by the research team and any required adjustments to the protocol will be discussed.

Adverse event reporting and harms {22}

Although adverse events and risks with this trial are low and not expected, participants will be instructed to communicate with the research team any discomfort or any unexpected adverse reaction or event during the intervention periods. All the information about adverse events reported will be recorded by the research team and will include information about the frequency, severity, date

of occurrence, duration, and action taken, as well as the effect that the adverse event had on the intervention and on the participant outcome.

Frequency and plans for auditing trial conduct {23}

The research team will frequently meet once a week to go over participant and intervention progress, new problems, and needed protocol modifications. These sessions will also be used to keep an eye on participant safety, outcome data, and adverse event data.

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

We intend to initially inform the sponsor and funder of any necessary protocol adjustments. After that, the principal investigator will transmit a copy to the research center's FCT project management database along with a notification to the ethical commission. In this manner, we can retain transparency and responsibility throughout the research study.

To ensure that all parties involved have access to correct and current information regarding the study, we will lastly update the protocol in the clinical trial registry.

Dissemination plans {31a}

For this project, we expected to publish at least three scientific papers in international peer-review journals within the first quartile and having significant impact factors: (1) research protocol and rationale publication; (2) results of our primary aims on CGM with different exercise times, separately for individuals with T2DM and those with overweight/obesity; (3) inter-individual response to standardized exercise performed at different times of the day in individuals with T2DM and overweight/obese older adults.

Furthermore, the findings from this project will be presented at national and international scientific meetings on exercise and diabetes. Dissemination to the stakeholders and the general public will be accomplished through seminars, social media posts, and summary reports that will be developed and presented by the FMH-UL, APDP, Karolinska Institute research groups, as well as short reports in lay language summarizing main findings and derived recommendations for our target populations and related health practitioners.

Discussion

This paper describes the protocol of the Ex-Timing project, a study that aims to test the effect of exercise timing on the inter-individual response variability in glycemic control and other related metabolic health parameters in two distinct populations: individuals with T2DM and age-matched sedentary overweight/obese individuals.

The metabolic benefits of exercise in individuals with T2DM are well-known [2–5]. Nevertheless, there are many sources of inter-individual variability that preclude not all individuals from achieving the benefits of exercise on glycemic control. The major source of inter-individual variability in the glycemic response to exercise known and studied is exercise dose, although other sources exist, which may be significantly relevant to consider when prescribing exercise [6]. One such understudied source is exercise timing, which may be particularly relevant when it comes to achieving improvements in glycemic outcomes in people with T2DM, given the influence of circadian rhythms on insulin sensitivity [10–12].

The existing literature on exercise timing has been derived from animal models or from a mixed population with more than one type of medication, which can lead to conflicting results and increased inter-individual variability [5, 13–17, 22]. For instance, Savikj et al. observed in a randomized crossover trial that afternoon HIIT was more effective than morning HIIT at improving blood glucose in males with T2DM, whereas morning HIIT had an acute, adverse effect, raising blood sugar [5]. Our study [22] and Mancilla et al. [15] corroborate these findings. On the other hand, Teo et al. found no significant differences in glycemic effects between morning and evening exercise, including postprandial glycemic response [17]. However, Ezagouri et al., in an animal model, report that exercise modifies the expression of some metabolic regulators and pathways, including insulin and glucose metabolism, in a time-dependent manner, with more dramatic modifications in the early group (ZT14) than in the late group (ZT22) [14].

The Ex-Timing project will provide increased insight and knowledge into the role of exercise timing on glycemic control and related metabolic outcomes by accounting for many of the shortcomings of the previous investigations through our use of a randomized crossover trial, where each individual will serve as their own control, with two groups: (1) individuals with T2DM on the most common mono-hyperglycemic drug therapy (i.e., metformin) [3] and (2) age-matched sedentary overweight/obese individuals, where glycemic control is known to deteriorate [16].

We may have some issues retaining the participants throughout the study since low compliance is one of the major risks in this investigation. To increase exercise compliance, we intend to establish a community outreach program in order to increase motivation and provide the conditions for the participants to continue exercising after the cessation of the intervention.

We anticipate that Ex-Timing will be a unique and an important approach to assess how the endogenous metabolic rhythms set forth by the cellular clock machinery may interact with exercise to potentially improve its effectiveness in individuals with T2DM and those at risk. Alongside established factors such as exercise dose, exercise timing may also be a relevant variable to consider within the scope of exercise prescription for individuals with T2DM. The findings from this study will contribute to our understanding of the inter-individual response to exercise and help in development of more individually tailored exercise prescription guidelines for individuals with T2DM and individuals with overweight/obesity.

Trial status

The current protocol version is dated 03/10/2023. The recruitment began on the 1st of September 2023. On the 14th of September, the pilot study kicked off and is currently ongoing. Due to some delay in the revision of the final draft of the manuscript, we were not able to submit the manuscript prior to the beginning of the pilot study.

Abbreviations

T2DM	Type 2 diabetes mellitus
HIIT	High-intensity interval training
AUC	Area under the curve
APDP	Associação Protectora dos Diabéticos de Portugal
PPO	Peak power output
VO2peak	Peak oxygen uptake
CGM	Continuous glucose monitoring
BMI	Body mass index
CPET	Cardiopulmonary exercise test
SD	Standard deviation
DXA	Dual-energy X-ray absorptiometry
USDA	United States Department of Agriculture
USA	United States of America
EHIS-PAQ	Domain-specific physical activity questionnaire
WHOQOL Questionnaire	World Health Organization Quality of Life Questionnaire
PSQI Questionnaire	Pittsburgh Sleep Quality Index Questionnaire
FRAQ	Falls Risk Awareness Questionnaire
CPF Scale	Composite Physical Function Scale
IPAQ	International Physical Activity Questionnaire
GPAQ	Global physical activity questionnaire
SBQ	Sedentary Behaviour Questionnaire

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13063-024-08335-y.

Additional file 1: SPIRIT checklist for *Trials*.

Acknowledgements
Not applicable.

Authors' contributions {31b}
LBS, JPM, JR, and CC contributed to the conception and design of the study. JPM, ECO, FJ, MCF, and RR were responsible for data acquisition, analysis, and interpretation. ECO and MHR contributed to the discussion and reviewed/edited the manuscript. JPM and MHR reviewed/edited the manuscript. LBS, JPM, JR, and CC researched data and revised it critically for important intellectual content. ECO and MHR drafted the manuscript. All authors gave approval of the final version and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved. All authors read and approved the final manuscript.

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Availability of data and materials {29}
Anonymized trial data will be available for non-commercial research purposes only upon request to the PI.

Declarations

Ethics approval and consent to participate {24}
The study has been approved by the ethics committee of the Associação Protectora dos Diabéticos de Portugal, with the approval number 014/2023, and it will be conducted in accordance with the Declaration of Helsinki for Human Studies. Written, informed consent to participate will be obtained from all participants.

Consent for publication {32}
Each participant will get a thorough oral and written explanation of the study prior to beginning the experiment. Before obtaining the signed informed consent, an opportunity to ask questions will be provided. We will be willing to provide a model consent form on request.

Competing interests {28}
The authors reported no conflicts of interest.

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