Research Article

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Impact of medically supervised fasting on the vitamin D, glycemic control, quality of life and need for medication among type 2 diabetes mellitus: Protocol for a randomized control trial (FAVIT Trial)

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Abstract

Objective – The objective of this study is to investigate the effect of prolonged medically supervised fasting (PMSF) for 6 months on vitamin D levels and diabetes-related clinical outcomes in type 2 diabetes mellitus (T2DM) patients.

Methods – This is an open-label, parallel arm, randomized control trial; 170 T2DM patients from a complementary medicine setting, aged between 20 and 70 years, with a body mass index >25 kg/m^2 and glycated hemoglobin (HbA1C) levels >6% and <12%, treated with lifestyle advice and/or oral hypoglycemic drugs, will be randomized to receive PMSF or a diabetic diet (DD), along with their usual care. The patients will be initially trained in PMSF and DD for a duration of 10 days in the study setting, followed by 5 months of practice at their respective residences. The PMSF will fast for five days a month for three consecutive months after the initial exposure, whereas the DD group will follow the same diet for 6 months. Other than fasting days, the PMSF group will follow the same DD as the control group. The primary outcome is an improvement in vitamin D levels, and the secondary outcomes include serum calcium, insulin levels, insulin resistance, blood cell counts, anthropometrics, quality of life, medication score, compliance rate, and disease perception.

Discussion – In this study, we hypothesize that PMSF would gradually increase vitamin D levels, which would increase calcium levels, which would promote insulin secretion and upregulate its function. This may help reduce the need for diabetic medication and result in diabetic remission.

Keywords: diabetes mellitus, fasting therapy, calorie restriction, vitamin D, insulin resistance, inflammation

1 Background and rationale

Type 2 diabetes mellitus (T2DM) is one of the leading causes of morbidity and mortality in India as well as across the globe, with a recent estimate suggesting nearly 537 million individuals living with T2DM [1]. There is a growing body of evidence suggesting that lifestyle changes like dietary modifications and physical activities have a role to play in the prevention and management of diabetes mellitus [2]. Recent literature discusses the existence of an inverse relationship between vitamin D deficiency and the progression of T2DM [3].

Vitamin D deficiency is another emerging public health issue that plays a substantial role in the pathogenesis and progression of various metabolic and immune dysfunctions [3,4]. Numerous reports suggest vitamin D deficiency has a direct role to play in T2DM. Vitamin D is thought to be a catalyst in stimulating the production of insulin by the pancreas. Khudayar et al. reported an 83.2% prevalence of vitamin D deficiency in their recent cross-sectional study of 525 T2DM patients, indicating the importance of considering vitamin D deficiency as a factor influencing the

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progression of T2DM [3]. This is substantiated by an earlier study that reported that hypovitaminosis D promotes insulin resistance and beta-cell dysfunction and that an improvement in vitamin D levels is associated with better glycemic control [5].

Furthermore, T2DM patients with hypovitaminosis D exhibit increased levels of glycated hemoglobin (HbA1C), inflammation, etc. [6,7]. Polymorphisms in vitamin D receptor genes have been linked to insulin secretion and resistance, making vitamin D an important risk factor in the development and progression of T2DM [8]. Vitamin D regulates calbindin, a calcium-binding protein found in pancreatic β-cells, which influences insulin synthesis and secretion [9,10]. Besides this, vitamin D also determines insulin sensitivity by promoting the expression of peroxisome proliferator-activated receptor delta, which has a regulatory role in skeletal muscles and adipose tissue [11,12].

Likewise, the presence of vitamin D is important for improving intestinal calcium absorption by facilitating active calcium transport [13]. This is achieved by improving the synthesis of the calcium-binding protein calbindin. Therefore, adequate vitamin D and calcium are inevitable in the management of T2DM, as they both have implications for insulin resistance, its secretion, and glucose control [14].

While the sun remains the single largest source of vitamin D for humans, the drastic changes in sun exposure habits and increased indoor activities have left the majority of the population vitamin D deficient [15,16]. Inflammation has been identified as the underlying mechanism in both vitamin D deficiency and type 2 diabetes [17,18]. Intervention to enhance vitamin D levels is required since vitamin D levels predict diabetes outcomes [19]. Therefore, lifestyle interventions that can improve vitamin D levels independently of sun exposure are warranted in the management of T2DM.

Therapeutic fasting is known to be beneficial in treating an array of chronic diseases like cardiovascular disease, metabolic syndromes, musculoskeletal disorders, and infections. Prolonged fasting therapy has also been shown to improve vitamin D levels in patients with a variety of chronic conditions, including T2DM [20]. The human body responds to therapeutic fasting by inducing physiologically relevant adaptations that result in metabolic homeostasis [21]. Fasting has been thought to promote the activation of activated protein kinase (AMPK) which is similar to the functions of anti-hyperglycemic drugs like metformin and biguanide [22,23]. AMPK plays a significant role in improving insulin sensitivity and glycemic control [23].

Therapeutic fasting has been shown to increase circulating vitamin D levels in the body through its action on adipose tissue [24]. Our group investigated the role of 10-day medically supervised fasting on the vitamin D levels of healthy female adults and found fasting to significantly improve the vitamin D levels in both healthy volunteers and patients with non-communicable diseases [20,25]. Another quasi-experimental study that used a hypocaloric diet in the management of obesity has also shown that calorie restriction improves vitamin D levels and has found the same to be associated with reduced insulin resistance [26].

A recent clinical trial on calorie restriction on 306 T2DM patients for 12 months has shown that maintaining a calorie 825–853 kcal/day for up to 5 months can help in achieving complete remission of T2DM and cessation of anti-diabetic drugs without any adverse events (AEs) [27]. Barring this one study, all other studies are conducted for a shorter duration with a smaller sample size. Therefore, the proposed study investigates the impact of prolonged medically supervised fasting (PMSF) for 4 months followed by 2 months of follow-up on the vitamin D levels, serum calcium levels, glycemic control, and the need for anti-diabetic medications in T2DM patients.

1.1 Primary study objective

1.1.1 To assess the long-term impact of PMSF on the vitamin D levels of T2DM patients.

1.1.1.1 Secondary study objectives

1. To assess the long-term impact of PMSF on the blood sugar levels and HbA1C levels of T2DM patients.
2. To assess the long-term impact of PMSF on the fasting insulin levels and insulin resistance of T2DM patients.
3. To assess the long-term impact of PMSF on the weight, body mass index (BMI), waist circumference, and hip circumference of T2DM patients.
4. To assess the long-term impact of PMSF on the quality of life of T2DM patients using the World Health Organization (WHO)-Quality of Life BREF questionnaire.
5. To assess the long-term impact of PMSF on the disease perception of T2DM patients using the Brief Illness Perception questionnaire.
6. To assess the long-term impact of PMSF on the medication scores of T2DM patients using a medication score diary.
7. To evaluate the feasibility of PMSF, measured as the percentage of compliance based on the self-report of the study participants in the PMSF group.

2 Methods

2.1 Study setting and design

The study is designed as an open-label, parallel-arm, randomized controlled trial and will be conducted at SantHirdaram Yoga and Nature Cure Hospital, a 125-bed inpatient complementary setting in Bhopal, India. The study protocol was approved by the Institutional Ethics Committee of SantHirdaram Medical College of Naturopathy and Yogic Sciences via F.No. 12/SHMCNYS-IEC/P34 and is being registered as a clinical trial in the Clinical Trial Registry of India (CTRI REF/2023/01/063091).

2.2 Recruitment

Participants will be recruited from the general pool of patients who visit SantHirdaram Yoga and Nature Cure Hospital, Bhopal, for their general health programs. Participants will also be invited to participate in this program through social media invitations and flyers. The participants will be fully informed regarding the disclosure of identifying data, trial procedures, and the total time commitment required, after which written informed consent will be obtained from the potentially eligible participants. After obtaining the consent, the participants will be screened for further eligibility.

2.3 Study population

The study will enroll 178 T2DM patients, both men and women, with a body mass index of >25 kg/m^2 and HbA1C levels of >6% and <12%, who are treated solely with lifestyle advice or with a combination of lifestyle advice and oral hypoglycemic drugs.

2.4 Inclusion criteria

All the participants must meet the following inclusion criteria in order to be eligible to participate in this study:

1. A diagnosis of T2DM;
2. Age >20 years and <70 years;
3. Body Mass Index ≥25;
4. Most recent HbA1C values >6.0% and <12%;
5. Treatment with lifestyle advice with or without oral hypoglycemic drugs.

2.5 Exclusion criteria

Participants who meet any of the following criteria will be excluded from taking part in this study:

1. Patients who are on insulin therapy;
2. Any recent history (<6 months) of myocardial infarction;
3. Any past history of syncope with calorie restriction/fasting;
4. Known cases of severe kidney, heart, hematological, or liver diseases;
5. Autoimmune diseases or patients who are on immunosuppressive drugs;
6. Pregnant women and lactating mothers;
7. Patients who are a part of any other diet modification or lifestyle programs;
8. Patients who are a part of any other clinical trial;
9. Patients who practiced fasting regularly by themselves prior to the study.

2.6 Screening

All the participants will be interviewed to obtain their medical history, followed by a physical examination to test their eligibility for participation. A fasting blood sample will be collected from all the study participants to determine their HbA1C levels and assess their further eligibility.

2.7 Intervention

Participants will be randomized to receive PMSF delivered in two phases: Phase A: In-house training for 10 days of PMSF in the study setting. Phase B: Virtually guided PMSF practice for 5 days at the participants' homes or to a control group who will initially receive a diabetic diet (DD) for 10 days in the study setting, which they will continue to follow at their respective homes throughout the study period. Both the group participants will continue their medication use during the study period as advised by their general practitioners, including the PMSF period.

2.8 PMSF

2.8.1 Phase A: in-house training for fasting in the study settings

During this time, the PMSF group will receive in-house, hands-on training to fast for 10 days, which will be divided

into three phases: the preparatory phase (days 1, 2, and 3), where a cooked diet will be given on day 1, followed by a boiled diet on day 2, and a raw diet on day 3 (approximately 1,000 kcal/day). The second phase will be the fasting phase (days 4–7), during which the patient will be given various juices with an approximate calorie count of 500 kcal/day, and the third phase will be the refeeding phase (days 7–10), where the patient will be given a raw diet followed by a boiled and cooked diet (approximately 1,000 kcal/day). Calorie intake is gradually reduced until day 3, then the participants fast for 4 days before gradually increasing from day 7 to day 10.

2.8.2 Phase B: virtually guided, medically supervised fasting at the participants' homes

After the initial exposure in the study setting, the participant will be guided to practice a 5-day fasting program that includes a 1-day preparatory phase (approximately 1,000 kcal), a 3-day fasting period (approximately 500 kcal), and a 1-day refeed (approximately 1,000 kcal) for three consecutive months. This program will be supervised by a licensed yoga and naturopathy physician through an audio–video software program (Zoom.us) where the patient will be given a consultation for any queries, and additionally, an online interactive channel (24 × 7) will be made available for the participants during the 5 days of virtually guided fasting.

After 4 months, the experimental group will be on a DD similar to the control group but may continue to practice 5-day fasting once a month for the next 2 months. The purpose of the 2-month follow-up is primarily to assess the long-term effects as well as the patients' adherence to PMSF. The dietary recall will be collected every month through an online consultation to document the consumption patterns during the DD. The experimental group participants will be on the same DD as those in the control group on the rest of the days when they are not fasting throughout the 6 months of the trial.

2.9 Control group

The control group will be on a DD that includes whole grains, fruits, nuts, seeds vegetables, salads, roti (Indian breads), and brown rice (approximately 1,500 kcal/day) during their inpatient stay (10 days as like that of PMSF group). The control group participants will be on the same DD (approximately 1,500 kcal/day) as suggested during their inpatient stay for the duration of the trial. The control group will be provided with one diet consultation per month to reiterate their diet plan and collect a food recall.

In addition to this, both groups will receive yoga and naturopathic interventions like hydrotherapy, mud therapy, massage, enema, etc., during their inpatient stay to support fasting therapy by promoting elimination. These therapies are recommended during fasting as per the international consensus on fasting therapy [28]. The detailed treatment plan is tabulated in supplemental file 1.

2.10 Randomization and treatment allocation

After screening using the inclusion criteria, the patients will be randomized into two groups at a ratio of 1:1. A simple randomization technique will be used for randomization using the computer-generated random numbers generated through the software Randomization Service for Multicenter Clinical Trials (www.randomizer.at). Allocation concealment will be done using the sequentially numbered, opaque, and sealed envelope technique.

2.11 Blinding

This study will be conducted as an assessor-blinded study, as the support staff will be collecting the data from the participants (anthropometric measures, medication scores, and research questionnaires), and the laboratory personnel will be blinded to allocation. The investigators and the participants cannot be blinded, as this is a dietary intervention study.

2.12 Data collection

Data collection will be performed by the support staff of the hospital, who will be trained on all the trial-related data collection methods both in the study setting and through online assessments. The data will be collected in person from the patient at the study setting for the baseline and post-intervention assessments during phase A. Following this, all the anthropometric measurements, vitals, medication scores, and AEs (if any) will be collected through a telephone call throughout phase B. The measures related to the quality of life, disease perception measures, and adherence scores will be collected electronically through Google Forms. The fasting blood sample will be collected from the home of each participant through a centrally operated laboratory

chain from month 2 through month 6. The investigators will store all the data (medical records, case reports, medication records, study-related forms, etc.) according to the GCP guidelines. The overview of data collection time points in relation to the intervention is depicted in Figure 1.

2.13 Outcomes

The overview of outcome assessments during the 6-month trial period is depicted in Table 1. Anthropometric measurements include height, weight, BMI, waist circumference, and hip circumference. The vitals include sitting blood pressure (measured using a digital sphygmomanometer) where an average of two measurements will be taken. The patients will be given a personal digital sphygmomanometer to measure their blood pressure and an average of two measurements will be taken. Fasting blood samples will be collected for laboratory tests at all time points (Table 1).

2.14 Primary outcome

The primary endpoint of the study is an improvement in the vitamin D levels from the baseline. The change will be compared between the PMSF group and the control group after 6 months. A successful outcome will be defined as a statistically significant ($P \leq 0.05$) increase in the vitamin D levels in the PMSF group compared to their control group.

2.15 Secondary outcomes

The following secondary outcome measures will be recorded at the baseline (day 0), the 10th day after the end of the first intervention, month 2 (day 5 of fasting), month 3 (day 5 of fasting), month 4 (day 5 of fasting), month 5 (30 days from the last fasting period), and month 6 (60 days from the last fasting period) in both groups.

1. weight, waist circumference, hip circumference, and body mass index;

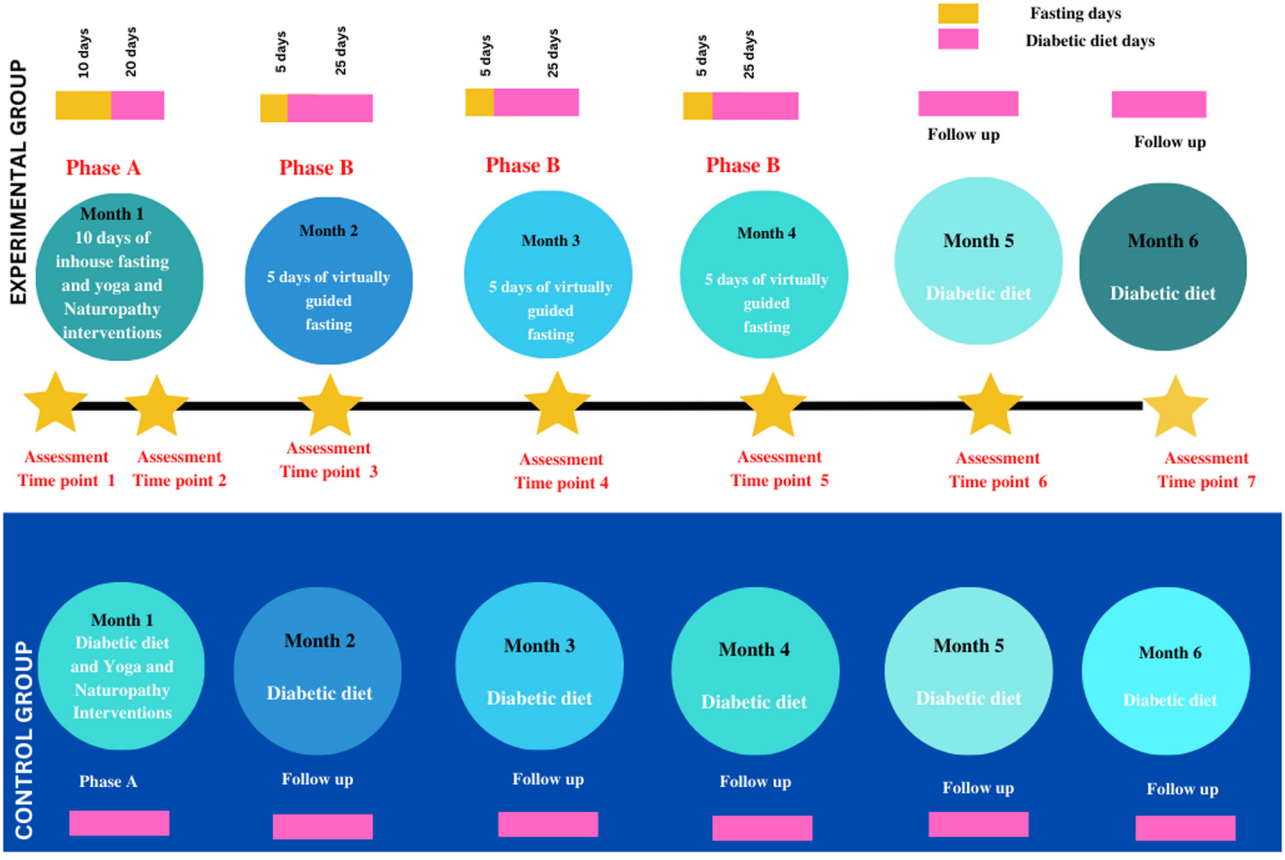

Figure 1: Timeline of assessments from baseline to follow-up in relation to the timing of the Intervention.

Table 1: Time points of assessments during the trial

Timepoints Assessments	T0 Month-0 before trial	T1 Month 1 baseline day 1 of inhouse patient stay	T2 Month 1 day 10 of inhouse patient stay	T3 Month 2	T4 Month 3	T5 Month 4	T6 Month 5	T7 Month 6
Recruitment	*							
Screening: medical history, HbA1C levels	*							
Informed consent	*							
Enrolment	*							
Allocation	*							
Intervention		*	*	*	*	*	Follow-up^	Follow-up^
Serum vitamin D		*	*	*	*	*	*	*
Serum total calcium		*	*	*	*	*	*	*
Fasting blood sugar		*	*	*	*	*	*	*
Fasting insulin		*	*	*	*	*	*	*
HOMA – homeostatic model assessment		*	*	*	*	*	*	*
HbA1C		*	*	*	*	*	*	*
Complete blood count		*	*	*	*	*	*	*
WHO-QoL-Bref		*	*	*	*	*	*	*
BIPQ		*	*	*	*	*	*	*
Medication dosage		*	*	*	*	*	*	*
Compliance VAS score		*	*	*	*	*	*	*
Blood pressure		*	*	*	*	*	*	*
Weekly food recall questionnaire		*	*	*	*	*	*	*
Anthropometrics		*	*	*	*	*	*	*
Assessment of safety/ reporting of adverse events		*	*	*	*	*	*	*

T – Time points; HbA1C – glycated hemoglobin; QOL – Quality of life; ^DD will be followed with or without 5-day fasting; *Data collected.

2. blood pressure and pulse rate;
3. fasting blood sugar;
4. serum total calcium;
5. fasting insulin;
6. HOMA – Homeostatic model assessment (for evaluating insulin resistance);
7. HbA1C – glycosylated hemoglobin;
8. complete blood count;
9. World Health Organization Quality of Life (WHO-QoL-Bref);
10. Brief Illness Perception Questionnaire (BIPQ).

Apart from the above, the following measures will be recorded at the pre-determined intervals:

2.15.1 Medication scores

The medication intake will be captured using a personal diary where the participants from both groups will be asked to record their medication doses at baseline, at the end of 10 days of inpatient stay, and then, every 30 days from the day of discharge from the inpatient setting up to 6 months.

2.15.2 Compliance scores

Fasting program compliance will be measured using a self-reported visual analog score on a 10 cm linear scale, with a higher score indicating greater ease in adhering to the fasting protocol. This VAS score will be collected from the PMSF at the end of each fasting program.

2.15.3 Weekly food recall questionnaire

A weekly food recall questionnaire will be used to assess the pattern of food intake in both groups and will be

collected in both groups by the end of each month from the day of discharge from the inpatient setting.

2.16 Power calculation

The sample size was calculated based on a previous study [20] which studied the impact of therapeutic fasting on vitamin D levels in patients with non-communicable diseases including T2DM. For an effect size (d) = 0.475; level of significance (α) = 0.05 and Power (β) = 80%, the sample size required was 142; however, considering an attrition rate of 20%, we calculated the final sample size required as n = 178, with each group requiring 89 participants.

2.17 Statistical analysis

The data collected in each phase will be entered into Microsoft Excel spreadsheets and will be imported to the International Business Machine Statistical Package for Social Sciences, Version 27 for cleaning and analysis. The analysis will be carried out employing the intention-to-treat principle including all the study samples who were randomized and included in intervention and control groups [29]. Missing data if any will be treated employing the Last Observation Carried Forward (LOCF) [30] and multiple imputation methods [31]. The continuous variables captured in the study will be assessed for the presence of normal distribution employing Kolmogorov–Smirnov test [32].

2.18 Baseline data

The baseline data across the sample characteristics and study variables will be compared between intervention and control groups. The sample characteristics that will be compared include age, gender, medical history, socioeconomic status, habits, previous food consumption patterns (quantified in calories), drug history, anthropometrics, and vitals. The study variables compared include the primary outcome measure (i.e., Vitamin-D level) and the secondary outcome measures (i.e., weight, waist circumference, hip circumference, BMI, blood pressure, pulse rate, FBS, serum total calcium, fasting insulin, HOMA, and HbA1C). Frequencies and percentages will be used to summarize categorical variables. Normally distributed continuous variables will be summarized using mean and standard deviation. The median and interquartile range will be used to summarize non-normal variables.

The statistical significance of the baseline differences between the groups will be assessed with a null hypothesis that at baseline, there is no significant difference between the groups across the study variables. The differences for categorical variables will be assessed employing a chi-squared test for homogeneity. The continuous variables adhering to the assumption of normality will be tested employing an independent-sample t-test. The continuous variables that do not adhere to the assumption of normality will be tested employing the Mann–Whitney U-test [33]

2.19 Primary and secondary outcomes

The primary and secondary outcomes will be compared between the intervention and control groups to ascertain the effect of intervention (i.e., PMSF) on the outcome variables studied. The measures in the outcome variables captured at the end of the 6th month (i.e., T7) will be compared between the intervention and control groups. The mean and standard deviation (for normally distributed variables) or median and interquartile range (for non-normal variables) will be compared. The independent sample t-test will be used to assess for significance of the difference between the primary and secondary outcome measures which are continuous. For the primary and secondary outcome measures which are not continuous, Mann–Whitney U-test will be used to assess if the outcome measures in intervention and control arms significantly differ from each other. Cohen's d will be measured as a standardized effect size to determine the impact of the intervention on study variables [34].

Multivariate procedures will be employed to determine the impact of intervention after adjusting for any potential baseline differences in the outcome variables across intervention and control groups. For the normally distributed study variables, Analysis of Covariance will be employed with the post-intervention measurement of the outcome variables (captured at T7) as the dependent variable, baseline measurement (captured at T0) as the fixed factor, and the intervention status as a covariate. Partial Eta-squared (η^2) will be computed as a measure of effect size. The non-normal outcome variables will be analyzed employing a generalized linear model, with the post-intervention measurement of the outcome variables (T7) as the dependent variable, baseline measurement (T0) as an offset factor, and intervention as a covariate. Adjusted mean difference will be computed as a measure of effect size.

2.20 Participant withdrawal

All the participants are free to walk out of the trial at any point in time without giving any reason for their withdrawal. The discontinued or withdrawn participants will not be replaced as the trial has included 20% additional samples to maintain the desired power in case of participants' withdrawal or loss to follow-up. All the participants randomized to intervention and control arms will be included in the final analysis using intention-to-treat analysis. For the participants who drop out of the study mid-way LOCF method will be employed to avoid missing data. The investigators can withdraw a participant from continuing the program in the case of any medical emergencies. The reason for the discontinuation will be documented, and all the data collected until the point of discontinuation will be analyzed.

2.21 Safety

The participants from both groups will be trained to check their random blood sugar (RBS) levels using a glucometer provided to them by the research team. RBS will be checked multiple times during the fasting period and as and when the patients feel any dizzinessor hypoglycemic symptoms. Furthermore, any incidental findings (new comorbidities) that may have a significant impact on the patient's health will be documented, both to ensure their safety and to assist the investigators in deciding whether to withdraw them from the trial.

2.22 AEs

In general, there are no foreseen serious adverse events (SAE) or AEs expected while delivering fasting therapy, as fasting is only known to induce a few self-limiting symptoms like headache, fatigue, nausea, vomiting, diarrhea, fever, and dizziness [20,35]. The patient will be trained to self-manage these symptoms during their inpatient stay. During the virtually guided, medically supervised fasting, the same will be monitored. The participants will be provided with an emergency contact line for reporting if there are any SAEs or AEs. Additionally, SAEs and AEs will be recorded during every telephone contact for data collection.

2.22.1 Medication use

The patients will continue with their anti-diabetic medications with constant monitoring of their blood glucose levels. All the changes in medication dose or addition of anti-diabetic medications made by the patient's general practitioner during the trial period will be documented.

2.22.2 Monitoring

The institutional ethics committee will serve as the monitory board and the investigators will be submitting a safety report to the committee every 6 months. No interim analysis will be performed.

3 Discussion

Maintaining optimal blood glucose levels through a healthy eating pattern, regular physical activity, and promoting weight loss has been shown to significantly attenuate the progression of T2DM and its complications [36,37]. Indian complementary medicine settings, especially the yoga and naturopathy facilities, use medically supervised therapeutic fasting as a first-line management strategy in many diseases, including T2DM [38]. Therapeutic fasting or calorie restriction interventions are known to be beneficial in the management of T2DM by improving glycemic control, preventing complications, reducing the need for medications, and offering remission [27,39].

While the benefit of fasting in T2DM is well known, the mechanisms behind its effectiveness are less explored. Numerous preclinical studies suggest various mechanisms by which fasting affects T2DM, such as the restoration of the autophagic reflex in pancreatic islets, [40] activation of neurogenin 3, a progenitor cell responsible for alpha and beta cell regeneration, [41] reduction of gut dysbiosis, [42] and increased adipocyte-specific glucose transporter 4 expression. Recent evidence suggests an increased prevalence of hypovitaminosis D among T2DM patients and suggests vitamin D plays a definite role in improving insulin sensitivity, beta cell function, reducing HbA1C levels, etc. [3–7].

Studies on PMSF suggest that PMSF improves vitamin D levels by its action on adipose tissues [20,24,25,43], which catalyze intestinal calcium absorption [44,45] and subsequently upregulate insulin function, secretion, and glycemic control [44,45]. To date, no long-term studies have been conducted to confirm whether PMSF can progressively maintain vitamin D and calcium levels in T2DM patients, as well as its role in reducing the need for antidiabetic medications and reversing T2DM. Further, adherence to long-term fasting or calorie restriction programs is considered difficult. Supervised dietary programs with adequate social support and monitoring are thought to improve adherence to long-term programs [46].

The fasting and vitamin D trial uses a supervised fasting program that is divided into two phases, the first of which trains patients to acclimate to the fasting program and the second of which they are virtually guided. This may help in overcoming adherence-related issues while delivering fasting regimens and may also provide insights into the feasibility of guiding or supervising fasting programs in remote mode. Additionally, this randomized control trial may provide mechanistic insights into the role of PMSF in T2DM management. Furthermore, the outcomes of this trial may help in devising clinical practice guidelines for introducing PMSF into T2DM prevention and management. The enrolment for this trial is expected to start in March 2024.

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Conflict of interest: The authors state no conflict of interest

Ethical approval: The research related to human use has been complied with all the relevant national regulations, and institutional policies and in accordance with the tenets of the Helsinki Declaration, and has been approved by the Institutional Ethics Committee of SantHirdaram Medical College of Naturopathy and Yogic Sciences via F.No. 12/SHMCNYS-IEC/P34. The trial is being registered as a clinical trial in the Clinical Trial Registry of India (CTRI REF/2023/01/063091).

Informed consent: Informed consent has been obtained from all individuals included in this study.

Data availability statement: Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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