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A technology assisted precision ketogenic diet intervention for cardio-renal-metabolic health in overweight or obese adults: Protocol for a randomized controlled trial

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ABSTRACT

Background: The obesity epidemic is a public health concern, as it is associated with a variety of chronic conditions. The ketogenic diet has drawn much scientific and public attention. However, implementation is challenging and its effect on cardio-renal-metabolic health is inconclusive. This study will assess the feasibility, acceptability, and preliminary efficacy of a technology-assisted ketogenic diet on cardio-renal-metabolic health.
Methods: This is a single center, 6-month, stratified, randomized controlled trial. A total of 60 overweight/obese adults (18+ years old) will be enrolled, including 20 without type 2 diabetes (T2D) and without chronic kidney disease (CKD); 20 with T2D, but without CKD; and 20 with early-stage CKD. Participants will be stratified based on health conditions and randomized into a ketogenic diet (n = 30) or a low-fat diet group (n = 30). Health education involving diet and physical activity will be delivered both digitally and in-person. Mobile and connected health technologies will be used to track lifestyle behaviors and health indicators, as well as provide weekly feedback. The primary outcome (weight) and the secondary outcomes (e.g., blood pressure, glycemic control, renal health) will be assessed with traditional measurements and metabolomics.
Discussion: Mobile and connected health technologies provide new opportunities to improve chronic condition management, health education attendance, planned lifestyle changes and engagement, and health outcomes. The advancement of bioinformatics technology offers the possibility to profile and analyze omics data which may advance our understanding of the underlying mechanisms of intervention effects on health outcomes at the molecular level for personalized and precision lifestyle interventions.

1. Introduction

Obesity is a major public health crisis worldwide. In 2014, more than 2.1 billion people, (~30% of the global population), were overweight or obese [1]. In the United States (US), the prevalence of obesity has increased rapidly in the adult population, from 3.4% in 1962 to 42.4% in 2017–2018 [2]. The aggregate medical costs of noninstitutionalized obese US adults rose from $212.4 billion US dollars in 2005 to $315.8 billion in 2020 [3], partially attributed to the relationship between excessive body fat and the development of chronic conditions, including but not limited to cardiovascular disease, type 2 diabetes (T2D), and chronic kidney disease (CKD) [4,5]. These interrelated health conditions

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including hypertension, insulin resistance, metabolic dyslipidemia, obesity, microalbuminuria, and/or reduced renal function can be referred to as Cardio-Renal-Metabolic (CRM) health [6]. Finding an effective strategy to improve CRM health for overweight and obese populations is urgently needed.

Lifestyle interventions targeting diet and physical activity have yielded favorable weight, cardiovascular, glycemic and renal outcomes [7–14]. Weight loss is associated with improved glycemic control, renal function and cardiovascular health in overweight/obese prediabetic or T2D patients [15,16]. However, the diet regimens to optimize health outcomes for overweight/obese adults is still elusive. For decades, a low-fat, low-calorie diet has been implemented to control weight, prevent and/or mange T2D, and delay or reduce complications of diabetes (e.g., cardiovascular disease, renal disease). Both the diabetes prevention program (DPP) and the Look AHEAD (Action for Health in Diabetes) lifestyle interventions apply a low-fat diet for weight control, prevention and management of diabetes as well as the prevention of diabetic complications such cardiovascular disease and kidney disease [15,16].

In contrast, the ketogenic diet is a very low-carb and high-fat diet regimen, which has drawn much attention from the public and the scientific communities regarding its effects on weight control, glycemic management, cardiovascular health and renal function [17–19]. A ketogenic diet can lead to metabolic processes such as gluconeogenesis and ketogenesis, and consequently influence various health outcomes such as body weight, lipid profile, and glucose metabolism in overweight/obese individuals with or without T2D [20,21]. A recent meta-analysis by Choi, Jeon and Shin [20] found that in overweight/obese adults, a ketogenic diet can reduce body weight by 7.78 and 3.81 kg with and without T2D, respectively, indicating T2D patients are more likely to benefit from a ketogenic diet for weight loss than their counterparts on a low-fat diet. Yuan and colleagues [21] analyzed data from 13 studies and reported that the ketogenic diet was associated with significant reduction in fasting blood glucose by 1.29 mmol/l (95% CI: 0.79, 1.78), hemoglobin A1c (HbA1c) by 1.07% (95% CI: 0.78, 1.37) and increased insulin sensitivity.

The benefits of a ketogenic diet on long-term glycemic control are unclear, and its effects on cardiovascular and renal outcomes are controversial [19]. One of the concerns is the potential impairment on renal health [22]. Crosby and colleagues [22] reviewed the ketogenic diet's effects on chronic disease and indicated that high protein consumption may facilitate hyperfiltration and lead to long-term damage in people with CKD. Additionally, for those without CKD, a ketogenic diet with animal-sourced foods may increase the risk of developing CKD through the consumption of animal fat and protein. However, a recent study reported that after three months on a ketogenic diet, significant weight loss was found in obese patients with mild kidney failure and no variations of renal function were found, concluding that a ketogenic diet is an effective and safe approach to improving weight, even in those with renal impairment [23]. A previous review has reported the effects of a ketogenic diet on cardiovascular health indicators as debatable, arguing that even though a ketogenic diet is associated with increases in high-density lipoprotein (HDL) cholesterols levels, it may also increase low-density lipoprotein (LDL) cholesterol levels, and the high fat content of a ketogenic diet may increase cardiovascular risks as well [24]. Given the controversial findings, innovative ways to assess the effects of ketogenic diet are needed.

The advances in "omics" and related analytical techniques have led to the tremendous scope of their application in lifestyle science. Previous studies identified that a ketogenic diet induced changes in the epigenome and gut microbiome in both human and animal models [25–27]. Additionally, the ketogenic diet is reported to improve mitochondrial function, which is closely related to CRM health [28,29]. Few studies have examined the metabolomic effects from a ketogenic diet, and the available literature is currently limited to animal models [30]. Examining the changes and alterations of metabolomic features may provide an understanding of the underlying mechanisms of a ketogenic diet on health outcomes. Modern metabolomic techniques (e.g., MALDI-MSI analysis, ZipChip-MS analysis) give the possibility to analyze and measure hundreds of small molecules to detect changes which are related not only to energetic metabolisms of ketogenic diet, but also lipid, nucleic and amino acid metabolisms [31,32]. Such techniques offer the promising possibility to obtain detailed pictures of the dynamic state of metabolism on the individual cell, tissue/organ and whole organism level, which could give a more complete understanding of the molecular organization and regulation of the organism under different conditions [31,33]. Recently, Sharma and his team identified novel metabolites (e.g., amino acids and organic acids) associated with kidney function, particularly in those with CKD and diabetes [29,34]. These novel markers may help assess intervention response, predict disease status, and identify precision therapeutic and intervention strategies.

Despite various reported potential health benefits of a ketogenic diet, implementing such a diet intervention is challenging. Significant barriers to engage people for attending education sessions include the lack of time, transportation, social support, and/or financial resources [35]. Adherence to the ketogenic diet regimen remains another barrier to implementation and assessing the efficacy on health outcomes in both clinical and research settings [36]. Therefore, the purpose of this study is to assess the feasibility and the preliminary efficacy of a technology-assisted lifestyle intervention with a ketogenic diet and physical activity on CRM health in a 6-month randomized controlled trial in 60 overweight/obese adults with or without T2D and CKD. The intervention will be enhanced by hybrid in-person and digital education sessions, mobile and connected health technologies for self-monitoring, and AI-generated personalized feedback. Traditional health biomarkers (e.g., HbA1c, lipid profile) and omics features (e.g., metabolites and mitochondrial DNA) will be used. We hypothesize that compared to a low-fat and low-calorie diet, a ketogenic diet is superior in weight control and CRM health in both traditional and omics markers.

2. Methods

2.1. Study design and study population

This study is a single center, 6-month, stratified, randomized controlled trial. A convenient sample size of 60 overweight/obese adults age 18+ years old will be enrolled, including 20 being overweight/obese without T2D or CKD; 20 being overweight/obese with T2D, but without CKD; and 20 being overweight/obese with early-stage CKD (10 with T2D and 10 without T2D). The sample size of 60 is determined by Kieser and Wassmer's recommendation of 40 for a pilot trial sample size [37]. Considering a 66% retention rate based on a previous 6-month ketogenic diet study, we will recruit 60 participants (30 in ketogenic diet group, 30 in low-fat group) for this study [38].

The inclusion criteria are: (1) 18+ years old; (2) overweight/obese [Body Mass Index (BMI) $\geq$ 25.0 kg/m^2]; (3) with/without T2D (self-reported); (4) with/without early-stage CKD (self-reported or evidence of CKD with 60 $\leq$ estimated glomerular filtration rate (eGFR) <90 mL/min/1.73m^2 or 30 mg/g $\leq$ albumin-to-creatinine ratio (ACR) < 1000 mg/g within the past 12 months or at site screening); (5) being able to read and write in English; and (6) owing a smartphone. Exclusionary criteria are: (1) unable or unwilling to provide informed consent; (2) non-English speaking; (3) unwilling to accept random assignment; (4) pregnant or breast feeding; (5) unable to walk without assistance; (6) type 1 diabetes; (7) under SGLT2 inhibitor treatment; (8) enrollment in other low-carb/ketogenic diet or weight loss programs; (9) triglyceride $\geq$500 mg/dL or with LDL cholesterol $\geq$129 mg/dL; (10) severe psychiatric disorders deemed by investigators which might interfere with study procedures; (11) severe chronic conditions (e.g. severe heart disease, renal disease, cognitive impairment) that would preclude them from participating; and (12) having plans to leave the city or US for over 2 weeks within 6 months of enrollment. If deemed eligible with telephone screening, a site visit will be scheduled for screening.

All participants will receive information on the program and give verbal consent before screening procedures to determine eligibility. Participants will provide written, informed consent (Supplementary file 2) once deemed eligible, and before entering the study. This study was approved by the University of Texas Health Science Center at San Antonio (UTHSCSA) institutional review board (IRB) (Protocol Number: HSC20190528H) and registered at http://ClinicialTrials.gov (NCT05071287). This study protocol version number is 4, dated March 9, 2021. Any significant modifications to the protocol will require a formal amendment and approval from the Office of the IRB, UTHSCSA.

2.2. Recruitment

We will recruit participants from several sources: (1) electronic invitations sent through the University Health System's (UHS) electronic medical record (EMR) messaging system, MyChart; (2) onsite recruitment for UTHSCSA patients; (3) UTHSCSA Find-a-Study system; (4) flyers posted at public places (e.g., UTHSCSA, public libraries, churches, etc.); and (5) direct clinician referrals. To recruit using EMR, we will send an IRB approved invitation message to potential participants who previously consented to be contacted about research studies for which they may be eligible. A list of potentially eligible patients will be generated via EMR platforms (e.g., Epic), including those who are overweight/obese based on BMI, and/or who have an International Classification of Diseasescode consistent with T2D.

2.3. Randomization

Eligible participants will be randomly assigned to either a ketogenic diet group ($n = 30$) or a low-fat/low-calorie diet group (n = 30) with a 1:1 allocation. Random allocation sequences will be generated by a statistician with stratification by T2D status (diagnosis of T2D; no diagnosis of T2D) and CKD status (evidence of CKD; no evidence of CKD), which will be uploaded to REDCap for randomization (Fig. 1). Concealment will be ensured as the REDCap system will not release randomization results until the participant provides written consent and is enrolled. Participants, the dietitian, and the research staff will not be blinded to the allocation due to the nature of the study. The principal investigators (PIs) and data statistician will be blinded to the study arms.

2.4. Intervention

The intervention will be 6 months. Enrolled participants will receive usual care throughout the study. The lifestyle intervention, involving diet and physical activity, is developed according to the DPP and Look AHEAD lifestyle intervention programs. The diet components of the intervention are modified for participants per assigned study arms. To ensure participant adherence to diet, physical activity goals, and further achievement of intervention aims, we will use mHealth tools to facilitate the delivery of behavior modification techniques.

2.4.1. Major components

There are 3 major components of the intervention (Table 1): lifestyle management education, mobile device assisted self-monitoring, and personalized feedback. Each enrolled participant will receive a package with digital health tools, a personalized diet handout, and program-related resources and materials. Participants will be instructed to install apps on their phone, connect the digital health tools, become familiar with the devices, and scheduled for their first individual intervention session.

2.4.2. Education sessions

Individual Intervention Sessions. Individual intervention sessions, held either in-person or virtually, will occur three times during the intervention (beginning, 3-months and 5-months). Protocol for the sessions will be established by a licensed dietician, who will train the research team to assist with them. Due to the intricacy of the ketogenic diet and complexity of questions that could arise, keto diet sessions being supervised or lead by the licensed dietitian will be encouraged. The first session will facilitate participant understanding of their personalized diet, weight and physical activity goals. Upon completion, participants will be instructed to initiate the diet regimen per their assigned arm, beginning day one in the intervention. Individual sessions will also occur at 3 months and 5 months. During these sessions, participant experiences and perceptions of the program will be collected in addition to any questions or concerns regarding the diet regimen and program being discussed and addressed.

Digital Lifestyle Education. Participants are more likely to be motivated and engaged in behavior changes if they are well-trained.

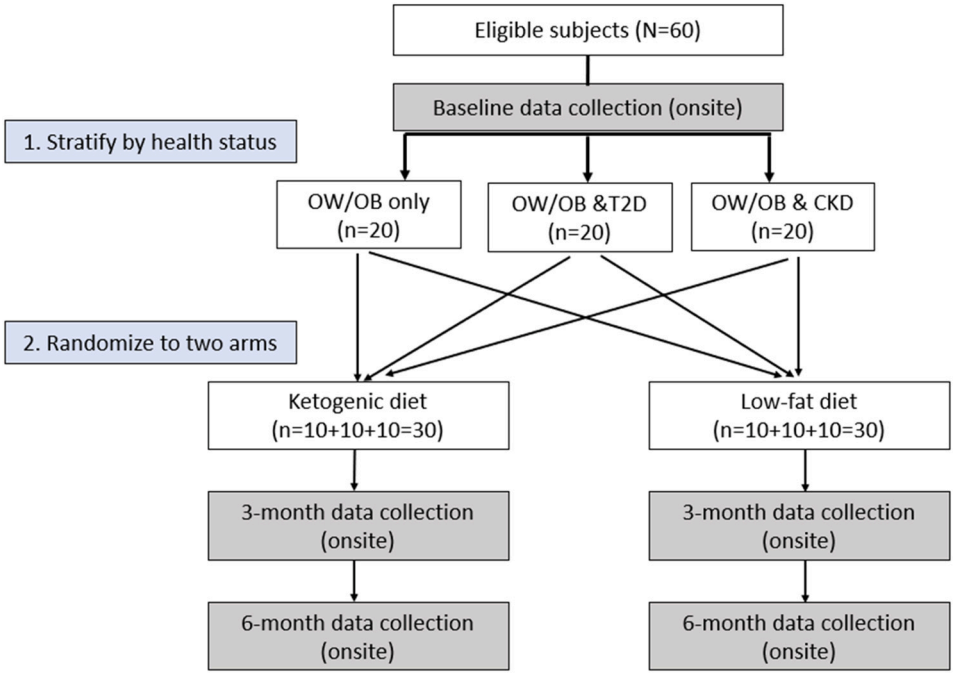

Fig. 1. Flow diagram of the randomization. Dark gray boxes indicate data collection visits occurring onsite. In addition to onsite data collection, lifestyle and health data will also be collected through mobile, digital, and electronic means throughout the intervention. OW: overweight; OB: obese; T2D: type 2 diabetes; CKD: chronic kidney disease; OW/OB only: participants being overweight/obese without T2D and without CKD; OW/OB&T2D: overweight/obese with T2D and without CKD; OW/OB&CKD: overweight/obese with early-stage CKD ($n = 10$ with T2D and n = 10 without T2D).

Table 1
Summary of intervention components.

Intervention Components	Ketogenic Diet group	Low-fat Diet group	Frequency and format
Lifestyle Management			
Education (goal setting, knowledge empowerment, self-monitoring, problem solving, etc.)			3 individual intervention sessions, virtual or in-person; 12 digital lifestyle education sessions
Diet	Ketogenic diet	Low-fat diet	Individual sessions and digital sessions
Physical activity	Yes	Yes	Individual sessions and digital sessions
Weight	Yes	Yes	Individual sessions and digital sessions
Self-Monitoring			
Diet	Yes	Yes	Daily
Physical activity	Yes	Yes	Daily
Glucose	Yes	Yes	Daily (those with diabetes) Weekly (those without diabetes)
Weight	Yes	Yes	Daily
Blood pressure	Yes	Yes	Weekly
Ketone	Yes	No	Daily during the first two weeks, then twice weekly thereafter
Personalized Feedback	Yes	Yes	Weekly

Note, other content of education sessions is presented in Table 2.

Digital platforms have great potential for delivering health education in a cost-effective manner, as demonstrated during the COVID-19 pandemic [39]. Therefore, in addition to the individual intervention sessions, we will use Articulate 360 (Articulate Global LLC, NY) e-learning software to provide education for behavior skills and instructions on how to use mHealth technologies. The aim is to help participants incorporate knowledge and skills and feel empowered to make behavior changes.

We will deliver one new asynchronous interactive digital lifestyle education session (Supplementary Fig. S1) each week during the first 3 months (total = 12 sessions). The sessions are aligned with best practices for lifestyle interventions and adapted from the group-based DPP Group Lifestyle Balance™ (GLB) Program to fit into the e-learning environment. General adaptations include 1) modification to be individual-based; 2) embedded JavaScript to track learning progress; 3) narrated animations; 4) interactive content including video clips, quizzes, free text reflections, webpage resources; and 5) reduced content length (<10–15 min per lesson). A registered dietitian developed the ketogenic diet and MyPlate content to replace the healthy eating sessions in the original curriculum. All lessons were reviewed by the study team. Table 2 is a summary of session topics.

Table 2
The outline of 12 digital lifestyle education sessions.

Week	Ketogenic Diet Group	Low-Fat Diet Group
0	Program Introduction	
1-1	What is ketogenic diet?	What is MyPlate?
1-2	Ketogenic diet adaptation and preparation	How to prepare for MyPlate?
2-1	Burn more calories than you take in	
2-2	Use digital tools to self-monitor	
3	Make sense of food labels	
4	All about carbohydrates	
5-1	Cholesterol	
5-2	Understanding fat	
6	Portion control	
7	Physical activity	
8	Grocery shopping	
9	Eating out healthy	
10	Problem solving Coping with social cues	
11	Mindful eating	
12	Holiday eating	

Personalized Ketogenic Diet. Participants in the ketogenic group will be on a very low-carb, high fat, ketogenic diet. We will recommend a carbohydrate, protein, and fat intake goal based on a 1.5:1 ketogenic ratio (1.5 g of fat to 1 g of carbohydrate and protein combined). Recommended daily calorie consumption will be individualized using the Mifflin-St. Jeor equation (using body weight) to predict resting metabolic rate (RMR). Total energy expenditure (TEE) will be calculated by multiplying the RMR by an activity factor [40]. The activity factor is estimated according to the level of activity of each individual and categorized into sedentary, low active, active or very active. The values of 1.00, 1.11, 1.25, and 1.48 are used for male, and 1.00, 1.12, 1.27, 1.45 for female, respectively [41]. The recommended amount of protein intake will be 1.0 to 1.2 g/kg of body weight per day using the Hamwi Formula to calculate ideal body weight with an adjustment for frame size [42]. Adults under the age of 65 years will be given a protein recommendation of 1.0 g/kg of body weight/day, and 1.2 g/kg of body weight/day for adults older than 65 years [43].

The macronutrient distribution will be ~10% energy intake from carbohydrates, ~10–20% from protein, and ~ 70%–80% from fat. A daily multivitamin as well as vitamin D will be recommended for participants during the study. They will also be advised to discuss supplementation and potential medication adjustments with their primary care provider or other health care professional.

Participants in the ketogenic diet group will receive a handout with personalized macronutrient goals, food equivalents, example meals, a ketogenic diet cookbook, and a food scale. To ensure adherence, participants will be instructed to self-monitor β-hydroxybutyrate using a Keto-Mojo blood glucose and ketone meter (Keto-Mojo, Napa, California) daily during the first 2 weeks and twice a week afterwards to ensure a metabolic state of ketosis (blood ketone: 0.5 to 3 mmol/L) is reached and maintained.

Personalized Low-Fat Diet. The low-fat control group will be instructed to restrict total calorie and fat consumption according to the Look AHEAD intervention lifestyle program [44,45] (Supplementary Table S1).

Participants will receive a handout with their personalized daily calorie and fat intake goals based on the weight measured at baseline. In addition, this group will receive resources for recipes through websites supplied during the first digital lifestyle education lesson and a food scale.

Physical activity. Participants in both groups will receive a weekly physical activity goal of 175 min of moderate-to-vigorous activities per week based on recommendations from Look AHEAD lifestyle program [44]. If participant physical activity is low initially, they will be instructed to increase activity gradually. An example is shown in supplementary Table S2.

2.4.3. Monitoring of lifestyle behaviors and health indicators

Self-monitoring behaviors and health indicators enable participants to identify the discrepancies between goals and actual performance, be aware of the problems and barriers related to diabetes self-management, and improve self-management [46]. The hardware, software, and corresponding function which will be used for self-monitoring is presented in Table 3.

To facilitate self-monitoring and the research team's remote monitoring, data collected from the above-mentioned devices can be automatically integrated and synchronized to an application programming interface (API) integration platform developed by the research team (Supplementary Fig. S2). Self-monitoring data can be presented as descriptive graphs (e.g., daily steps, blood glucose readings) and aggregated tables (e.g., energy intake calculated from food intake, weekly self-monitoring data summaries).

Table 3
mHealth components for self-monitoring.

mHealth Component	Function
Hardware	
Fitbit Inspire 2 (Fitbit LLC, San Francisco, CA, USA)	Fitness tracker to track physical activity and food intake (daily)
Withings Body Scale (Withings LLC, Issy-les-Moulineaux, Ile-de-France)	Weight scale to track weight (daily)
Keto-Mojo + (Keto-Mojo, Napa, CA, USA)	2-in-1 blood glucose and blood ketone monitor - (Fasting blood glucose daily for those with diabetes, weekly for those without diabetes) - (Fasting ketone status for those in the keto diet daily for the first two weeks, then twice weekly thereafter)
Omron 3 Series Wrist Blood Pressure Monitor (Omron Healthcare Inc., Kyoto, Japan)	Blood pressure monitor (weekly)
Etekcity Digital Kitchen Scale	Measuring food portions (daily) *Kitchen scale readings not transmitted or collected
Software	
Fitbit App (Fitbit LLC, San Francisco, CA, USA)	App to help participants self-monitor activity and food intake
Keto-Mojo App (Keto-Mojo, Napa, CA, USA)	App to help participants self-monitor blood glucose and blood ketone (KD group*)
Withings Health Mate App (Withings LLC, Issy-les-Moulineaux, Ile-de-France)	App to help participants self-monitor body weight and blood pressure

2.4.4. Personalized feedback

Providing feedback will reveal a participant's performance towards prescribed behavior and health outcome goals as well as promote self-regulation. By providing personalized feedback, participants may better understand their progress on behavior change and health status. During the study, participants will receive personalized feedback from the research team through push notifications enabled by TigerConnect and through email (Supplementary Fig. S3).

2.4.5. Intervention fidelity

To ensure intervention fidelity, we will implement the recommendations from the National Institutes of Health (NIH) Behavior Change Consortium [47] in study design, provider training, treatment delivery, treatment receipt, and enactment of treatment skills. The research staff will be trained before study initiation and receive relevant training as needed throughout the study. On a weekly basis, (1) the research team will meet weekly to update intervention delivery and adherence, and (2) two research staff members will monitor the digital lesson logins and viewing rates from participants through Articulate 360, assess participants' lifestyle and health indicator tracking through the API integration platform, and send personalized messages to participants.

2.5. Measurements

This pilot study will assess the feasibility, acceptability, and explore the preliminary efficacy of the intervention on health outcomes as outlined in Table 4. In addition, lifestyle behavior changes will be tracked by mobile devices as described in Section 2.4.3 (self-monitoring). All primary and secondary measures will be collected on site in a research laboratory at School of Nursing, UTHSCSA.

2.5.1. Feasibility and acceptability

Feasibility and acceptability will be measured by factors such as percent of completed daily food logs and the number of: days with step and activity data, blood glucose readings, ketone readings (keto group only), blood pressure readings, digital health lessons watched and number of times watched, dropouts or participants lost to follow-up; reasons for leaving the study; and number of participants screened and enrolled.

In addition, focus group discussions for participants who were assigned to the ketogenic diet group will also be conducted at 6 months to assess the acceptability, experience and perceptions of the intervention and the study. An interview guide will be developed to lead the focus group discussion. The interview guide will include questions such as "how was your overall experience with a ketogenic diet?" and "what did you think about the weekly personalized feedback from the study team?" Probe questions such as "which was the best part of/about… (topic)" or " did you notice an impact on others (family or friends)" will be asked wherever appropriate. All interviews will be led by trained research staff, and recorded using a digital recorder.

Table 4
Summary of study measurements and time of data collection.

Study Variables	Time of collection		
	Baseline	3-month	6-month
Primary outcomes: Weight	X	X	X
Secondary outcomes: BMI, HbA1c, Blood pressure, blood lipids, eGFR, urine ACR, urine and plasma targeted metabolites, and mitochondrial function	X	X	X
Other measures: Demographics, medical history, medication usage, cognitive function, patient-reported outcomes and diabetes specific surveys (demographics only at baseline)	X	X	X
Feasibility and acceptability: Enrollment rate, retention rate, compliance to study activities and lifestyle regimen, perceptions and experience of the study, etc.	X	X	X
Mobile device tracked data: Physical activity, diet, glucose, blood pressure, ketosis (keto group only), body weight	Throughout the study		

2.5.2. Primary outcome

The primary outcome is weight. Weight will be measured twice in kilograms (kg) without shoes, wearing light clothing, using an electronic weight scale at baseline, 3 months, and 6 months.

2.5.3. Secondary outcomes

Secondary outcomes include BMI, blood pressure, blood lipids, glycemic control (measured by HbA1c), and renal health (eGFR and ACR). Height will be measured twice in centimeters without shoes using a stadiometer. BMI will be calculated by weight divided by height squared. Fasting blood and urine samples will be collected. Part of the blood and urine samples will be sent to Quest Diagnostics (Dallas, TX) for analysis and attainment of HbA1C, lipid profile, eGFR and urine ACR, the remainder will be processed to produce buffy coat and platelet aliquots which will be stored at -80^{oC} until further metabolomic processing. Likewise, the remaining urine sample will be aliquoted and further stored at -80 °C until further processing.

Metabolomic analysis will be used to identify the effect of a ketogenic diet on metabolism by measuring platelet and urine metabolites. Targeted metabolite approaches will be conducted to assess the preliminary efficacy of the intervention using a ZipChip capillary electrophoresis system from 908 Devices, coupled to a high resolution orbitrap MS/MS. Over 50 previously identified metabolites, including amino acid panels, organic acid panels and other relevant metabolites (e.g., acetoacetic acid, citric acid, succinic acid, pyruvic acid, 3-hydroxybutyric acid, pyroglutamic acid, creatinine, adenine), will be assessed in both platelet and urine samples. We will also extract mtDNA to assess mitochondrial function via mtDNA copy numbers and mtDNA oxidative damage.

2.5.4. Other measures

Demographics, medical history, medications, cognitive function, and patient-reported outcomes will be recorded. Cognitive function will be assessed by the Montreal Cognitive Assessment (MoCA), a cognitive screening tool with high sensitivity and specificity often used as a validated clinical tool to detect mild cognitive impairment [21,22]. Patient-reported outcomes will be assessed by Patient-Reported Outcomes Measurement Information System 29 v 2.0 (PROMIS®-29 v 2.0), a validated and reliable measure of health change for interventions [23].

2.6. Safety and confidentiality

This lifestyle intervention may cause side effects. We will monitor and document any side effects and adverse events throughout the study. Participants can withdraw anytime from the trial in the event any severe side effects occur, as determined by the study team, or if he/she is not willing to continue participating in the study. Unanticipated adverse events will be reported to the IRB with appropriate responses to the events.

Study-related information, including all paper data and completed consent forms, will be securely stored at the study site in locked file cabinets accessible to authorized personnel only. All biospecimens will be labeled with a coded identification (ID) number to maintain confidentiality. All records with identifiable information will be stored separately from the deidentified records. All local databases will be secured on the institution's server with password-protected access.

The mobile devices used for this study have the capacity to store a limited amount of recent data. All collected data will be synced with the vendors' database through Wi-Fi via a mobile app (Fitbit, Withings, Keto-Mojo). The dashboard used to aggregate and display participants' data from Fitbit, Withings, Omron, and Keto-Mojo is on the institution's server behind the institution's firewall.

A senior data statistician will be appointed to serve as the data safety officer, who will be in place throughout the study who has no direct involvement with the study procedures. The PIs will meet with the data safety officer every 3 months to review data on recruitment, enrollment, retention, and intervention adherence. Any potential adverse event that requires a modification to the protocol and/or intervention will be immediately reported to the Safety Officer by the PIs.

2.7. Planned statistical analyses

To assess intervention effect, we will consider changes in both the primary outcome (e.g., weight) and secondary outcomes (e.g., HbA1c, ACR, eGFR targeted metabolites) from baseline to 6-months. For non-metabolite outcomes (eGFR, etc.), linear mixed-effect regression models adjusted for covariates (e.g., strata, age, race, education, gender, physical activity levels) will be adopted to assess the association between group assignment and changes of health outcomes after intervention (primary/secondary outcomes and other health-related outcomes). While there is no formal power calculation for this study, 95% confidence intervals and the 2-sided $\alpha = 0.05$ will be used to identify significance. The metabolomic results will be analyzed using LIMMA [48] to account for stratification, covariates, and shared patterns of variability in multiplex assays. To manage multiple metabolite testing, we will use the Benjamini-Hochberg [49] false discovery rate (FDR) procedure for metabolites with an FDR cutoff of $<10\%$. The distribution of outcomes will be examined, and transformation will be performed to approximate the Gaussian distribution. Intention-to-treat analysis will be adopted for this clinical trial. We will include data from participants who completed baseline and at least one follow-up data collection. Descriptive statistics such as mean (±standard deviation) for continuous variables and frequency (%) for categorical variables will be provided to describe the distributions of study variables. Data will be analyzed using SAS 9.4 and R version 4+ (Vienna, Austria).

Data from focus group discussions will be recorded and transcribed. Thematical content analysis will be used to explore participants' acceptability and experience of the ketogenic diet and the intervention.

2.8. Dissemination policy

The results of the trial will be submitted to peer-reviewed scientific journals for publication and presented at international, national and/or regional scientific conferences, symposia, and seminars. Aggregated data will be presented, and no indefinable data will be disclosed at the dissemination activities.

3. Discussions

Seeking effective and sustained interventions and treatments to address obesity, manage comorbid conditions, and improve CRM health cannot be overstated [1–5]. The ketogenic diet might be a promising approach for CRM health, however, the findings of its effects on CRM health are still controversial and innovative strategies to improve the adherence and compliance are scarce. This pilot clinical trial addresses this research gap by testing the efficacy of a 6-month, technology-assisted, ketogenic diet lifestyle intervention on CRM health (e.g., weight, lipid profile, glycemic control and renal health) using both traditional and innovative omics indicators in overweight/obese adults with/without T2D and CKD.

To our knowledge, this is one of the first studies to assess a technology assisted ketogenic diet intervention on weight control and related health conditions. This technology assisted ketogenic diet intervention approach may also alleviate the intensive staff workload involved in ketogenic diet interventions. The involvement of digital lessons may promote the adherence to health education, which is particularly needed amid and beyond the COVID-19 pandemic. Individualized goal setting and using multiple mobile and wearable devices to monitor lifestyle behaviors (e.g., physical activity, diet intake) and health indicators (e.g., weight, glucose levels, ketosis) may help participants improve adherence by becoming aware of the link between lifestyle and health indicators.

This trial will also provide preliminary results to estimate sample size and improve intervention and study procedures for future larger clinical trials and lay the groundwork to understanding the effects of ketogenic diet on CRM health at the molecular level. By using both traditional and novel markers, the findings may provide research and clinical implications regarding intervention efficacy and safety of a ketogenic diet on health outcomes for individuals who are overweight/obese and have multiple chronic health conditions.

The current study has several limitations. First, CKD will be defined by one-time assessment of eGFR and ACR readings, which has been frequently used to define CKD in research [38,50]. Second, this pilot study used a convenient sample size and is not powered to determine the impact of the intervention on assessed health outcomes. In addition, study participants are recruited from a single geographic region. Lastly, due to the nature of the intervention and the staffing arrangement, other than the PIs and data analysist, the intervention arms will not be blinded to other study staff such as the data collectors. However, all staff will be following the standard study protocol, including the measurement protocol; in addition, most of the data we collect is objective (e.g., lab results) or self-reported, and influence from a data collector is at low risk.

4. Conclusion

Mobile and connected technologies provide new opportunities to improve the management of complex chronic conditions. These new approaches may improve health education attendance, planned lifestyle changes, engagement, and health outcomes. Additionally, as the technology to profile and analyze omics data advances, these new markers may advance our understanding of the underlying mechanisms of

intervention effects on health outcomes at molecular levels, and guide precision lifestyle interventions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cct.2022.106845.

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