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Title: Protocol for a randomized controlled trial on the feasibility and effects of ten-hour time-restricted eating on cardiometabolic disease risk among career firefighters doing 24-hour shiftwork: The Healthy Heroes Study

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Abstract

Introduction: Career firefighters experience chronic circadian rhythm disruption, which increases their risk of cardiometabolic disease. The recent discovery that eating patterns regulate circadian rhythmicity in metabolic organs has raised the hypothesis that maintaining a consistent daily cycle of eating and fasting can support circadian rhythms and reduce disease risks. Preclinical animal studies and preliminary clinical trials have shown promising effects of time-restricted eating on reducing disease risk without compromising physical performance. However, there is a lack of randomized controlled trials on time-restricted eating (TRE) on shift workers including firefighters. This study aims to investigate the feasibility and efficacy of ten-hour TRE on health parameters that contribute to cardiometabolic disease risks among career firefighters who work a 24-hour shift schedule.

Methods and analyses: The Healthy Heroes Study is a randomized controlled parallel open-label clinical trial with 150 firefighters over 1 year. Firefighters are randomized with a 1:1 ratio to either the control or intervention group. The control group receives behavioral nutritional counseling (standard of care, "SOC"). The intervention group receives the same SOC with the addition of a self-selected 10-h TRE window. After the 2-week baseline, participants enter a 3-month monitored intervention period, followed by a 9-month self-guided period with follow-up assessments. The impact of TRE on blood glucose levels, body weight, body composition, biomarkers (neuroendocrine, inflammatory, and metabolic), sleep, and mood are evaluated. These assessments occur at baseline, the end of the 3-month intervention period, and follow-ups at 6-, 9-, and 12-months. Temporal calorie intake is monitored with the smartphone application myCircadianClock throughout the study. Continuous glucose monitors, wrist-worn actigraphy, and questionnaires are used to monitor glucose levels, activity, sleep, and light exposure.

Ethics and Dissemination: The study has been approved by the Institutional Review Boards of the University of California, San Diego, and the Salk Institute for Biological Studies.

Article Summary

Strengths and Limitations

- In accordance with the funding agency's recommendation to avoid unintended health-based discrimination at work, the eligibility criteria do not exclude healthy firefighters with normal values of metabolic health (relevant data can be analyzed upon stratification of healthy or non-healthy parameters).
- Mediterranean diet is used as the SOC as it is known to improve metabolic health.
- The career firefighters in San Diego Fire and Rescue adopt a 24-h shift schedule that is also followed among 74% of fire departments in the US; however, the feasibility, adoptability, and efficacy of a 10-hr TRE among volunteer firefighters, and those with a shift schedule different from 24 h may not be generalized from this study.
- The study uses the myCircadianClock app to monitor and guide participants, which reduces the burden of frequent clinic visits and can be used for large scale adoption of the results at the national and international level.
- The study uses continuous measurements of activity, sleep, and interstitial glucose levels with integrative analyses of these data streams to offer deep insight into the impact of shift work on blood glucose regulation.

Introduction

Shift workers constitute up to 20% of the workforce in industrial countries and they are indispensable to the functioning of modern societies. Firefighters are shift workers who often work at night when our circadian rhythm instructs our body to sleep. Chronic disruption of circadian (~24-hour, h) daily rhythms among shift workers, including firefighters, increases the risk of obesity, diabetes, cardiovascular diseases, insomnia, and cancer [1-9]. However, pragmatic lifestyle intervention to counteract the adverse health effects of shift work is lacking. Recent progress in circadian science has raised the possibility of novel interventions for reducing the disease risk of firefighters.

While the major emphasis on reducing circadian disruption has been on restoring sleep, the impact of food timing on health has opened new avenues to lessen the adverse effects of circadian disruption. Preclinical studies have shown that restricting all food intake to a consistent 8-12 h window- without reducing calories - can prevent and reverse obesity, diabetes, digestive disorders, liver disease, and cardiovascular disease [10]. Restricting the timing of food without explicitly reducing calories is called Time-Restricted Eating (TRE). More importantly, TRE does not compromise physical fitness, but rather improves motor coordination and endurance [11 12], critical attributes for firefighters. Pre-clinical animal models revealed cellular and molecular changes by which TRE improves health. TRE enhances the circadian clock to optimize health by coordinating the timing of digestive hormones, metabolic enzymes, and storage depots in the metabolism of sugar and fat for optimal function [13 14]. Abnormal sugar and fat metabolism are implicated in numerous diseases including obesity, diabetes, and cardiovascular diseases [15].

The scope and feasibility of TRE in shift workers have not been studied. Random eating patterns are widespread among both non-shift and shift workers [11 16]. Non-shift workers can adopt a 10-h eating window and self-sustain the new behavior that reduced body weight and improved sleep [11]. Such an eating pattern intervention also indirectly improves nutrition quality by reducing excessive caloric intake by up to 20%, most of which comes from an unhealthy energy-dense diet.

There is increasing evidence that TRE or other forms of fasting can lead to reduced cardiovascular diseases and even cancer [17]. More importantly, the American Heart Association [18] and the National Nutrition Task Force [19] have emphasized the importance of daily eating-fasting rhythm in disease prevention.

However, TRE has never been tried among firefighters in a randomized controlled trial (RCT). Nearly 75% of firefighters nationwide are overweight or obese [20-22]. Comorbidities associated with obesity are prevalent among firefighters [22]. Obesity jeopardizes their safety and well-being as well as public safety. Obesity is also a significant risk factor for subsequent disability [23 24]. Therefore, we hypothesize that TRE can improve blood glucose regulation, reduce obesity, and attenuate comorbidities associated with impaired glucose homeostasis or obesity.

In a randomized controlled trial, this protocol will test the efficacy of eating time intervention relative to standard behavioral counseling on firefighters' health. The firefighters' cardiometabolic disease risks will be tested through a series of blood tests and questionnaires. A customized smartphone app developed in our lab will be used to guide participants to adopt the new eating pattern and log their lifestyle data.

Methods

Overview

In this RCT, firefighters from San Diego County are randomly assigned to a control group of behavioral nutrition counseling (standard of care, "SOC") or the intervention group of SOC with the addition of adopting a 10-h eating window for 3 months (TRE). Participants are followed up for one year. Participants in the TRE group may eat outside the 10-h window up to 2 days/week to allow social commitments that are deemed necessary for sustaining their emotional health. The research team also works with participants to help adjust for challenging schedules. The impact of TRE on blood glucose levels, NMR-lipid profile, biomarkers, body weight, body composition, sleep, and mood will be evaluated. Questionnaires will be administered for self-reported health and wellness assessments. Participants use an electronic diary (smartphone myCircadianClock application ("mCC app")) to log their caloric intake. Sleep and activity are passively measured with actiwatches at baseline and every 3-months following. ***We hypothesize*** *that imposing eating-fasting cycles (TRE) will restore the equilibrium between catabolic and anabolic processes, which will promote glucose and lipid homeostasis, strengthen neuroendocrine signals, improve the regulation of circadian rhythms leading to a reduction of cardiometabolic disease risks, and improved sleep and subjective quality of life.*

The first participant was enrolled in the study on May 8, 2018. Due to COVID-19 and severe fire seasons in California, the study timeline was delayed. The study is expected to be

completed in January 2021, but we anticipate further delay if the California fire season lengthens beyond October 1, 2020, and the COVID-19 pandemic continues.

Recruitment

Participants were recruited via flyers/pamphlets, a short informational video describing the study, emails from SDFR providing study information, recruitment events, and speaking to firefighters in the fire stations.

Enrollment and Randomization

150 firefighters (75 in the SOC group and 75 in the TRE group) from the San Diego County will be enrolled in the study. All participants receive a unique coded identifier to maintain patient confidentiality. Participants are screened and must meet inclusion and exclusion criteria (**Table 1**) before study enrollment. The statistician dictated the randomization of participants. We anticipate a 20% drop-out rate (the study is ongoing).

The firefighters are assured that study participation or withdrawal from the study has no bearing on their employment or receiving any benefit from the fire department. Individual participant's data and any identifiable data will not be explicitly shared with the fire department.

Due to the low-risk of harm, a Data Safety Monitoring Board was not appointed for this study. Instead, a Data Safety Monitoring Plan is provided (supplement).

Table 1. Study Enrollment Criteria

Inclusion Criteria:
• Firefighter or work a 24-hr shift schedule with San Diego Fire and Rescue or other fire departments in San Diego County. • Age: 21-65 years. • Own a smartphone (Apple iOS or Android OS). • If participants are on cardiovascular medications (HMG-CoA reductase inhibitors (statins), other lipid-modifying drugs (including over the counter drugs such as red yeast rice and fish oil), anti-hypertensive, anti-diabetes drugs), no dose adjustments will be allowed during the study period.
Exclusion Criteria:
• Insulin-dependent diabetes mellitus

- Presence of acute chronic inflammatory or autoimmune disease (defined by acute symptoms or C-reactive protein >10 mg/L), malabsorption syndromes, liver disease, or kidney disease (stage 3 or greater).
- Uncontrolled thyroid disease
- Intake of drugs likely to interfere with study endpoints, including corticosteroids, anabolic steroids, anti-psychotics, antiretroviral drugs, and immunosuppressive drugs (within 3 months of starting the study).
- The presence or recent history of anemia (hematocrit <33% within 3 months of starting the study).
- History of bariatric surgery.
- Pregnant or breast-feeding women.
- Current or recent (within 12 months of starting the study) pregnancy or breastfeeding, or intention of becoming pregnant in the next 6 months.
- Any cancer other than non-melanoma skin cancer in the last 3 years.
- On a special or prescribed diet for other reasons (e.g. Celiac disease).
- Depression as determined by the Beck Depression Inventory (BDI).
- Planned international travel during the study period.
- Insufficient logging on the mCC app (does not log at least 2 entries a day for 10 of 14 days) during baseline will exclude from being randomized into the intervention period.
- Inability or unwillingness to adhere to the study protocol and instructions from study personnel.

Outcomes

The primary, secondary and other outcomes are listed in Table 2. Since the inclusion/exclusion criteria do allow recruitment of participants whose outcome measures are within the reference range, we anticipate each arm will have participants with heterogeneous health parameters. Therefore, in addition to the comparison of all participants in the SOC and TRE arms, we will also do sub-analyses of outcome measures for participants who are outside the reference range at the beginning of the intervention period.

Table 2. Study Outcomes	
Primary Outcome Measures	1. Evaluate the impact of TRE on glucose homeostasis. The primary endpoint will be the change in glucose levels assessed via fasting blood glucose and continuous glucose monitors (CGM). Data from CGMs will be analyzed to determine changes in glucose response within individuals and a daily average for glucose value will be computed. 2. Assess the feasibility and adherence of TRE. This will be measured by the percentage of days logged that participants ate within their TRE window and end of study surveys.
Secondary Outcome Measures	3. Assess changes in metabolic and neuroendocrine biomarkers in response to TRE. Cardiometabolic homeostasis will be measured with blood biochemistry (including, but not limited to: fasting glucose, HbA1c, cholesterol, triglycerides, NMR lipoprofile, HbA1c, and hs-CRP). Neuroendocrine markers include insulin and leptin. 4. Systolic blood pressure (mmHg) in response to TRE 5. Diastolic blood pressure (mmHg) in response to TRE 6. Body weight (kg) in response to TRE
Other Outcomes	7. Body mass index (kg/m^2) 8. Waist and hip circumference (cm) 9. Hip (cm)/waist (cm) ratio 10. Body composition including but not limited to the fat percentage (%), fat mass (kg), and lean mass (kg) 11. Questionnaires (SF-36, ESS, PSQI, BDI)
Sub-analyses	12. For each outcome measure (1-11), sub-analyses will be done on participants in both arms who are outside the reference healthy range for the respective measures.

Intervention

Groups. There are two groups in this study. The SOC group is given nutritional guidelines to follow the Mediterranean diet and are advised to continue their habitual daily eating pattern. The second group will implement the 10-h TRE intervention with SOC.

The study statistician will generate the study randomization table before the start of the study using the SPSS program using block sizes of 4 and 8. He will be contacted by the study coordinator when a subject is ready to be randomized. Participants will be randomized into the interventional TRE + SOC arm or the SOC only arm. It is not possible to blind the research team from the intervention group allocation as the eating window had to be known to assess adherence throughout the study.

Time-restricted eating

Participants assigned to the TRE group are instructed to consume all foods and beverages (except water) within a consistent self-selected time window of 10 h/day for the 3-month intervention and continue through the 12-month follow-up visit. Participants may consume caffeine (without cream or sugar) outside the eating window as needed, and log it in the mCC app.

Remote engagement with the participants/Education material

Lifestyle or behavioral intervention studies typically require frequent in-person study visits to adopt the suggested intervention. Such frequent physical visits can be burdensome for firefighters. Therefore, the mCC app is used to deliver educational materials, reminders/encouragement, and answer frequently asked questions.

During the 2-week baseline period, all participants receive text notifications reminding them to log their caloric intake in the app to improve logging compliance. After the participants are randomized to the SOC or TRE group, each group receives 2-3 notifications/week as messages within the app. These notifications are group-specific and intended to increase the participants' understanding of the SOC or TRE treatment. After the 3-month monitored intervention, upon entering the self-guided 9-month follow-up period, participants receive weekly educational material as ~800-1000-word articles on the impact of SOC or TRE on their overall health. Participants also receive short 4-5 question surveys that serve the dual function of raising their

awareness of general health and assessing their understanding of how shift work affects their health. The participants can use the contact section of the app to communicate with the research team.

Assessment of adherence

If participants in the control group restrict their eating window to less than their habitual ≥12 h/day or if the eating window of participants in the TRE group deviates from their self-selected 10-h eating window ≥4 days during the first week, the participant will be contacted via telephone to ensure that the participant has understood the concept of their group assignment. Participants in the TRE group choose their self-selected window at clinic visit two and enter it in the app. During the intervention, the TRE participants can change their eating window if they are not satisfied with the originally selected window. These changes are only made when necessary and approved by the research team.

Adherence to logging will be calculated as the percent of days that participants logged at least 2 calorie-containing items with a minimum for 5 h apart. For the TRE arm, non-adherence to TRE will be assessed by the percentage of days that participants logged more than 1 h outside (before or after) their designated eating window. Eating window will be determined by the 95% interval of all calorie-containing ingestion events during baseline, the 3-month intervention period, and within the two weeks leading up to follow-up visits at 6-, 9-, and 12-months. The eating window will be calculated for both groups. Adherence criterion is not applied in the control group. Adherence analysis is based on Wilkinson et al., 2020 [25].

Patient and Public Involvement

To assess and improve the participants' experience in the study, participants are asked about the successes and challenges they face with adherence to the intervention. These assessments were taken at clinic and fire station visits, and through short surveys via the mCC app.

Members of San Diego Fire and Rescue, including The SDFR Chief, Healthy and Wellness Officers, and the SDFR Union Representative, have been involved throughout the study development including applications for funding, protocol development, participant recruitment, and are active members of an advisory committee.

Visits

The study includes 6 clinic visits from the participants, 2 fire station visits from the research team, and 1 phone call from the dietician (See **Figure 1, Table 3**). For clinical visits 4 and 5, participants are mailed an actiwatch to wear for 2 weeks before the clinic visit.

Table 3. Overview of Study Visits

Measure/Event	Pre	CV1	CV2	Call	FSV1	CV3	CV4	CV5	FSV2	CV6
Week of Study	Pre	0	2	8	10	14	28	41	50	52
Inclusion/Exclusion Screening	X	X								
Medical History		X								
Informed Consent		X								
Pregnancy test (fertile women only)		X								
mCC app Instructions		X								
Randomized to Intervention Group and select 10-h eating window if TRE			X							
Vitals, Body weight, body composition		X				X	X	X		X
Blood draw		X				X	X	X		X
CGM applied		X			X				X	
Actigraphy		X			X	X	X	X	X	X
SF-36		X				X	X	X		X
BDI-II		X				X	X	X		X
PSQI			X			X	X	X		X
ESS			X			X	X	X		X
24-h Dietary Recall			X	X						X
End of Study Survey										X

Clinic Visit 1(Day 1)- After visit 1, participants enter a 2-week baseline period where they are instructed to use the mCC app to document all oral intake (logging water is optional). Vitals, body weight, body composition, and fasting blood draw will be obtained. A CGM is applied and an actiwatch is provided to wear for 2-weeks.

Clinic Visit 2 (End of week 2)- Participants are randomized into either the SOC or TRE group. Participants return to the clinic to return CGM and actigraphy. If randomized to the TRE group, participants will select a 10-h eating window and set it on the app. All participants meet with the dietician who provides behavioral nutritional counseling and obtains a 24-h dietary recall.

Monitored Intervention Period (Weeks 3-14)-Participants enter the 3-month monitored intervention period in which they will either be engaged in the SOC or the TRE intervention starting at clinic visit 2.

Phone Call- (Week 8)- All participants will speak with the dietician to reinforce good nutritional practices with the Mediterranean diet over the phone and provide a 24-h dietary recall.

Fire Station Visit 1 (Week 12)- A research team member will go to the fire station to provide the firefighters with a CGM and actigraphy device, which they will wear for 2 weeks.

Clinic Visit 3 (Week 14)-Participants will return the CGM and actigraphy device. Vitals, body weight, body composition, and fasting blood draw will be obtained. They will be asked to complete the same questionnaires from Visits 1 and 2. This will conclude the 3-month monitored intervention period, and it will also initiate the 9-month self-guided period.

During the self-guided period (Months 3-12), there will be 3 clinic visits and one fire station visit. During these 9 months, they are asked to use the mCC app for at least 2 consecutive weeks every month.

Clinic Visit 4 (Month 6) and Clinic Visit 5 (Month 9)- Participants will use the mCC app for at least 2 consecutive weeks per month. They will also complete the same questionnaires, vitals, body weight/composition, and fasting blood draws as they did at clinic visits 1 and 3.

Fire Station Visit 2 (Month 12)- The research team will go to the fire station to provide the firefighters with a CGM and an actigraphy device, which they will wear for 2 weeks.

Clinic Visit 6 (End of Month 12)- At this final visit, all assessments will be repeated, CGM and actigraphy device will be returned, and the dietician will also conduct a 24-h dietary recall.

COVID-19 Protocol

Due to COVID-19, the Altman Clinical and Translational Research Institute (ACTRI) temporarily closed from April-July 2020, after which it remains accessible only to essential therapeutic research studies. During this period, clinic visits were conducted at the SDFD Wellness center, where its nurses are trained and provided supplies by the UCSD research staff. Two weeks before these visits, participants were sent the actigraphy and questionnaires via US mail. The actigraphy will be worn for 2 weeks and then returned to the staff at their clinic visit. Starting in June, we phased out questionnaires by mail and switch to administering questionnaires online. Additionally, participants received a questionnaire in the mail asking if any changes to medications or sleeping habits, travel plans, and specific dates worked over the past two weeks, as these were questions that we asked in-person.

For the 3-month and 12-month visits, participants were also mailed a new CGM in the mail along with the watch and surveys 2 weeks prior. In place of fire station visits, Zoom was used to video-chat with the participant and give them instructions on how to apply and activate the CGM using the reader. They remained blind to the CGM data.

Data Collection and Measurements

Data will be collected during clinic visit days and in free-living conditions.

Data collection during clinic visits.

All clinical testing (vitals, blood draw, questionnaires) will be performed at the UCSD - ACTRI. Blood is processed at the UCSD Clinical Laboratory. All participants are advised to fast overnight and visits are scheduled between 8 am-12 pm.

Anthropometric and vital signs. At every clinic visit the following measurements will be made by standardized and hospital-grade equipment: height, weight, body temperature, blood pressure, and heart rate. Body composition will be assessed by a standardized Tanita scale (DC 430U; Tokyo, Japan).

Blood tests. All participants will have their blood drawn at the ACTRI by certified nurses. Venous blood samples are collected in the fasting state at all six clinic visits. The laboratory tests for cardiometabolic function (comprehensive metabolic panel (CMP), complete blood count (CBC), thyroid stimulating hormone (TSH), hemoglobin A1c (HbAa1c), high sensitivity C-reactive

protein (hs-CRP), triglycerides (TG), low density lipid (LDL), high density lipid (HDL), cholesterol assessed via NMR lipoprofile, insulin) are done by a certified analytical laboratory contracted by UCSD.

De-identified blood samples (specifically, serum and plasma) obtained during this study are biobanked at UCSD or the Salk Institute for possible further biochemical testing. Samples will not be shared with researchers outside of those associated with this protocol. Samples are biobanked indefinitely.

Questionnaires. Participants are asked to complete the following questionnaires:

- Clinic Visits 1,3,4,5,6:
  - Short Form-36 Quality of Life (SF-36): To measure overall functional health score as well as separate physical and mental health dimension components
  - Beck Depression Inventory-II (BDI): To screen for depression
  - Epworth Sleep Scale (ESS): To assess daytime sleepiness (CV2 instead of CV1)
  - Pittsburgh Sleep Quality Index (PSQI): To evaluate sleep in seven major areas – subjective sleep quality, sleep latency, sleep duration, sleep disturbances, sleep efficiency, use of sleeping aids, and daytime dysfunction. (CV2 instead of CV1)

- Clinic Visit 2
  - ESS
  - PSQI
  - 24-h food recall: To assess nutrient consumption, amounts, and timing based on the prior day's intake.
- Week 8 phone call
  - 24-h food recall
- Clinic Visit 6
  - End of Study Surveys: To obtain self-reported impressions of study and receive feedback.

Data collection during free-living conditions (outside of the clinic)

*The myCircadianClock application (mCC app) for self-logging lifestyle parameters***.** The mCC app is designed to run on both Android and iOS devices that account for more than 90% of all smartphones and uses HIPAA-compliant Amazon Web Server (AWS) for server-side operations. During TRE, participants set their daily eating periods and receive alerts and reminders specific to TRE protocols. Participants may choose to receive an automated alert 15 or 30 minutes before the end of the eating interval to finish their last meal of the day. All participants can log their food, sleep, and exercise. For food entries, the user can annotate the food picture with food names and other descriptors (portion size, leftovers, etc.). The app is customized with push notifications, reminders, and educational materials that are structured to guide the firefighters throughout the study, enable self-monitoring, and improve adherence and retention.

Continuous glucose monitoring (CGM). All participants are fitted with the Abbott Freestyle LibrePro CGM and will be instructed on its use. CGM measures interstitial fluid glucose, using a subcutaneous sensor placed in the upper arm area, every 15 minutes for up to 14 days. Participants wear the CGM for 2 weeks at a time at baseline (weeks 1-2), end of the 3-month intervention (weeks 12-14), and 1-year follow-up (weeks 50-52). CGMs estimate blood glucose levels with high accuracy that correlates with those obtained from either venous or capillary blood [26]. Changes in the average daily glucose and standard deviation will be calculated.

Wrist-worn actigraphy. To measure habitual physical activity and sleep, participants wear a Phillips accelerometer (Spectrum Plus™) on their non-dominant arm for two weeks at a time during the study period (baseline, and 2-weeks leading up to the end of the 3-month intervention, and follow-up visits at 6-, 9-, and 12-months). Wrist accelerometers have an acceptable correlation (r=0.90) between daily physical activity and activity counts reported by accelerometers [27] and have been used reliably in NHANES [28]. The actigraphy data will be used to measure sleep onset, sleep duration, and sleep efficiency.

Statistical analysis plan.

Data from randomized participants will be analyzed with an intention-to-treat protocol. All collected participants' data will be included. A sub-analysis will be performed to assess changes in participants who had health factors out of normal range at baseline. Data will be reported with

standard descriptive statistics as mean ± standard deviation for normally distributed data, and as median with (Q1:Q3) for non-normally distributed data. Analysis will be performed by examination of the distribution of variables to assess their means, standard deviations, and skewness. Continuous measures will be tested for normality and homogeneity of variance. Non-normally distributed variables will be transformed to meet the normal distribution assumption for linear models. Randomization will be assessed by performing a series of Wilcoxon-rank sum tests, Chi-square, or Fisher's exact tests to compare the groups on demographic and baseline clinical variables. Any variables on which the groups differ initially will be explored as covariates in subsequent analyses. Daily averages will be computed for the baseline and end of intervention. A mixed-effect model will be used [29-31]. Data will be analyzed using SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). All analyses will be two-tailed, where applicable, with α =0.05, which will be considered statistically significant.

Sample Size Calculation

No TRE studies have been done on firefighters. Most of the published TRE studies of comparable length of intervention are not RCT. Therefore, we used weight loss as a surrogate outcome for calculating sample size. The required sample size was calculated using G*Power software and for the mixed model approach using the RMASS program provided by Hedeker (http://tigger.uic.edu/~hedeker/ml.html). We are confident that the sample size of 150 provides us with a minimum power of 80% to detect a medium effect size for the primary hypotheses. Medium effect sizes were selected based on previous studies that are reviewed earlier. For the mixed model, the medium effect size is defined as a between-group difference increasing linearly from 0 at baseline to .5 SD units at the last time point. The minimum power estimation is based on sample size calculation for 10% and 20% attrition, correlations of 0.2, 0.5, and 0.8 between the repeated measures, and for medium and large effect sizes.

Missing Data

Missing data will be examined to assess randomness. The pattern of missing data will be examined according to the procedure recommended by Little and Rubin [32] which includes comparing group differences in the primary outcomes of subjects with versus without missing data. This allows

inclusion of subjects with missing data or those who terminated the study early, without relying on data imputation procedures.

Ethics and Dissemination

The study has been approved by the Institutional Review Boards of the University of California, San Diego, and Salk Institute for Biological Studies, La Jolla. Modifications to the protocol due to COVID-19 were allowed without full approval because there was no increased risk to the participants and the imminent and unique nature of the situation. Protocol modifications due to COVID-19 will be reported to the IRB. Any change in endpoints or analysis plan that occur will be amended in ClinicalTrials.gov.

Informed consent will be obtained by the study coordinators or research personnel associated with this protocol. Informed consent procedures will be supervised either by the study coordinator, supervising physicians, or the principal investigator. All research personnel giving informed consent will have undergone proper training and obtained the required certificates.

Results from the study will address whether 10-h TRE is effective and feasible in improving health outcomes among career firefighters who do 24-h shift work. Results will be disseminated through peer-reviewed manuscripts, reports, and presentations. Findings, regardless of the result, will be reported as described. All co-authors will comply with the International Committee of Medical Journal Editors and no professional writers will be engaged.

Discussion

This study is the first workplace RCT to introduce TRE among the US career shift workers to promote healthy circadian behavioral change within the existing work schedule and culture. The RCT design with a self-guided follow-up period of the trial will demonstrate the feasibility of adopting TRE among fire service personnel without changing their work schedules. The complementary in-clinic and free-living measurements of various vital, behavioral, biochemical, and physiological parameters at multiple time points throughout the study will help assess the impact of TRE on the multidimensional aspects of health. The use of a smartphone app to collect longitudinal data on nutrition and use of the same medium for bidirectional engagement with the participants with relevant and actionable educational materials is also a unique strength of this

study. If successful, this digital infrastructure will allow rapid dissemination and implementation of the TRE program among fire service personnel.

Why a 10-h TRE? Although TRE with a wide range of eating windows from 6- to 12-h has been shown to offer health benefits in pre-clinical animal models and humans, we chose a 10-h window for several reasons. In both animal models and a recently completed study on patients with metabolic syndrome, 10 h of eating and 14 h of fasting reduced adiposity, improved blood pressure, blood glucose, and blood cholesterol [25]. Therefore, we reasoned 10-h TRE may also benefit the firefighters. A 10-h TRE may also be a feasible and sustainable window for many firefighters. In SDFD and many other fire departments with communal eating at fire stations, breakfast and dinner are typically prepared within a 10 h window with the dinner being served at ~6 pm, which makes adopting to TRE easier. However, adopting this habit at home may be a challenge as the firefighters often want to share meals with their family during off days, and sustained 10-h TRE may involve the participation of the family to incorporate a TRE lifestyle.

Can it be adopted among fire services? If successful, TRE may have a relatively lower barrier to implementation relative to other approaches to reduce circadian disruption. Circadian rhythms are disrupted by light at night, sleep disruption, and erratic eating patterns. Methods to reduce the circadian disruptive impact of light or to improve sleep will likely involve both education and infrastructure investments to optimize lighting to support circadian rhythm or to improve sleep. On the other hand, if TRE is found beneficial, it can be implemented with minimal to no alterations in infrastructure.

Author Contributors

- SP, PT, ENCM, AZ conceived the study concept, developed study design and protocol, applied for funding, and initiated writing the manuscript.
- ENCM, AZ, HCL, AS, NG, AR, AP coordinated the project.
- AZ, AS, HCL, AP, AR, NG, ENCM screened/recruited participants and conducted clinic and fire station visits.
- AZ, AS, HCL, AP, AR, NG, XW, ENCM collected data.
- SG and JF developed and conducted the statistical analysis plan. SP, PT, and ENCM interpreted data.
- SP and PT are the co-principal investigators and the grant holders.
- All authors contributed and approved the final version of the manuscript.

Human Rights and Informed Consent

Ethical approval and oversight of this clinical study were approved and provided by the University of California, San Diego (UCSD) Institutional Review Board Human Research Protections Program (IRB#172083) and the Salk Institute for Biological Studies Institutional Review Board (IRB#18-0001). The study is registered on ClinicalTrials.gov (NCT03533023). All participants provided informed written consent.

Declaration of conflicting Interest. SP is the author of the book "The Circadian Code" for which he collects nominal author royalty. PT is a consultant for Amgen, Esperion, Boehringer Ingelheim, Novo Nordisk, and Sanofi, and is a shareholder for Epirum Bio. ENCM, AZ, HCL, AS, AR, AP, NG, XW, JF, and SG have no conflict of interest to disclose.

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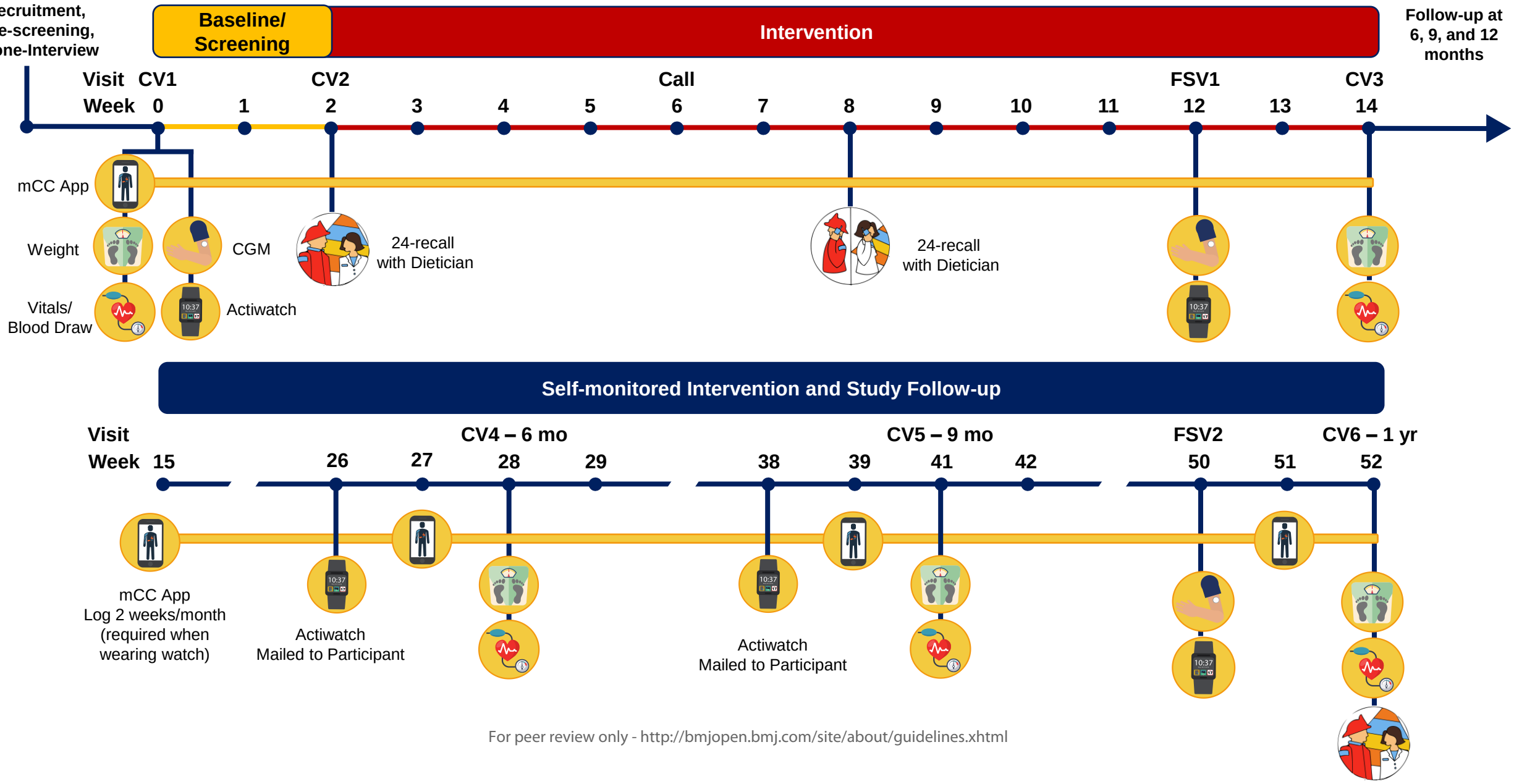
Figure Legend

Figure 1. Study Design and Timeline. mCC, myCircadianClock; CGM, continuous glucose monitor.



Supplemental Information

1. Data safety monitoring plan
2. Privacy and confidentiality
3. Data management

1. Data Safety and Monitoring Plan

The interventions in the study have minimal risk, but any adverse events will be documented. The PI reviews study conduct such as accrual, drop-outs, protocol deviations on a monthly basis. The PI reviews AEs individually real-time and in aggregate on a weekly basis. The PI reviews serious adverse events (SAEs) in real-time. The PI ensures all protocol deviations, AEs, and SAEs are reported to the IRB according to the applicable regulatory requirements.

An internal data quality inspection will be performed. The PI will do a random 10% data audit 1-2x/year. If necessary, re-training of data collectors will be conducted.

2. Privacy and Confidentiality

All study forms including consents, HIPAAs, and case report forms (CRFs), which will include elements from the present and past history, patient demographics, including age, current medications and lab draws, will be stored in in a locked cabinet in Dr. Taub's office in the ACTRI building. Only approved study personnel will have access to this information. To minimize the potential loss of confidentiality, patients will be assigned a unique number as their subject identifier code. The unique subject code will be used to label all study documents.

3. Data management

Data will be collected using standardized paper forms and will be identified with the study's ID of the participant. The codes that link the name of the participant and the study ID will be kept confidential by the Principal Investigator in a secured cabinet. Data will be entered in the computer independently by UCSD certificated and trained data entry staff, and discrepancies corrected by a supervisor based on source documents.

Specimens: Some portions of the specimens collected may be sent to a central laboratory outside of UCSD for testing. All samples will be labeled with a unique specimen code and the only way to link the specimens with the subject code and any personal health identifiers will be on a physical sheet of paper locked in a cabinet in Dr. Taub's office in the ACTRI building.

mCC app data: The mCC app is designed uses HIPAA compliant Amazon Web Server (AWS) for server-side operations. Two large capacity Linux servers (main server and backup) with load balancing are used to capture the data that the smartphone users transmit. The data will be stored on AWS S3 (Simple Storage Service). These two servers will remain online 24h a day, 7 days a week.

BMJ Open

Protocol for a randomized controlled trial on the feasibility and effects of ten-hour time-restricted eating on cardiometabolic disease risk among career firefighters doing 24-hour shiftwork: The Healthy Heroes Study

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Title: Protocol for a randomized controlled trial on the feasibility and effects of ten-hour time-restricted eating on cardiometabolic disease risk among career firefighters doing 24-hour shiftwork: The Healthy Heroes Study

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Abstract

Introduction: Career firefighters experience chronic circadian rhythm disruption, increasing their risk of cardiometabolic disease. The recent discovery that eating patterns regulate circadian rhythmicity in metabolic organs has raised the hypothesis that maintaining a consistent daily cycle of eating and fasting can support circadian rhythms and reduce disease risks. Preclinical animal studies and preliminary clinical trials have shown promising effects of time-restricted eating (TRE) to reduce disease risk without compromising physical performance. However, there is a lack of research on TRE in shift workers including firefighters. This study aims to investigate the feasibility and efficacy of ten-hour TRE on health parameters that contribute to cardiometabolic disease risks among career firefighters who work a 24-hour shift schedule.

Methods and analyses: The Healthy Heroes Study is a randomized controlled parallel open-label clinical trial with 150 firefighters over 1 year. Firefighters are randomized with a 1:1 ratio to either the control or intervention group. The control group receives Mediterranean Diet nutritional counseling (standard of care, "SOC"). The intervention group receives the same SOC and a self-selected 10-h TRE window. After the 2-week baseline, participants enter a 3-month monitored intervention, followed by a 9-month self-guided period with follow-up assessments. The impact of TRE on blood glucose, body weight, body composition, biomarkers (neuroendocrine, inflammatory, and metabolic), sleep, and mood are evaluated. These assessments occur at baseline, the end of the intervention, and 6-, 9-, and 12-month follow-ups. Temporal calorie intake is monitored with the smartphone application myCircadianClock throughout the study. Continuous glucose monitors, wrist-worn actigraphy, and questionnaires are used to monitor glucose levels, activity, sleep, and light exposure.

Ethics and Dissemination: The study was approved by the Institutional Review Boards of the University of California, San Diego, and the Salk Institute for Biological Studies. ClinicalTrials.gov: NCT03533023. Results will be disseminated through peer-reviewed manuscripts, reports, and presentations.

Article Summary

Strengths and Limitations

- In accordance with the funding agency's recommendation to avoid unintended health-based discrimination at work, the eligibility criteria do not exclude healthy firefighters with normal values of metabolic health (relevant data can be analyzed upon stratification of healthy or non-healthy parameters).
- Mediterranean diet is used as the SOC as it is known to improve metabolic health.
- The career firefighters in San Diego Fire and Rescue adopt a 24-h shift schedule that is also followed among 74% of fire departments in the US; however, the feasibility, adoptability, and efficacy of a 10-hr TRE among volunteer firefighters, and those with a shift schedule different from 24 h may not be generalized from this study.
- The study uses the myCircadianClock app to monitor and guide participants, which reduces the burden of frequent clinic visits and can be used for large-scale adoption of the results at the national and international levels.
- The study uses continuous measurements of activity, sleep, and interstitial glucose levels with integrative analyses of these data streams to offer deep insight into the impact of shift work on blood glucose regulation.

Introduction

Shift workers constitute up to 20% of the workforce in industrial countries and they are indispensable to the functioning of modern societies. Firefighters are shift workers who often work at night when our circadian rhythm instructs our body to sleep. Chronic disruption of circadian (~24-hour, h) daily rhythms among shift workers, including firefighters, increases the risk of obesity, diabetes, cardiovascular diseases, insomnia, and cancer [1-9]. However, pragmatic lifestyle intervention to counteract the adverse health effects of shift work is lacking. Recent progress in circadian science has raised the possibility of novel interventions for reducing the disease risk of firefighters.

While the major emphasis on reducing circadian disruption has been on restoring sleep, the impact of food timing on health has opened new avenues to lessen the adverse effects of circadian disruption. Preclinical studies have shown that restricting all food intake to a consistent 8-12 h window- without reducing calories - can prevent and reverse obesity, diabetes, digestive disorders, liver disease, and cardiovascular disease [10]. Restricting the timing of food without explicitly reducing calories is called Time-Restricted Eating (TRE). More importantly, TRE does not compromise physical fitness, but rather improves motor coordination and endurance [11 12], critical attributes for firefighters. Pre-clinical animal models revealed cellular and molecular changes by which TRE improves health. TRE enhances the circadian clock to optimize health by coordinating the timing of digestive hormones, metabolic enzymes, and storage depots in the metabolism of sugar and fat for optimal function [13 14]. Abnormal sugar and fat metabolism are implicated in numerous diseases including obesity, diabetes, and cardiovascular diseases [15].

The scope and feasibility of TRE in shift workers have not been studied. Random eating patterns are widespread among both non-shift and shift workers [11 16]. Non-shift workers can adopt a 10-h eating window and self-sustain the new behavior that reduced body weight and improved sleep [11]. Such an eating pattern intervention also indirectly improves nutrition quality by reducing excessive caloric intake by up to 20%, most of which comes from an energy-dense diet.

There is increasing evidence that TRE or other forms of fasting can lead to reduced cardiovascular diseases and even cancer [17]. More importantly, the American Heart Association [18] and the National Nutrition Task Force [19] have emphasized the importance of daily eating-fasting rhythm in disease prevention.

However, research on TRE is lacking among firefighters. Nearly 75% of firefighters nationwide are overweight or obese [20-22]. Comorbidities associated with obesity are prevalent among firefighters [22]. Obesity jeopardizes their safety and well-being as well as public safety. Obesity is also a significant risk factor for subsequent disability [23 24]. Therefore, we hypothesize that TRE can improve blood glucose regulation, reduce obesity, and attenuate comorbidities associated with impaired glucose homeostasis or obesity.

In a randomized controlled trial, this protocol will test the efficacy of eating time intervention relative to Mediterranean diet behavioral counseling on firefighters' health. The firefighters' cardiometabolic disease risks will be tested through a series of blood tests and questionnaires. A customized smartphone app developed in our lab will be used to guide participants to adopt the new eating pattern and log their lifestyle data.

Methods

Overview

In this RCT, firefighters from San Diego County are randomly assigned to a control group of Mediterranean diet nutrition counseling (standard of care, "SOC") or the intervention group of SOC with the addition of adopting a 10-h eating window for 3 months (TRE). Participants are followed up for one year. Participants in the TRE group may eat outside the 10-h window up to 2 days/week to allow social commitments that are deemed necessary for sustaining their emotional health. The research team also works with participants to help adjust for challenging schedules. The impact of TRE on blood glucose levels, NMR-lipid profile, biomarkers, body weight, body composition, sleep, and mood will be evaluated. Questionnaires will be administered for self-reported health and wellness assessments. Participants use an electronic diary (smartphone myCircadianClock application ("mCC app")) to log their caloric intake. Sleep and activity are passively measured with actiwatches at baseline and follow-up assessments every 3 months. ***We hypothesize*** *that imposing eating-fasting cycles (TRE) will restore the equilibrium between catabolic and anabolic processes, which will promote glucose and lipid homeostasis, strengthen neuroendocrine signals, improve the regulation of circadian rhythms leading to a reduction of cardiometabolic disease risks, and improve sleep and subjective quality of life.*

The first participant was enrolled in the study on May 8, 2018. Due to COVID-19 and severe fire seasons in California, the study timeline was delayed. The study is expected to be

completed in February 2021, but we anticipate further delay due to any unforeseen natural disaster and the course of the COVID-19 pandemic.

Recruitment

Participants were recruited via flyers/pamphlets, a short informational video describing the study, emails from SDFR providing study information, recruitment events, and speaking to firefighters in the fire stations.

Enrollment and Randomization

150 firefighters (75 in the SOC group and 75 in the TRE group) from the San Diego County will be enrolled in the study. All participants receive a unique coded identifier to maintain patient confidentiality. Participants are screened and must meet inclusion and exclusion criteria (**Table 1**) before study enrollment. The statistician dictated the randomization of participants. We anticipate a 20% drop-out rate (the study is ongoing).

The firefighters are assured that study participation or withdrawal from the study has no bearing on their employment or receiving any benefit from the fire department. Individual participant's data and any identifiable data will not be explicitly shared with the fire department.

Due to the low-risk of harm, a Data Safety Monitoring Board was not appointed for this study. Instead, a Data Safety Monitoring Plan is provided (supplement).

Table 1. Study Enrollment Criteria

Inclusion Criteria:
• Firefighter or work a 24-hr shift schedule with San Diego Fire and Rescue or other fire departments in San Diego County. • Age: 21-65 years. • Own a smartphone (Apple iOS or Android OS). • If participants are on cardiovascular medications (HMG-CoA reductase inhibitors (statins), other lipid-modifying drugs (including over-the-counter drugs such as red yeast rice and fish oil), anti-hypertensive, anti-diabetes drugs), no dose adjustments will be allowed during the study period.
Exclusion Criteria:
• Insulin-dependent diabetes mellitus

- Presence of acute chronic inflammatory or autoimmune disease (defined by acute symptoms or C-reactive protein >10 mg/L), malabsorption syndromes, liver disease, or kidney disease (stage 3 or greater).
- Uncontrolled thyroid disease
- Intake of drugs likely to interfere with study endpoints, including corticosteroids, anabolic steroids, anti-psychotics, antiretroviral drugs, and immunosuppressive drugs (within 3 months of starting the study).
- The presence or recent history of anemia (hematocrit <33% within 3 months of starting the study).
- History of bariatric surgery.
- Pregnant or breast-feeding women.
- Current or recent (within 12 months of starting the study) pregnancy or breastfeeding, or intention of becoming pregnant in the next 6 months.
- Any cancer other than non-melanoma skin cancer in the last 3 years.
- On a special or prescribed diet for other reasons (e.g. Celiac disease).
- Depression as determined by the Beck Depression Inventory (BDI).
- Planned international travel during the study period.
- Insufficient logging on the mCC app (does not log at least 2 entries a day for 10 of 14 days) during baseline will exclude from being randomized into the intervention period.
- Inability or unwillingness to adhere to the study protocol and instructions from study personnel.

Outcomes

The primary, secondary and other outcomes are listed in Table 2. Since the inclusion/exclusion criteria do allow the recruitment of participants whose outcome measures are within the reference range, we anticipate each arm will have participants with heterogeneous health parameters. Therefore, in addition to the comparison of all participants in the SOC and TRE arms, we will also do sub-analyses of outcome measures for participants who are outside the reference range at the beginning of the intervention period.

Table 2. Study Outcomes	
Primary Outcome Measures	1. Evaluate the impact of TRE on glucose homeostasis. The primary endpoint will be the change in glucose levels assessed via fasting blood glucose and continuous glucose monitors (CGM). Data from CGMs will be analyzed to determine changes in glucose response within individuals and a daily average for glucose value will be computed. 2. Assess the feasibility and adherence of TRE. This will be measured by the percentage of days logged that participants ate within their TRE window and end-of-study surveys.
Secondary Outcome Measures	3. Assess changes in metabolic and neuroendocrine biomarkers in response to TRE. Cardiometabolic homeostasis will be measured with blood biochemistry (including, but not limited to: fasting glucose, HbA1c, cholesterol, triglycerides, NMR lipoprofile, and hs-CRP). Neuroendocrine markers include insulin and leptin. 4. Systolic blood pressure (mmHg) in response to TRE 5. Diastolic blood pressure (mmHg) in response to TRE 6. Body weight (kg) in response to TRE
Other Outcomes	7. Body mass index (kg/m^2) 8. Waist and hip circumference (cm) 9. Hip (cm)/waist (cm) ratio 10. Body composition including but not limited to the fat percentage (%), fat mass (kg), and lean mass (kg) 11. Questionnaires (SF-36, ESS, PSQI, BDI)
Sub-analyses	12. For each outcome measure (1-11), sub-analyses will be done on participants in both arms who are outside the reference healthy range for the respective measures.

Intervention

Groups. There are two groups in this study. The SOC group is given nutritional guidelines to follow the Mediterranean diet and is advised to continue their habitual daily eating pattern. The second group will implement the 10-h TRE intervention with SOC.

The study statistician will generate the study randomization table before the start of the study using the SPSS program using block sizes of 4 and 8. He will be contacted by the study coordinator when a subject is ready to be randomized. Participants will be randomized into the interventional TRE + SOC arm or the SOC only arm. It is not possible to blind the research team from the intervention group allocation as the eating window had to be known to assess adherence throughout the study.

Time-restricted eating

Participants assigned to the TRE group are instructed to consume all foods and beverages (except water) within a consistent self-selected time window of 10 h/day for the 3-month intervention and continue through the 12-month follow-up visit. Participants may consume caffeine (without additional nutritional content such as cream, sugar, or artificial sweeteners) outside the eating window as needed, and log it in the mCC app.

Remote engagement with the participants/Education material

Lifestyle or behavioral intervention studies typically require frequent in-person study visits to adopt the suggested intervention. Such frequent physical visits can be burdensome for firefighters. Therefore, the mCC app is used to deliver educational materials, reminders/encouragement, and answer frequently asked questions.

During the 2-week baseline period, all participants receive text notifications reminding them to log their caloric intake in the app to improve logging compliance. After the participants are randomized to the SOC or TRE group, each group receives 2-3 notifications/week as messages within the app. These notifications are group-specific and intended to increase the participants' understanding of the SOC or TRE treatment. After the 3-month monitored intervention, upon entering the self-guided 9-month follow-up period, participants receive weekly educational material as ~800-1000-word articles on the impact of SOC or TRE on their overall health.

Participants also receive short 4-5 question surveys that serve the dual function of raising their awareness of general health and assessing their understanding of how shift work affects their health. The participants can use the contact section of the app to communicate with the research team.

Assessment of adherence

If participants in the control group restrict their eating window to less than their habitual ≥12 h/day or if the eating window of participants in the TRE group deviates from their self-selected 10-h eating window ≥4 days during the first week, the participant will be contacted via telephone to ensure that the participant has understood the concept of their group assignment. Participants in the TRE group choose their self-selected window at clinic visit two and enter it in the app. During the intervention, the TRE participants can change their eating window if they are not satisfied with the originally selected window. These changes are only made when necessary and approved by the research team. Changes to the eating window will be documented and reported with results.

Adherence to logging will be calculated as the percent of days that participants logged at least 2 calorie-containing items with a minimum of 5 h apart. For the TRE arm, non-adherence to TRE will be assessed by the percentage of days that participants logged more than 1 h outside (before or after) their designated eating window. The eating window will be determined by the 95% interval of all calorie-containing ingestion events during baseline, the 3-month intervention period, and within the two weeks leading up to follow-up visits at 6-, 9-, and 12-months. The eating window will be calculated for both groups. The adherence criterion is not applied in the control group. Adherence analysis is based on Wilkinson et al., 2020 [25].

Patient and Public Involvement

To assess and improve the participants' experience in the study, participants are asked about the successes and challenges they face with adherence to the intervention. These assessments were taken at clinic and fire station visits, and through short surveys via the mCC app.

Members of San Diego Fire and Rescue, including the SDFR Chief, Healthy and Wellness Officers, and the SDFR Union Representative, have been involved throughout the study development including applications for funding, protocol development, participant recruitment, and are active members of an advisory committee.

Visits

The study includes 6 clinic visits from the participants, 2 fire station visits from the research team, and 1 phone call from the dietician (See **Figure 1, Table 3**). For clinical visits 4 and 5, participants are mailed an actiwatch to wear for 2 weeks before the clinic visit.

Table 3. Overview of Study Visits

Measure/Event	Pre	CV1	CV2	Call	FSV1	CV3	CV4	CV5	FSV2	CV6
Week of Study	Pre	0	2	8	10	14	28	41	50	52
Inclusion/Exclusion Screening	X	X								
Medical History		X								
Informed Consent		X								
Pregnancy test (fertile women only)		X								
mCC app Instructions		X								
Randomized to Intervention Group and select 10-h eating window if TRE			X							
Vitals, Body weight, body composition		X				X	X	X		X
Blood draw		X				X	X	X		X
CGM applied		X			X				X	
Actigraphy		X			X	X	X	X	X	X
SF-36		X				X	X	X		X
BDI-II		X				X	X	X		X
PSQI			X			X	X	X		X
ESS			X			X	X	X		X
24-h Dietary Recall			X	X						X
End of Study Survey										X

Clinic Visit 1(Day 1)- After visit 1, participants enter a 2-week baseline period where they are instructed to use the mCC app to document all oral intake (logging water is optional). Vitals, body

weight, body composition, and fasting blood draw will be obtained. A CGM is applied and an actiwatch is provided to wear for 2-weeks.

Clinic Visit 2 (End of week 2)- Participants are randomized into either the SOC or TRE group. Participants return to the clinic to return CGM and actigraphy. If randomized to the TRE group, participants will select a 10-h eating window and set it on the app. All participants meet with the dietician who provides Mediterranean Diet nutritional counseling and obtains a 24-h dietary recall.

Monitored Intervention Period (Weeks 3-14)-Participants enter the 3-month monitored intervention period in which they will either be engaged in the SOC or the TRE intervention starting at clinic visit 2.

Phone Call- (Week 8)- All participants will speak with the dietician to reinforce good nutritional practices with the Mediterranean diet over the phone and provide a 24-h dietary recall.

Fire Station Visit 1 (Week 12)- A research team member will go to the fire station to provide the firefighters with a CGM and actigraphy device, which they will wear for 2 weeks.

Clinic Visit 3 (Week 14)-Participants will return the CGM and actigraphy device. Vitals, body weight, body composition, and fasting blood draw will be obtained. They will be asked to complete the same questionnaires from Visits 1 and 2. This will conclude the 3-month monitored intervention period, and it will also initiate the 9-month self-guided period.

During the self-guided period (Months 3-12), there will be 3 clinic visits and one fire station visit. During these 9 months, they are asked to use the mCC app for at least 2 consecutive weeks every month.

Clinic Visit 4 (Month 6) and Clinic Visit 5 (Month 9)- Participants will use the mCC app for at least 2 consecutive weeks per month. They will also complete the same questionnaires, vitals, body weight/composition, and fasting blood draws as they did at clinic visits 1 and 3.

Fire Station Visit 2 (Month 12)- The research team will go to the fire station to provide the firefighters with a CGM and an actigraphy device, which they will wear for 2 weeks.

Clinic Visit 6 (End of Month 12)- At this final visit, all assessments will be repeated, CGM and actigraphy device will be returned, and the dietician will also conduct a 24-h dietary recall.

COVID-19 Protocol

Due to COVID-19, the Altman Clinical and Translational Research Institute (ACTRI) temporarily closed from April-July 2020, after which it remains accessible only to essential therapeutic research studies. During this period, clinic visits were conducted at the SDFD Wellness center, where its nurses are trained and provided supplies by the UCSD research staff. Two weeks before these visits, participants were sent the actigraphy and questionnaires via US mail. The actigraphy will be worn for 2 weeks and then returned to the staff at their clinic visit. Starting in June 2020, we phased out questionnaires by mail and switched to administering questionnaires online. Additionally, participants received a questionnaire in the mail asking if any changes to medications or sleeping habits, travel plans, and specific dates worked over the past two weeks, as these were questions that we asked in-person.

For the 3-month and 12-month visits, participants were also mailed a new CGM in the mail along with the watch and surveys 2 weeks prior. In place of fire station visits, Zoom was used to video-chat with the participant and give them instructions on how to apply and activate the CGM using the reader. They remained blind to the CGM data.

Data Collection and Measurements

Data will be collected during clinic visit days and in free-living conditions.

Data collection during clinic visits.

All clinical testing (vitals, blood draw, questionnaires) will be performed at the UCSD - ACTRI. Blood is processed at the UCSD Clinical Laboratory. All participants are advised to fast overnight and visits are scheduled between 8 am-12 pm.

Anthropometric and vital signs. At every clinic visit the following measurements will be made by standardized and hospital-grade equipment: height, weight, body temperature, blood pressure, and heart rate. Body composition will be assessed by a standardized Tanita scale (DC 430U; Tokyo, Japan).

Blood tests. All participants will have their blood drawn at the ACTRI by certified nurses. Venous blood samples are collected in the fasting state at all six clinic visits. The laboratory tests for cardiometabolic function (comprehensive metabolic panel (CMP), complete blood count (CBC),

thyroid-stimulating hormone (TSH), hemoglobin A1c (HbA1c), high sensitivity C-reactive protein (hs-CRP), triglycerides (TG), low-density lipid (LDL), high-density lipid (HDL), cholesterol assessed via NMR lipoprofile, insulin) are done by a certified analytical laboratory contracted by UCSD.

De-identified blood samples (specifically, serum and plasma) obtained during this study are biobanked at UCSD or the Salk Institute for possible further biochemical testing. Samples will not be shared with researchers outside of those associated with this protocol. Samples are biobanked indefinitely.

Questionnaires. Participants are asked to complete the following questionnaires:

- Clinic Visits 1,3,4,5,6:
    - Short Form-36 Quality of Life (SF-36): To measure overall functional health score as well as separate physical and mental health dimension components
    - Beck Depression Inventory-II (BDI): To screen for depression
    - Epworth Sleep Scale (ESS): To assess daytime sleepiness (CV2 instead of CV1)
    - Pittsburgh Sleep Quality Index (PSQI): To evaluate sleep in seven major areas – subjective sleep quality, sleep latency, sleep duration, sleep disturbances, sleep efficiency, use of sleeping aids, and daytime dysfunction. (CV2 instead of CV1)

- Clinic Visit 2
    - ESS
    - PSQI
    - 24-h food recall: To assess nutrient consumption, amounts, and timing based on the prior day's intake.
- Week 8 phone call
    - 24-h food recall
- Clinic Visit 6
    - End of Study Surveys: To obtain self-reported impressions of the study and receive feedback.

Data collection during free-living conditions (outside of the clinic)

*The myCircadianClock application (mCC app) for self-logging lifestyle parameters***.** The mCC app is designed to run on both Android and iOS devices that account for more than 90% of all smartphones and uses HIPAA-compliant Amazon Web Server (AWS) for server-side operations. During TRE, participants set their daily eating periods and receive alerts and reminders specific to TRE protocols. Participants may choose to receive an automated alert 15 or 30 minutes before the end of the eating interval to finish their last meal of the day. All participants can log their food, sleep, and exercise. For food entries, the user can annotate the food picture with food names and other descriptors (portion size, leftovers, etc.). The app is customized with push notifications, reminders, and educational materials that are structured to guide the firefighters throughout the study, enable self-monitoring, and improve adherence and retention.

Continuous glucose monitoring (CGM). All participants are fitted with the Abbott Freestyle LibrePro CGM and will be instructed on its use. CGM measures interstitial fluid glucose, using a subcutaneous sensor placed in the upper arm area, every 15 minutes for up to 14 days. Participants wear the CGM for 2 weeks at a time at baseline (weeks 1-2), end of the 3-month intervention (weeks 12-14), and 1-year follow-up (weeks 50-52). CGMs estimate blood glucose levels with high accuracy that correlates with those obtained from either venous or capillary blood [26]. Changes in the average daily glucose and standard deviation will be calculated.

Wrist-worn actigraphy. To measure habitual physical activity and sleep, participants wear a Phillips accelerometer (Spectrum Plus™) on their non-dominant arm for two weeks at a time during the study period (baseline, and 2-weeks leading up to the end of the 3-month intervention, and follow-up visits at 6-, 9-, and 12-months). Wrist accelerometers have an acceptable correlation (r=0.90) between daily physical activity and activity counts reported by accelerometers [27] and have been used reliably in NHANES [28]. The actigraphy data will be used to measure sleep onset, sleep duration, and sleep efficiency.

Statistical analysis plan.

Data from randomized participants will be analyzed with an intention-to-treat protocol. All collected participants' data will be included. A sub-analysis will be performed to assess changes in participants who had health factors out of the normal range at baseline. Data will be reported

with standard descriptive statistics as mean ± standard deviation, or 95% confidence interval for normally distributed data, and as median with (Q1:Q3) for non-normally distributed data. Analysis will be performed by examination of the distribution of variables to assess their means, standard deviations, and skewness. Continuous measures will be tested for normality and homogeneity of variance. Non-normally distributed variables will be transformed to meet the normal distribution assumption for linear models. Randomization will be assessed by performing a series of Wilcoxon-rank sum tests, Chi-square, or Fisher's exact tests to compare the groups on demographic and baseline clinical variables. Any variables on which the groups differ initially will be explored as covariates in subsequent analyses. The 95% eating window will be computed for the baseline and end of intervention. A mixed-effect model will be used [29-31]. Data will be analyzed using SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). All analyses will be two-tailed, where applicable, with α=0.05, which will be considered statistically significant.

Sample Size Calculation

To date, there are no published RCTs assessing TRE as an intervention in firefighters. Most of the published TRE studies of comparable length of intervention are not RCT. Therefore, we used weight loss as a surrogate outcome for calculating sample size. The required sample size was calculated using G*Power software and for the mixed model approach using the RMASS program provided by Hedeker (http://tigger.uic.edu/~hedeker/ml.html). We are confident that the sample size of 150 provides us with a minimum power of 80% to detect a medium effect size for the primary hypotheses. Medium effect sizes were selected based on previous studies that are reviewed earlier. For the mixed model, the medium effect size is defined as a between-group difference increasing linearly from 0 at baseline to .5 SD units at the last time point. The minimum power estimation is based on sample size calculation for 10% and 20% attrition, correlations of 0.2, 0.5, and 0.8 between the repeated measures, and for medium and large effect sizes.

Missing Data

Missing data will be examined to assess randomness. The pattern of missing data will be examined according to the procedure recommended by Little and Rubin [32] which includes comparing group differences in the primary outcomes of subjects with versus without missing data. This allows

inclusion of subjects with missing data or those who terminated the study early, without relying on data imputation procedures.

Ethics and Dissemination

The study has been approved by the Institutional Review Boards of the University of California, San Diego, and Salk Institute for Biological Studies, La Jolla. Modifications to the protocol due to COVID-19 were allowed without full approval because there was no increased risk to the participants and the imminent and unique nature of the situation. Protocol modifications due to COVID-19 will be reported to the IRB. Any change in endpoints or analysis plan that occur will be amended in ClinicalTrials.gov.

Informed consent will be obtained by the study coordinators or research personnel associated with this protocol. Informed consent procedures will be supervised either by the study coordinator, supervising physicians, or the principal investigator. All research personnel giving informed consent will have undergone proper training and obtained the required certificates.

This paper was reported using the SPIRIT reporting guidelines [33]. Results from the study will address whether 10-h TRE is effective and feasible in improving health outcomes among career firefighters who do 24-h shift work. Results will be disseminated through peer-reviewed manuscripts, reports, and presentations. Findings, regardless of the result, will be reported as described. Participants will receive their data at the end of the one-year follow-up. This includes summaries of sleep, continuous glucose monitors (when available), blood work, and food entries from baseline, the end-of-intervention, and the 6-,9-, and 12-month follow-up assessments. All co-authors will comply with the International Committee of Medical Journal Editors and no professional writers will be engaged.

Discussion

This study is the first workplace RCT to introduce TRE among the US career shift workers to promote healthy circadian behavioral change within the existing work schedule and culture. The RCT design with a self-guided follow-up period of the trial will demonstrate the feasibility of adopting TRE among fire service personnel without changing their work schedules. The complementary in-clinic and free-living measurements of various vital, behavioral, biochemical, and physiological parameters at multiple time points throughout the study will help assess the

impact of TRE on the multidimensional aspects of health. The use of a smartphone app to collect longitudinal data on nutrition and the use of the same medium for bidirectional engagement with the participants with relevant and actionable educational materials is also a unique strength of this study. If successful, this digital infrastructure will allow rapid dissemination and implementation of the TRE program among fire service personnel.

Why a 10-h TRE? Although TRE with a wide range of eating windows from 6- to 12-h has been shown to offer health benefits in pre-clinical animal models and humans, we chose a 10-h window for several reasons. In both animal models and a recently completed study on patients with metabolic syndrome, 10 h of eating and 14 h of fasting reduced adiposity, improved blood pressure, blood glucose, and blood cholesterol [25]. Therefore, we reasoned 10-h TRE may also benefit the firefighters. A 10-h TRE may also be a feasible and sustainable window for many firefighters. In SDFD and many other fire departments with communal eating at fire stations, breakfast and dinner are typically prepared within a 10 h window with the dinner being served at ~6 pm, which makes adopting to TRE easier. However, adopting this habit at home may be a challenge as the firefighters often want to share meals with their family during off days, and sustained 10-h TRE may involve the participation of the family to incorporate a TRE lifestyle.

Can it be adopted among fire services? If successful, TRE may have a relatively lower barrier to implementation relative to other approaches to reduce circadian disruption. Circadian rhythms are disrupted by light at night, sleep disruption, and erratic eating patterns. Methods to reduce the circadian disruptive impact of light or to improve sleep will likely involve both education and infrastructure investments to optimize lighting to support circadian rhythm or to improve sleep. On the other hand, if TRE is found beneficial, it can be implemented with minimal to no alterations in infrastructure.

Author Contributors

- SP, PT, ENCM, AZ conceived the study concept, developed study design and protocol, applied for funding, and initiated writing the manuscript.
- ENCM, AZ, HCL, AS, NG, AR, AP coordinated the project.
- AZ, AS, HCL, AP, AR, NG, ENCM screened/recruited participants and conducted clinic and fire station visits.
- AZ, AS, HCL, AP, AR, NG, XW, ENCM collected data.
- SG and JF developed and conducted the statistical analysis plan. SP, PT, and ENCM interpreted data.
- SP and PT are the co-principal investigators and the grant holders.
- All authors contributed and approved the final version of the manuscript.

Human Rights and Informed Consent

Ethical approval and oversight of this clinical study were approved and provided by the University of California, San Diego (UCSD) Institutional Review Board Human Research Protections Program (IRB#172083) and the Salk Institute for Biological Studies Institutional Review Board (IRB#18-0001). The study is registered on ClinicalTrials.gov (NCT03533023). All participants provided informed written consent.

Declaration of conflicting Interest. SP is the author of the book "The Circadian Code" for which he collects nominal author royalty. PT is a consultant for Amgen, Esperion, Boehringer Ingelheim, Novo Nordisk, and Sanofi, and is a shareholder for Epirum Bio. ENCM, AZ, HCL, AS, AR, AP, NG, XW, JF, and SG have no conflict of interest to disclose.

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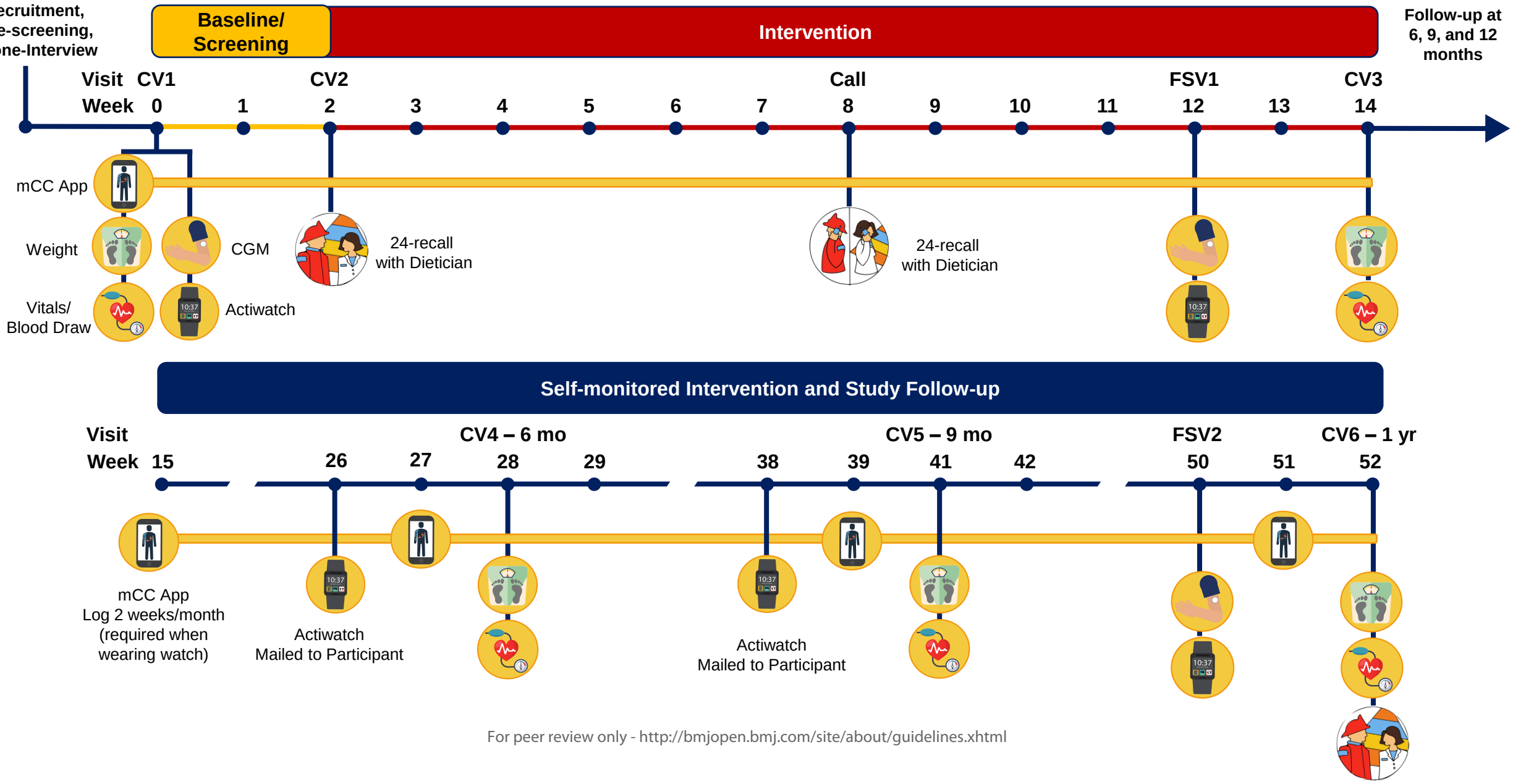
Figure Legend

Figure 1. Study Design and Timeline. mCC, myCircadianClock; CGM, continuous glucose monitor.



Supplemental Information

1. Data safety monitoring plan
2. Privacy and confidentiality
3. Data management

1. Data Safety and Monitoring Plan

The interventions in the study have minimal risk, but any adverse events will be documented. The PI reviews study conduct such as accrual, drop-outs, protocol deviations on a monthly basis. The PI reviews AEs individually real-time and in aggregate on a weekly basis. The PI reviews serious adverse events (SAEs) in real-time. The PI ensures all protocol deviations, AEs, and SAEs are reported to the IRB according to the applicable regulatory requirements.

An internal data quality inspection will be performed. The PI will do a random 10% data audit 1-2x/year. If necessary, re-training of data collectors will be conducted.

2. Privacy and Confidentiality

All study forms including consents, HIPAAs, and case report forms (CRFs), which will include elements from the present and past history, patient demographics, including age, current medications and lab draws, will be stored in in a locked cabinet in Dr. Taub's office in the ACTRI building. Only approved study personnel will have access to this information. To minimize the potential loss of confidentiality, patients will be assigned a unique number as their subject identifier code. The unique subject code will be used to label all study documents.

3. Data management

Data will be collected using standardized paper forms and will be identified with the study's ID of the participant. The codes that link the name of the participant and the study ID will be kept confidential by the Principal Investigator in a secured cabinet. Data will be entered in the computer independently by UCSD certificated and trained data entry staff, and discrepancies corrected by a supervisor based on source documents.

Specimens: Some portions of the specimens collected may be sent to a central laboratory outside of UCSD for testing. All samples will be labeled with a unique specimen code and the only way to link the specimens with the subject code and any personal health identifiers will be on a physical sheet of paper locked in a cabinet in Dr. Taub's office in the ACTRI building.

mCC app data: The mCC app is designed uses HIPAA compliant Amazon Web Server (AWS) for server-side operations. Two large capacity Linux servers (main server and backup) with load balancing are used to capture the data that the smartphone users transmit. The data will be stored on AWS S3 (Simple Storage Service). These two servers will remain online 24h a day, 7 days a week.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	1, 2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	#3	Date and version identifier	Supplement: Informed Consent (each page)
Funding	#4	Sources and types of financial, material, and other support	1
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 20
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A

Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	13
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6,7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-15
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	10
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	10
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from	8

		baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	11, Fig 1, Table 3
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	16, 17
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce	9

		predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6, 9
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			

Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13-15
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	15
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Supplement
Statistics: outcomes	#20a	Statistical methods for analyzing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15-16

Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	7
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomized analysis), and any statistical methods to handle missing data (eg, multiple imputation)	16,17
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Supplement
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Supplement

Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Supplement
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	19
Protocol amendments	#25	Plans for communicating important protocol modifications (e.g, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	17
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorized surrogates, and how (see Item 32)	17
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Supplement

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	19
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2, 17
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	17
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A

Appendices

Informed consent materials	#32	Model consent form and other related documentation given to participants and authorized surrogates	Supplement
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	14, supplement