

**Chrononutrition-based hypocaloric diets with varying macronutrient and protein distribution in shift workers with prediabetes or type 2 diabetes: Protocol for a randomized controlled trial**

Carmen Rodrigo-Carbó<sup>1,2</sup>, Mikel Tous-Espelosin<sup>3,4</sup>, Irene Gracia-Rubio<sup>1,6\*</sup>, Sofía Pérez-Calahorra<sup>5\*\*</sup>, Ángela Lezcano-Enciso<sup>1</sup>, Rebeca Monjón-González<sup>1</sup>, Isabel Lamiquiz<sup>7</sup>, Itziar Lamiquiz-Moneo<sup>1,6†</sup>, Rocío Mateo-Gallego<sup>1,2†</sup>

<sup>1</sup>Miguel Servet University Hospital, Aragon Health Research Institute, CIBERCV. Unidad de Investigación Traslacional. Hospital U. Miguel Servet (Planta 0). P.º de Isabel la Católica, 1-3, 50009, Zaragoza, Spain.

<sup>2</sup>Department of Physiatry and Nursing, Faculty of Health and Sport Science, University of Zaragoza. Pl. Universidad, 3, 22002, Huesca, Spain.

<sup>3</sup>GIZartea, Kirola eta Ariketa Fisikoa Ikerkuntza Taldea (GIKAFIT), Society, Sports, and Exercise Research Group, Department of Physical Education and Sport, Faculty of Education and Sport-Physical Activity and Sport Sciences Section, University of the Basque Country (EHU), Vitoria-Gasteiz, Álava.



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<sup>4</sup>Bioaraba Health Research Institute, Physical Activity, Exercise, and Health Group, Vitoria-Gasteiz, Basque Country, Spain.

<sup>5</sup>Department of Physiatry and Nursing, Faculty of Health Science, University of Zaragoza. C. Domingo Miral, s/n, 50009, Zaragoza, Spain.

<sup>6</sup>Department of Human Anatomy and Histology, University of Zaragoza. C. Domingo Miral, s/n, 50009, Zaragoza, Spain.

<sup>7</sup>Department of Psychology and Sociology, Faculty of Social and Labor Sciences, University of Zaragoza, Spain

**\*Corresponding author:** Irene Gracia-Rubio; Department of Human Anatomy and Histology, University of Zaragoza. C. Domingo Miral, s/n, 50009, Zaragoza, Spain; igraciarubio@unizar.es; +34 976761667

**\*\*Co-corresponding author:** Sofía Pérez-Calahorra; Department of Physiatry and Nursing, Faculty of Health Science, University of Zaragoza. C. Domingo Miral, s/n, 50009, Zaragoza, Spain. spperezc@unizar.es; +34 876554458.

<sup>¶</sup>Both authors equally contributed as senior authors.

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**Abstract**

Shift work is associated with an increased risk of metabolic disorders, including type 2 diabetes, largely due to circadian misalignment, irregular meal patterns, and suboptimal diet quality. Chrononutrition, which focuses on aligning nutrient intake with circadian rhythms, has emerged as a promising strategy to improve metabolic health. Protein intake plays a key role in glucose homeostasis, and high-protein hypocaloric diets have shown benefits in people with type 2 diabetes. However, the effects of higher evening protein intake in shift workers remain unclear. This trial aims to analyze the effect of three hypocaloric diets differing in macronutrient composition and distribution throughout the day on glycemic control, body composition, and other secondary outcomes in shift workers with overweight or obesity and prediabetes or type 2 diabetes. This is a 12-week, three-arm, parallel-group, single-blind randomized controlled trial including 126 shift workers. Participants are randomized equally to: (A) a high-protein diet with protein-enriched dinner (50–60% of daily protein); (B) a high-protein diet with protein-restricted dinner (10–20% of daily protein); or (C) a normoproteic control diet with usual protein distribution. Primary outcomes include changes in glycemic control and DXA-derived body composition. Secondary outcomes are lipid profile, sleep quality, and quality of life. Other clinical and lifestyle parameters are evaluated to monitor changes throughout the intervention. Assessments are performed at baseline, week 6, and week 12. All analyses will follow the intention-to-treat principle. This study will provide new evidence on how evening protein intake may influence metabolic health in populations exposed to circadian disruption.

**Key words:** Chrononutrition; Shift work; Hypocaloric diet, High-protein diet, Type 2 Diabetes

## Abbreviations

ADA – American Diabetes Association

AAMI – Association for the Advancement of Medical Instrumentation

AUC – Area Under the Curve

BMI – Body Mass Index

CEICA – Comité de Ética de la Investigación de la Comunidad Autónoma de Aragón

CKD-EPI – Chronic Kidney Disease Epidemiology Collaboration

CRP – C-reactive protein

CV – Cardiovascular

DBP – Diastolic Blood Pressure (por la metodología de presión arterial)

DXA – Dual-energy X-ray absorptiometry

ELISA – Enzyme-Linked Immunosorbent Assay

ESH – European Society of Hypertension

GLP-1 – Glucagon-like peptide-1

GIP – Glucose-dependent insulintropic polypeptide

HbA1c – Glycated hemoglobin A1c

HDL-C – High-density lipoprotein cholesterol

HOMA-IR – Homeostasis Model Assessment of Insulin Resistance

HP – High-protein

ISO – International Organization for Standardization

LDL-C – Low-density lipoprotein cholesterol

NP – Normoproteic

PSQI – Pittsburgh Sleep Quality Index

SBP – Systolic Blood Pressure

SF-12 – 12-Item Short-Form Health Survey

SPIRIT – Standard Protocol Items: Recommendations for Interventional Trials

T2D – Type 2 Diabetes

VLCD – Very Low-Calorie Diet

## INTRODUCTION

Shift work, increasingly common in industrialized societies, poses significant challenges to human health (1). It is defined as a labor practice that includes a wide range of schedules such as evening or night shifts, irregular or rotating shifts, weekend work, or on-call duties (2). According to the Sixth European Working Conditions Survey, approximately 21% of workers engage in permanent or rotating shift work, and 19% perform shifts that include night work (3). This irregularity often results in circadian misalignment, which contributes to metabolic, cardiovascular, and psychological disorders, with type 2 diabetes (T2D) being among the most frequently reported metabolic disorders associated with circadian misalignment (4–6).

Disruption of the circadian system, commonly referred to as chronodisruption, has been identified as a key mediator of the health effects associated with shift work. Irregular light exposure, sleep deprivation, and misalignment between endogenous biological rhythms and behavioral cycles (e.g., feeding and activity) contribute significantly to metabolic dysregulation in shift workers, especially in those who work night shifts (7–9). Furthermore, shift workers often exhibit poorer dietary habits, including increased intake of energy-dense snacks, irregular meal timing, and reduced consumption of fresh, balanced meals. These patterns are driven by fatigue, lack of structured mealtimes, stress, and limited access to healthy food during night shifts. Social isolation during meals and skipping family meals may further compromise diet quality (1,10). Altogether, these lifestyle disruptions exacerbate the physiological consequences of circadian misalignment, including impaired glucose metabolism, increased insulin resistance, and elevated risk of weight gain and T2D (11,12). These findings highlight the urgent need for dietary strategies tailored to shift-working populations that may reduce the adverse health impacts of circadian misalignment by addressing irregular meal timing and inappropriate or mis-timed distribution of nutrients.

Among dietary strategies targeting metabolic improvement in this population, hypocaloric diets have been shown to promote weight loss and improve glycemic outcomes in adults with overweight/obesity and type 2 diabetes (13,14). However, intervention evidence specifically in shift workers remains limited, although recent trials suggest beneficial effects on body composition and metabolic parameters (15). High-protein diets, particularly within energy-restricted interventions, have been associated with improvements in glycemic control and other cardiometabolic parameters in individuals with T2D (16,17). These findings are consistent with the evidence-based recommendations of the European Association for the

Study of Diabetes, which indicate that higher protein intakes may be recommended in the short term within energy-restricted weight-loss diets in individuals with T2D and overweight/obesity, provided that renal function is preserved (18).

The mechanisms underlying the metabolic benefits of high-protein diets are not yet fully elucidated, but they may involve the insulinotropic effect of amino acids, better maintenance of muscle mass after weight loss, and enhanced satiety, among others (19). Essential amino acids (especially branched chain amino acids) stimulate mammalian target of rapamycin complex, which is a key regulator of muscle growth and protein synthesis. Besides, proteins stimulate incretin secretion (GLP-1 and GIP), which are essential in glucose homeostasis by enhancing insulin secretion and suppressing glucagon release (20,21). In shift workers, evidence from one controlled trial indicates that high-protein diets may reduce glucose and insulin levels and improve psychometric performance, while high-carbohydrate diets have been associated with increased fatigue and suboptimal glycemic control (22).

Recent evidence suggests that adding protein to carbohydrate-containing meals may modulate postprandial glucose responses (23,24). Diurnal variation in glucose homeostasis has been well documented; oral glucose tolerance and insulin sensitivity are higher in the morning, so carbohydrate consumption early in the day is likely to elicit a more favorable postprandial glycaemic response. Thus, within the broader framework of chrononutrition, the alignment of food intake with circadian physiology may be relevant for metabolic regulation (25,26). Shift workers often exhibit higher postprandial glucose levels during night shifts (27), and some evidence suggests a high-protein meal consumed at evening/night time may lead to more favorable postprandial responses than a standard meal (24). Accordingly, allocating a greater proportion of protein to the evening/night-time period may be a relevant strategy to explore in shift workers. Despite these insights, previous studies on shift workers are limited by small sample sizes, short intervention periods, and a lack of focus on individuals with T2D. Moreover, the potential role of the distribution of protein intake across the day, particularly the proportion consumed during the evening/night-time meal window, remains insufficiently studied in this population. Therefore, further research is needed to explore whether different patterns of protein distribution within a hypocaloric diet may influence glycemic control and body composition in this population. We hypothesize that within hypocaloric high-protein diets, allocating a greater proportion of daily protein intake to the evening/night-time period may lead to greater improvements in glycaemic control and body composition than allocating

a smaller proportion of protein to that period in shift workers with impaired glucose metabolism and overweight or obesity.

This study protocol describes a three-arm, randomized controlled trial in shift workers with overweight or obesity and prediabetes or T2D, comparing the effects of three distinct hypocaloric diets with varying macronutrient composition and protein distribution across the day on metabolic outcomes. The findings may contribute to the development of more tailored nutritional strategies for shift-working populations.

Therefore, this protocol describes a three-arm, randomized controlled trial designed to evaluate the effects of three distinct hypocaloric diets—differing in macronutrient composition and distribution of protein intake across the day—on metabolic outcomes in shift workers with prediabetes or T2D and overweight or obesity. The **primary objective** is to assess changes in glycaemic parameters -mainly HbA1c concentration- and body composition. **Secondary objectives** include evaluating the impact of the interventions on lipid profile, adipokine concentration, sleep quality, and health-related quality of life. The study will also assess chronotype, physical activity, sedentary behavior, and dietary intake as additional variables relevant to the interpretation of the study results. All outcomes will be measured over a 12-week intervention period.

## MATERIALS AND METHODS

### Study design

This study is a 12-week, three-arm, parallel-group, single-blind randomized controlled trial conducted in adult shift workers with prediabetes or T2D and overweight or obesity. The study started in September 2024 and is expected to be completed in December 2025. Eligible participants must be engaged in shift work that includes, on average, at least one night shift per month, either as part of a rotating schedule or as fixed night work. Participants are individually randomized in a 1:1:1 ratio to one of three dietary interventions: (A) high-protein with protein-enriched dinner (50-60% of daily protein); (B) high-protein with protein-restricted dinner (10-20% of daily protein); or (C) normoproteic control diet with usual protein distribution. The study comprises four visits: a prescreening visit, a baseline visit, and follow-up assessments at 6 and 12 weeks post-randomization, during which all study data are collected. A visual summary of the study design is provided in **Figure 1**.

The randomization sequence is generated using R statistical software by an investigator not involved in participant recruitment or outcome assessment, ensuring allocation concealment. To minimize assessment bias, investigators involved in DXA measurements and laboratory analyses will be different from those delivering the dietary intervention and will remain unaware of participants' group allocation. Randomization is stratified by sex and by type of shift pattern, categorized as either high-frequency rotating shifts within the same week or fixed night shifts and weekly rotational blocks.

The intervention period lasts 12 weeks. Participants attend three in-person visits: one at baseline (week 0), one at week 6 (midpoint follow-up), and one at week 12 (end of intervention). In addition, a pre-screening visit is conducted prior to enrollment to verify eligibility and obtain informed consent. Participants also have access to the study dietitian throughout the trial via phone, WhatsApp, or email to resolve questions and reinforce dietary guidance.

The study is conducted at the Clinical Research Unit of the Institute for Health Research Aragón, located within the Miguel Servet University Hospital in Zaragoza (Spain). All assessments are standardized and performed under controlled conditions. The trial is registered at ClinicalTrials.gov (identifier: NCT06912555) and has been approved by the institutional ethics committee (Research Ethics Committee of the Autonomous Community of Aragon; PI23-558). Written informed consent will be obtained from all participants prior to enrollment. In addition, at the first visit—together with the study explanation and the participant information sheet and prior to signing the informed consent—participants are clearly informed that they could withdraw from the study at any time, whether for personal or medical reasons, because the dietary intervention is not suitable for them, or for any other reason they consider appropriate. All withdrawals and their reasons are recorded throughout the study. The study protocol has been developed in accordance with the principles of the Declaration of Helsinki and adheres to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines (28).

### **Study population**

Recruitment will be carried out through a coordinated, multi-step process designed to ensure transparency and voluntary participation. Participant recruitment will be achieved by publicising the study through local media and displaying information posters in healthcare facilities and large companies with substantial shift-working staff. The study announcement

will include a dedicated telephone number and email address through which interested individuals may contact the research team. Upon initial contact, potential participants will receive detailed information regarding the study's objectives, procedures, and eligibility criteria. Those expressing interest and meeting the basic requirements will be invited to a pre-screening visit at the Clinical Research Unit to confirm eligibility.

Inclusion criteria include: a) women and men aged  $\geq 18$  years; b) body mass index (BMI) between 27.5 and 40 kg/m<sup>2</sup>; c) diagnosis of prediabetes (fasting glucose 100–125 mg/dL and/or HbA1c 5.7–6.4%) or T2D (fasting glucose  $\geq 126$  mg/dL and/or HbA1c  $\geq 6.5\%$  and/or currently taking metformin), according to the American Diabetes Association criteria (29); d) current employment in shift work that includes, on average, at least one night shift per month, either as part of a rotating schedule or as fixed night work, and expected to continue throughout the study period; e) provision of written informed consent after having read the participant information sheet and clarified any doubts. Participants who meet any of the following criteria will be excluded: a) treatment with any antidiabetic agents (oral or injectable), including insulin, within the past two months, except for metformin or dipeptidyl peptidase-4 inhibitors, which must have been taken at a stable dose for at least six months prior to enrollment; b) use of lipid-lowering or glucose-modifying medications or functional foods (e.g., phytosterols, red yeast rice) unless taken in a stable regimen for a minimum of six months prior to enrollment and expected to remain unchanged throughout the study; c) presence of uncontrolled chronic or endocrine diseases (e.g., cardiovascular, renal, hepatic, or thyroid disorders) that could interfere with study outcomes, in the judgment of the investigators; d) body weight variation of  $\geq 5\%$  during the three months preceding enrollment; e) unstable use of vitamin or nutritional supplements within the six months prior to the study; f) regular alcohol consumption exceeding 30 g of ethanol per day; g) pregnancy or intention to become pregnant during the study period; h) any other circumstance that, in the opinion of the investigators, may compromise adherence to the nutritional intervention (e.g., frequent travel, inability to attend scheduled visits) or interfere with study results (e.g. any serious medical condition).

### **Randomization and blinding**

Participants will be randomly assigned in a 1:1:1 ratio to one of the three dietary intervention arms using computer-generated block randomization. Stratification will be performed based on sex (male/female) and type of shift pattern (high-frequency rotating shifts within the same

week vs. weekly rotating or fixed night shifts), in order to ensure balanced allocation across key variables. The randomization sequence will be generated using the `block_ra()` function from the `randomizr` package in R, with a fixed seed to ensure reproducibility.

The study follows a single-blind design. Participants will be unaware of the specific dietary group to which they are assigned, including the caloric prescription. Although investigators will withhold information on energy content, protein amount, and specific diet allocation, complete blinding cannot be ensured because participants may infer their assignment from the menus provided. Investigators responsible for statistical analysis and outcome assessment will remain blinded to group allocation throughout the trial. The dietitians responsible for delivering the nutritional intervention will not be blinded, due to the practical and methodological requirements of dietary counseling and adherence monitoring. Group allocation will be concealed at the time of enrollment. Randomization will be managed by a researcher not involved in recruitment, data collection, or outcome evaluation, and assignment will be recorded in a masked database to maintain allocation concealment.

### **Dietary intervention**

All participants receive an individually tailored hypocaloric diet designed to induce a moderate caloric restriction of approximately 30% of their estimated total energy expenditure, equivalent to a reduction of 400–600 kcal/day. Total energy expenditure is calculated individually for each participant based on validated predictive equations and clinical assessment. Caloric intake is never prescribed below 1,200 kcal/day, in order to ensure that all nutritional requirements are met.

Participants are randomized to one of three dietary intervention arms, which differ in both total protein intake and the proportion of protein allocated to the evening/night-time period:

- **HP Diet A (High-protein, protein-dominant dinner):** A hypocaloric diet providing 1.3–1.5 g of protein per kilogram of body weight per day, with approximately 60% of the total daily protein allocated to the evening meal (consumed between 8:00 PM and 3:00 AM).
- **HP Diet B (High-protein, protein-restricted dinner):** A hypocaloric diet also providing 1.3–1.5 g protein/kg/day, but with only ~15% of total daily protein intake assigned to the evening meal (8:00 PM to 3:00 AM).

- **NP Diet C (Normoproteic control diet):** A hypocaloric diet providing 0.8 g of protein per kilogram of body weight per day, with protein intake distributed according to the participant's usual habits and preferences, aiming for a relatively homogeneous intake across the day.

A predefined evening/night-time window (8:00 PM to 3:00 AM) was established to standardize the intervention across participants with heterogeneous shift schedules and eating habits and to capture the main intake occurring during the latter part of the day/night. The trial was designed to compare protein allocation within this standardized evening/night-time window rather than the effects of specific clock times within that interval. The exact timing of intake will be recorded, although the study was not designed to compare narrower time points.

Macronutrient distribution remains consistent across groups (30% of fat and the rest of calories coming from carbohydrates), aside from the variations in protein content and evening/night-time protein allocation. Each participant receives a detailed dietary prescription that includes structured meal plans specifying recommended food groups and quantities, sample menus with specific dishes to facilitate adherence, and individualized nutritional counseling. A sample daily menu for each diet is provided in **S1 Appendix**.

Dietary adherence will be monitored using both dietary records and an objective biomarker. Participants will complete food records that include the timing of each eating occasion, allowing assessment of whether protein intake is consumed within the prescribed evening/night-time meal window and in the assigned proportion. In addition, urinary urea will be used as an objective marker of protein intake to evaluate whether participants are achieving the prescribed protein targets. Together, these measures will be used to assess adherence to both the amount and distribution of protein intake during the intervention.

## **Outcomes**

The primary outcomes of this trial are glycaemic parameters -mainly HbA1c concentration- and body composition change after 12 weeks of intervention. HbA1c is a well-established marker of long-term glycaemic control and a reliable indicator of the risk of chronic diabetes-related complications; accordingly, it constitutes the principal therapeutic target in individuals with prediabetes and T2D. Additional glycaemic outcomes include fasting plasma glucose, fasting insulin, and the homeostasis model assessment of insulin resistance (HOMA-

IR). Body composition outcomes include total body fat, fat-free mass, and visceral adipose tissue variation after 12 weeks post-randomization.

Secondary outcomes comprise lipid profile (total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and non-HDL cholesterol), adipokines concentration (asprosin, visfatin, and apelin), sleep quality, and health-related quality of life. Other clinical, biochemical and lifestyle parameters will be recorded to monitor health changes throughout the intervention in order to identify potential confounders. Clinical variables include blood pressure and chronotype; biochemical variables involve hepatic, renal, or thyroid function assessment, among others; and lifestyle parameters include dietary characteristics and objectively measured physical activity and sedentary behavior.

## **Data collection and assessment procedures**

### *Study visits*

As outlined in **Figure 1** participants will attend a pre-screening visit at which eligibility criteria will be verified and written informed consent obtained. If no laboratory analysis has been performed within the preceding two months, blood will be drawn and the relevant clinical and demographic variables recorded. During the randomization visit, participants will also take part in a structured dietary interview with the study dietitian to assess individual food preferences and eating habits. This information will be used to tailor the dietary plan to improve adherence while maintaining the assigned macronutrient targets; the dietary plan will be delivered within 2–3 days of the randomization visit. Clinical, anthropometric, biochemical, and lifestyle data will be collected at baseline and at the end of the 12-week intervention. A mid-intervention visit at week 6 will include all assessments except for biochemical parameters and dual-energy X-ray absorptiometry (DXA). In addition, biweekly email or phone contact will be maintained to reinforce adherence, promote follow-up, and minimize dropout.

### *Clinical data*

Demographic and medical history data, including age, sex, smoking status, medication and supplement use, and blood pressure, will be collected at all study visits. Blood pressure will be measured according to standardized procedures with an automated sphygmomanometer (Omron HEM-907-E, OMRON Healthcare Europe B.V., Hoofddorp, Netherlands), following

international standard procedures (30). Participants will be sitting quietly for  $\geq 5$  min in a temperature-controlled, silent room with back and arm supported, feet flat on the floor, legs uncrossed, and the mid-cuff of the measured arm aligned with the heart. They will be asked to refrain from talking, moving, or using electronic devices during the procedure and to abstain from caffeine, smoking, and vigorous exercise for  $\geq 60$  min beforehand. Researchers will perform two different measurements obtained 1 min apart. If systolic or diastolic values differ by  $\geq 5$  mmHg, a third reading will be taken and the mean of the two closest values recorded for analysis (31).

#### *Anthropometric and body composition data*

Body composition will be measured via DXA (software version 8.1; Lunar Prodigy, General Electrics, Chicago, IL, USA) in accordance with manufacturer instructions and international guidelines. Participants will be asked to wear loose, lightweight, and non-metallic clothing and remove all jewellery or metal objects. During the scan, they will lie supine with arms extended alongside the torso, palms facing downward and separated by 5–10 cm, legs slightly abducted, and ankles secured with Velcro straps. If the patient's body size exceeds the scan field, a validated hemi-body protocol will be applied (32,33). The DXA output will include total body weight, total and regional fat mass and fat-free mass, abdominal subcutaneous and visceral fat. Height will be measured to the nearest 0.1 cm using a calibrated wall-mounted stadiometer (Seca 813 and Seca 217, Seca Deutschland, Hamburg, Deutschland). BMI will be calculated by dividing body weight in kilograms by the square of height in meters ( $\text{kg}\cdot\text{m}^{-2}$ ). Waist circumference will be measured with anthropometric tape midway between the lowest rib and the iliac crest (Seca 201, Seca Deutschland, Hamburg, Deutschland).

#### *Biochemical analyses*

Blood and urine samples will be obtained after an overnight fast ( $\geq 8$  hours) at baseline and at the end of the 12-week intervention. Analyses will be performed through the routine clinical laboratory services of the Miguel Servet University Hospital. Glucose concentrations will be quantified with the hexokinase method on a Beckman Coulter AU analyser (Beckman Coulter, CA, USA), while HbA1c will be measured by high-performance liquid chromatography (Tosoh, Tokyo, Japan). Insulin resistance will be estimated with the HOMA-IR index, calculated as fasting glucose (mg/dL)  $\times$  insulin ( $\mu\text{U}/\text{mL}$ ) / 405. Total cholesterol, triglycerides, and HDL-C will be assessed by enzymatic colorimetry on the same Beckman platform; LDL-C will be derived with the Friedewald equation if triglycerides are  $<400$

mg/dL. Apolipoproteins A1 and B will be determined by kinetic nephelometry on the Beckman Coulter AU, and high-sensitivity C-reactive protein by immunoturbidimetry. Iron, uric acid, and albumin will be analysed colorimetrically, whereas vitamin B12 will be assayed by luminescence immunoassay. Serum and urinary creatinine will be obtained with the Jaffe's reaction, and liver transaminases, together with urinary urea, will be quantified kinetically. Microalbuminuria will be measured by turbidimetry, while glomerular filtration rate will be estimated with the CKD-EPI equation (34).

Moreover, two additional blood tubes will be collected at each time point to obtain anonymized serum aliquots, which will be stored at  $-80^{\circ}\text{C}$  for batch analysis at study completion. The concentrations of adipokines will be determined in these samples using an enzyme-linked immunosorbent assay after study completion.

#### *Dietary intake, physical activity, sedentary behavior, and sleep quality assessment*

Participants will complete 5-day dietary records, including two days during morning or afternoon shifts, two days during night shifts, and one weekend or holiday, at baseline, week 6, and week 12. Dietary data will be analyzed using EasyDiet software (Biocentury, S.L.U, Barcelona, Spain), which is based on Spanish food composition tables.

Sleep quality assessment will be measured in two different ways: 1) the Pittsburgh Sleep Quality Index (PSQI), a widely used self-report questionnaire that assesses sleep quality over a one-month time interval, validated in Spanish (35); and 2) using a 3-axis accelerometer (ActiGraph wGT3X-BT, Pensacola, FL, USA), which participants will be instructed to wear on the non-dominant wrist using an adjustable Velcro strap (36). The device will be worn continuously for eight consecutive days, removing it only for water-based activities (e.g., showering or swimming). Participants will be provided with a sleep and activity log, in which they will record their work shift schedule, bedtime, and wake time, device removal times, and any structured physical activity beyond routine walking. Each participant will receive oral instructions on how to wear the actigraph, keeping general recommendations and fulfilling the diary log, indicating the time of reference measurements (i.e., time to: wake up, get up, breakfast, lunch, nap, physical activity, dinner, asleep at night) and also to track all times when the device will be removed and replaced. On the eighth day, the actigraph will be returned to the investigators, and both the actigraph and the daily log will be collected. Accelerometer data will be downloaded, cleaned, and analyzed using the manufacturer's software (Actilife 6 desktop). For the analysis of physical activity and sedentary behaviour,

the triaxial accelerometer data will also be used. Participants will be asked to maintain their usual physical activity levels throughout the study. No specific exercise intervention or standardized exercise prescription will be provided. Physical activity will be considered in the statistical analyses where appropriate.

#### *Chronotype assessment*

The morningness–eveningness preference of each participant will be evaluated at baseline using the Horne & Östberg self-assessment questionnaire (37), a validated tool widely used to determine individual circadian typology.

#### *Quality of life*

Health-related quality of life will be assessed using the 12-Item Short-Form Health Survey (SF-12), a validated and widely used instrument that provides physical and mental health summary measures. The SF-12 has demonstrated good reliability and validity in both clinical and general populations (38,39). The Spanish version of the questionnaire has also been validated and shows appropriate psychometric properties, enabling accurate interpretation in this setting (40,41). Participants will complete the paper-based questionnaire during study visits, and responses will be processed using standardized scoring procedures.

#### *Safety monitoring*

Although adverse effects are not expected due to the nature of the dietary intervention, participants will be instructed to report any symptoms or concerns throughout the study, including symptoms or episodes compatible with hypoglycemia, despite the low expected risk in this study population. All adverse events, regardless of suspected causality, will be documented and reviewed by the clinical team across the study.

#### **Data management**

All study data will be pseudonymized using a unique participant identification code. The correspondence between personal identifiers and study codes will be kept separately and will only be accessible to authorized members of the research team. Paper documents will be stored in locked cabinets within restricted-access research facilities, accessible only to researchers with authorized card access. Biological samples will be labelled with the participant study code only and stored at  $-80^{\circ}\text{C}$  in restricted-access laboratory facilities. Electronic files and study databases will be managed from a password-protected computer

connected to the hospital's internal network and will be accessible only to authorized study personnel. All data and samples will be handled in accordance with the study protocol approved by the corresponding Research Ethics Committee.

### **Sample size**

The sample size was determined based on the expected change in HbA1c, one of the study's primary outcomes. HbA1c reflects average glycaemia over approximately 2–3 months (42), and higher levels are strongly associated with an increased risk of diabetes-related (particularly microvascular) complications (43). Current American Diabetes Association Standards of Care emphasize individualized HbA1c targets as a central goal of glycaemic management in populations with both prediabetic and T2D (44). Based on previous dietary intervention studies in adults with T2D, the standard deviation of HbA1c was estimated at approximately 1 percentage point (17,45). Because no directly comparable long-term intervention studies are available in shift workers, the expected between-group difference was estimated conservatively based on the comparison between the two most contrasted interventions (group A vs. group C), rather than on evening protein allocation alone. A difference of 0.5 percentage points in HbA1c was considered clinically relevant and biologically plausible in light of prior evidence showing improved glycaemic control with higher-protein diets in T2D and more favorable nocturnal postprandial responses after high-protein meals (17,24,45). The sample size calculation was therefore based on the hypothesis that the reduction in HbA1c in intervention group A would be at least 0.5 percentage points greater than that observed in group C. Assuming a one-sided test, with a 90% confidence level ( $Z_{\alpha} = 1.645$ ) and 80% statistical power ( $Z_{\beta} = 0.842$ ), the required sample size was estimated at 36 participants per group. To account for an anticipated 15% dropout rate, the final sample size was set at 42 participants per group, resulting in a total of 126 participants across the three study arms.

### **Statistical analysis**

All participants who complete the study will be included in the data analysis, independent of reported dietary compliance or weight loss, according to intention-to-treat analysis. Data will be expressed as mean  $\pm$  standard deviation or median (25th–75th percentile), depending on whether the variables follow a normal or non-normal distribution, respectively. The normality of data distribution will be assessed using the Shapiro–Wilk test. Differences that occur during the study within each diet group will be evaluated by the Friedman or repeated

measures ANOVA test, when analyzing non-parametric and parametric variables, respectively. We will calculate differences between diet groups (group  $\times$  time interaction) using a generalized linear model including log-transformed data, if applicable. Other statistical analyses will be performed using conventional methods appropriate to the distribution of each variable. All analyses will be conducted using R software (version 3.5.0), with a significance level set at  $P < 0.05$ . Any change in glucose-lowering medication during the 12-week intervention will be recorded and considered in the analyses. Participants with medication changes during follow-up will be retained in the intention-to-treat analysis, and additional sensitivity analyses excluding these cases will be performed where appropriate.

### **Ethical considerations**

This study will be conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and the guidelines of Good Clinical Practice. The protocol (Version 3, dated December 16, 2023) and the participant information and consent document (Version 3.0, dated December 15, 2023) were reviewed and approved by the Clinical Research Ethics Committee of Aragón (CEICA) on January 10, 2024 (Act No. 01/2024; Reference: PI23/558). A copy of the ethical approval is available in **S1 Appendix**. The committee deemed the study ethically acceptable, including the justification of foreseeable risks and burdens, the appropriateness of the informed consent procedures, the suitability of the research team and facilities, and the adequacy of proposed data protection and compensation measures.

Written informed consent will be obtained from all participants prior to inclusion. Participants will be informed about the voluntary nature of the study, the right to withdraw at any time, and the confidentiality of their data. The full informed consent materials are provided in **S1 Appendix**.

The trial is registered at ClinicalTrials.gov (Identifier: NCT06912555) and adheres to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) guidelines. The completed SPIRIT checklist is included in **S1 Appendix**.

### **Discussion**

To our knowledge, this protocol describes the first randomized controlled trial designed to evaluate the effects of three hypocaloric dietary interventions, varying in overall macronutrient distribution and protein distribution across the day, on glycemic control, body

composition, and metabolic health in shift workers with prediabetes or T2D and overweight or obesity. This population faces significant metabolic risks due to chronic circadian misalignment and irregular eating patterns, for which chrononutrition has emerged as a promising but underutilized intervention strategy (46), particularly given the observed disruptions in eating windows and macronutrient intake during night shift (47), and the broader impact on metabolic and psychosocial health (48). A review conducted by Henry CJ et al. emphasizes that while intermittent fasting and macronutrient variation have been studied individually, their integration with chronobiological principles in shift workers remains an emerging field (49). Furthermore, although recent tools have been developed to assess temporal eating patterns in shift-working populations, clinical trials incorporating nutrient-specific timing based on chronotype, particularly protein, are still lacking (46,50). By combining nutrient composition with evening/night-time protein allocation strategies, our trial seeks to fill this gap in the evidence.

This trial has several methodological strengths. First, the 12-week intervention period is based on extensive evidence showing that this duration is sufficient to produce significant improvements in HbA1c, fasting glucose, and lipid profiles in patients with T2D undergoing dietary treatment (45,51). Second, the randomized controlled design with stratified allocation by sex and shift type strengthens internal validity and ensures balanced group assignment. Third, the intervention arms are calorie-matched, differing only in macronutrient composition and temporal protein distribution, enabling a clear assessment of these dietary factors.

Moreover, the study employs a wide range of validated assessment tools and gold-standard measurements. Body composition is assessed using dual-energy X-ray absorptiometry (DXA), which is considered the gold standard for quantifying fat and lean mass (52). Physical activity and sedentary behavior, and sleep patterns, are objectively measured using triaxial accelerometers, which are widely used and validated in clinical research (53). Dietary intake is analyzed using structured 5-day food records entered into the EasyDiet software, and chronotype is assessed using the Horne–Östberg questionnaire (37). Additional validated questionnaires, such as the PSQI (35) and the SF-12 for health-related quality of life (38), provide standardized, reliable measures of key lifestyle and psychosocial variables.

While the study is designed to be methodologically robust, certain limitations should be acknowledged. Recruitment may be slower than anticipated due to the relatively strict inclusion criteria and the need to engage a working population with irregular schedules.

Although a 15% dropout rate has been anticipated in the sample size estimation, the rotating nature of shift work, personal constraints, and potential disruptions to routine may still pose challenges to study retention. Additionally, the 12-week intervention period, although supported by previous evidence for eliciting clinically relevant metabolic improvements, does not allow for evaluation of long-term outcomes or sustainability of dietary changes. Furthermore, because the expected between-group difference in HbA1c was estimated in the absence of directly comparable long-term trials in shift workers, the study may have limited power to detect smaller effects. Lastly, although dietary intake will be assessed through standardized 5-day food records, self-reporting remains subject to potential bias, even with the support of structured tools and investigator guidance.

Results from this trial will be disseminated through peer-reviewed publications, conference presentations, and institutional communication channels, including collaboration with the hospital's press office.

Any substantial protocol modifications, including changes to eligibility criteria, outcomes, or analysis plans, will be documented and reported to the CEICA and updated in the trial registry (ClinicalTrials.gov: NCT06912555). The study may be terminated early only in the event of serious adverse events, funding discontinuation, or other ethical concerns, in which case all relevant stakeholders and participants will be notified.

In summary, this trial will provide novel evidence on the effects of hypocaloric diets with distinct macronutrient profiles and protein distribution patterns in a population at high metabolic risk: shift workers with prediabetes or T2D. By incorporating circadian-related considerations into established dietary strategies, the study addresses a significant gap in the current literature. The findings may inform future dietary recommendations tailored to circadian biology and work schedules, contributing to more effective and personalized nutritional approaches for managing metabolic disorders in shift-working populations.

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## **Conflict of interest Statement**

The authors declare that they have no conflicts of interest to disclose.

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## **Authorship contributions**

Rocío Mateo-Gallego, Itziar Lamiquiz-Moneo and Irene Gracia-Rubio: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing - original draft; and Writing - review & editing. Carmen Rodrigo-Carbó: Formal analysis; Investigation; Methodology; Resources; Writing - original draft; and Writing - review & editing. Mikel Tous-Espelosin, Sofía Pérez-Calahorra, Isabel Lamiquiz-Moneo, Rebeca Monjón-Gonzalez and Ángela Lezcano-Enciso: Formal analysis; Investigation; Methodology; and Writing - review & editing.

## **Supporting information**

S1 Appendix. Informed consent materials

S1 Appendix. Ethical approval from the Research Ethics Committee of Aragón (CEICA)

S1 Appendix. Checklist. SPIRIT 2013 checklist: Recommended items to address in clinical trial protocol and related documents.

S1 Appendix. Example daily menus of each dietary intervention

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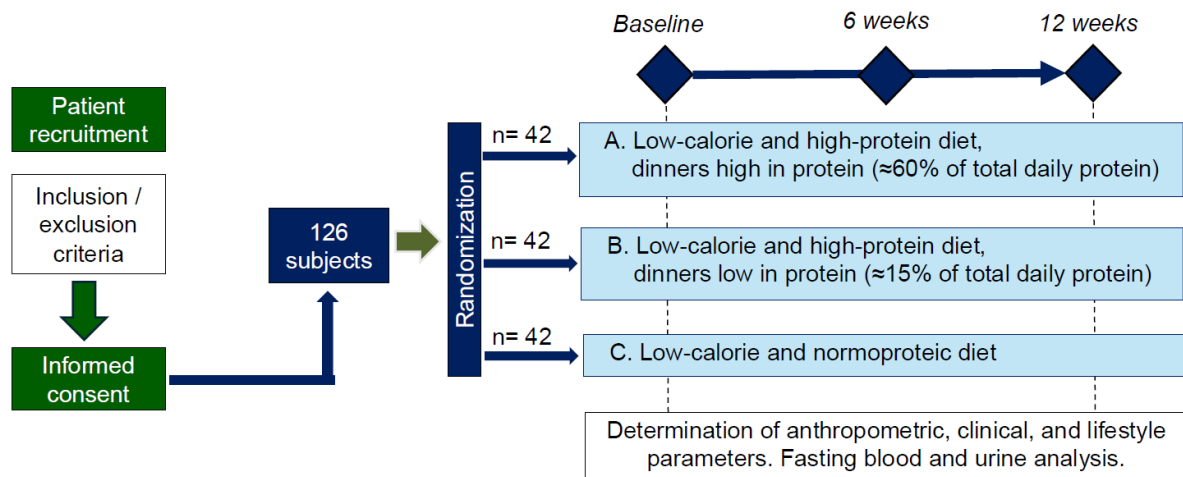
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**Figure 1.** Overview of the study design.

Eligible participants ( $n = 126$ ) are randomized into one of three hypocaloric dietary intervention arms (A, B, or C) differing in total protein content and its distribution across meals. The intervention lasts 12 weeks, with assessments at baseline, week 6, and week 12. All participants undergo anthropometric, clinical, and lifestyle evaluations, as well as fasting blood and urine analyses.