ANCILLARY REVIEWS

Which ancillary reviews do I need and when do I need them? Refer to HRP-309 for more information about these ancillary reviews.			
Select yes or no	**Does your study…**	*If yes…*	***Impact on IRB Review***
☐ **Yes** ☒ **No**	Include Gillette resources, staff or locations	*Gillette Scientific review and Gillette Research Administration approval is required. Contact: research@gillettechildrens.com*	**Required prior to IRB submission**
☒ **Yes** ☐ **No**	Involve Epic, or Fairview patients, staff, locations, or resources?	*The Fairview ancillary review will be assigned to your study by IRB staff Contact:* ancillaryreview@Fairview.org	**Approval must be received prior to IRB committee/ designated review.** **Consider seeking approval prior to IRB submission.**
☒ **Yes** ☐ **No**	Include evaluation of drugs, devices, biologics, tobacco, or dietary supplements or data subject to FDA inspection?	*The regulatory ancillary review will be assigned to your study by IRB staff Contact: medreg@umn.edu* *See: https://policy.umn.edu/research/indide*	
☒ **Yes** ☐ **No**	Require Scientific Review? Not sure? See guidance on next page.	*Documentation of scientific merit must be provided. Contact: hrpp@umn.edu*	
☐ **Yes** ☒ **No**	Relate to cancer patients, cancer treatments, cancer screening/prevention, or tobacco?	*Complete the CPRC application process. Contact: ccprc@umn.edu*	
☒ **Yes** ☐ **No**	Include the use of radiation? (x-ray imaging, radiopharmaceuticals, external beam or brachytherapy)	*Complete the AURPC Human Use Application and follow instructions on the form for submission to the AURPC committee. Contact: barmstro@umn.edu*	**Approval from these committees must be received prior to IRB approval;** **These groups each have their own application process.**
☒ **Yes** ☐ **No**	Use the Center for Magnetic Resonance Research (CMRR) as a study location?	*Complete the CMRR pre-IRB ancillary review Contact: ande2445@umn.edu*	
☐ **Yes** ☒ **No**	Include the use of recombinant or synthetic nucleic acids, toxins, or infectious agents?	*Complete the IBC application via eprotocol.umn.edu Contact:*	
☐ **Yes** ☒ **No**	Include the use of human fetal tissue, human embryos, or embryonic stem cells?	*Contact OBAO for submission instructions and guidance*	

☒ **Yes** ☐ **No**	Include PHI or are you requesting a HIPAA waiver?	*If yes, HIPCO will conduct a review of this protocol.* *Contact: privacy@umn.edu*	
☒ **Yes** ☐ **No**	Use data from the Information Exchange (IE)?	*The Information Exchange ancillary review will be assigned to your study by IRB staff* *Contact: ics@umn.edu*	**Approval must be received prior to IRB approval.** **These groups do not have a separate application process but additional information from the study team may be required.**
☐ **Yes** ☒ **No**	Use the Biorepository and Laboratory Services to collect tissue for research?	*The BLS ancillary review will be assigned to your study by IRB staff.* *Contact: cdrifka@umn.edu*	
☐ **Yes** ☒ **No**	Have a PI or study team member with a conflict of interest?	*The CoI ancillary review will be assigned to your study by IRB staff* *Contact: becca002@umn.edu*	
☒ **Yes** ☐ **No**	Need to be registered on clinicaltrials.gov?	*If you select "No" in ETHOS, the clinicaltrials.gov ancillary review will be assigned to your study by IRB staff* *Contact: kmmccorm@umn.edu*	
☒ **Yes** ☐ **No**	Require registration in OnCore?	*If you select "No" or "I Don't Know" in ETHOS, the OnCore ancillary review will be assigned to your study by IRB staff* *Contact: oncore@umn.edu*	**Does not affect IRB approval.**

PROTOCOL COVER PAGE

Protocol Title	Prolonged Daily Fasting as a Viable Alternative to Caloric Restriction in At-Risk Obese Humans
Principal Investigator/Faculty Advisor	Name: Lisa Chow, MD, MS
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	Telephone Number:
	Institutional Email Address:
Scientific Assessment	Nationally-based, federal funding organizations
IND/IDE # (if applicable)	Exempt (IND 147364)
IND/IDE Holder	Dr. Lisa Chow
Investigational Drug Services # (if applicable)	Exempt (IND 147364)
Version Number/Date:	Version 11.0, 6/20/2022

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
1	05/11/2020	• Removed Overnight Stay, • Shortened hyperinsulinemic-euglycemic clamp to 4 hours (previously 6 hours) • CGM is now optional • removed supervised meals • change U13palmitate to D5glycerol (still FDA exempt) • changed dietary recall to 3 at baseline and 3 at end (rather than 2 at baseline, 2 at midpoint, 2 at end) • Reduced compensation to $400 due to less participant burden • Added strategies to improve recruitment in case of recruitment shortfalls	Yes
2	06/30/2020	• Reduced in person visits from 8 to 5 • 2 visits are virtual (Visit 0 and Visit 4) • Visit 6 and visit 7 are combined • Insulin resistance inclusion criterion has been removed • Initial dietician in person counseling has been changed to a telephone counseling session • The glucose sensor (CGM) will be a study activity	Yes
3	07/27/2020	• EConsent/HIPAA Form, delivered and signed via REDCap, will be provided as an option for consent process dependent upon participant preference	Yes

		• SF-36 survey (Quality of Life) added to V3 and V6 • Added 'prior to visit fasting' language to a letter we send to the participants	
4	08/17/2020	• It was clarified in the consent form and protocol that the fasting labs obtained in Visit 1 determine eligibility. • In the protocol, the study procedures are made more explicit, such as conducting the remote/virtual visits using Zoom or Doximity. • In the consent/HIPAA form, consent language has been added specific to the use of the mCC app. This language was provided by the Salk Institute to replace Salk's online version of the mCC app's consent that we had been previously using. • The protocol has been updated to reflect the addition of the mCC app consent language (that Salk had been using online and they would like us now to incorporate into our consent form) to the main consent/HIPAA form.	Yes
5	12/22/2020	• It was clarified in the protocol on page 51 the study document retention requirements the study will follow. • Additional MRI language was added to page 13 of the consent form that addresses notifing the	Yes

		participants of significant new findings.	
6	1/5/21	We have expanded the eligibility criteria to include participants with at least an 12 hour eating window.	No
7	2/2/21	We have updated our exclusion criterion to reflect the exclusion of people on beta blockers or medications known to affect weight such as TZD, insulin, GLP-1 agonists, phentermine, or sibutamine. Our previous criteria of excluding for statin or diuretic use has now been removed as this will not affect our outcome parameters as initially believed. Changing this criteria will not increase risk and will broaden our participant pool.	No
8	2/18/2021	We are including ResearchMatch as a recruitment tool. We have also updated the calculations for the continuous infusion of D5glycerol (a naturally occurring and stable isotope) and the timing of the bolus for D5 glycerol and 6,6 2H2 glucose performed during V3 and V6. This does not impact participant safety. This is to accommodate for slight variability in the compounding by the compounding pharmacy and available pumps at CRU.	No
9	8/31/2021	To increase retention in the non-TRE (control) group, we will offer a complimentary dietary session after the intervention and Visit 6 have been completed to point out dietary changes the participant may wish to consider to support good nutrition and health body	No

		weight. A resource book may also be provided.	
10	6/20/2022	We are eliminating the inclusion criterion “Self-reported absence of known sleep apnea” since it is not scientifically relevant to our study aims and by eliminating it, we will be able to expand eligibility.	No

Table of Contents

ABBREVIATIONS/DEFINITIONS

- BMI (body mass index)
- Co-I (co-investigator)
- CR (caloric restriction)
- CGM (continuous glucose monitoring)
- CMRR (Center for Magnetic Resonance Research)
- DXA (Dual X-ray absorptiometry)
- D5glycerol (D5glycerol with 5 hydrogens labeled with deuterium – a stable isotope
- EHR (electronic health record)
- FFA (free fatty acid)
- FFM (fat free mass)
- H: Hypothesis
- HFF (hepatic fat fraction)
- HbA1c (hemoglobin A1c)
- HOMA-IR (homeostatic model of insulin-resistance)
- mCC (MyCircadianClock)
- MRI (magnetic resonance imaging)
- MRS (magnetic resonance spectroscopy)
- NAFLD (non alcoholic fatty liver disease)
- NCC (Nutrition Coordinating Center),
- NDSR (Nutrition Data System for Research_
- Non-TRE (non time restricted eating)
- OGTT (oral glucose tolerance test)
- mCC (my Circadian Clock)
- QOL (quality of life)
- RQ (respiratory quotient)
- SD (Standard deviation)
- TRE (time restricted eating)
- U13C palmitate (palmitate with all carbons labeled with 13C - a stable isotope)
- UMMC - University of Minnesota Medical Center
- WT (wild type)
- VO2max (maximum oxygen consumption)

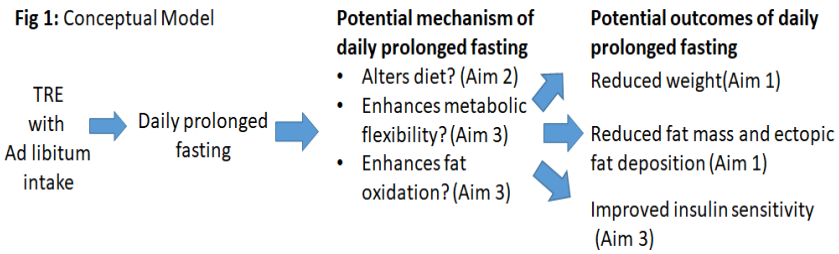
1.0 Objectives

1.1 Purpose: Obesity is reaching epidemic proportions, affecting 36% of the adult population in the United States. There is intense interest in dietary management to treat obesity and its associated complications. The first line of obesity treatment is caloric restriction (CR), although recidivism is common. For moderate CR, attrition rates of 20% are often reported, therefore weight loss options beyond CR are urgently needed.

Time restricted eating (TRE) shifts the paradigm in obesity treatment. TRE arose from synergizing food intake with the circadian rhythm. In animals, this meant limiting food intake (~ 8 hours, isocaloric) to their active phase, resulting in many metabolic benefits (proportional to fasting duration) such as reduction in adiposity, liver steatosis, hyperlipidemia and insulin resistance compared to mice fed *ad libitum.*

In America, humans have constant access to food. As the majority of Americans are perpetually eating (median eating window:14.8 hours), TRE presents another approach towards obesity treatment. In humans, TRE imposes a daily eating window (~8-10 hours) resulting in a daily 14-16 hour fast. Current human TRE studies report loss of weight and fat mass, with equivocal effects on glucose metabolism. These studies have limitations, including lack of randomization, restriction to single-sex, or provision of all food (limiting translation).

We performed a pilot study randomizing healthy, overweight (≥25 kg/m^2) participants with an eating window of ≥14 hours to either TRE with ad libitum intake (n=11, self-selected 8 hour window) or unrestricted eating (non-TRE: n=9) for 12 weeks **(Preliminary data**). All oral intake was documented (image, time) using a mobile phone app with good compliance [Mean (SD): 86.6% (11.8) of days]. The TRE group narrowed their eating window to 9.9 hours (2.0), a significant decline of 5.4 hours (2.2) from baseline ($p<0.0001$). In contrast, the non-TRE group did not significantly alter their eating window. At end-intervention, TRE reduced weight [-3.7% (1.8)], fat mass [-4.0% (2.9)], visceral fat [-11.1% (13.4)], fasting glucose [-7.7% (6.9)] and fasting triglyceride levels [-23.6% (21.7)] relative to baseline (all $p<0.05$). These measures were unchanged in non-TRE. Several key findings were seen: 1) TRE reduced weight, fat mass and visceral fat compared with non-TRE; 2) Greater restriction of the eating window was associated with greater fat mass/visceral fat loss; 3) TRE improved self-reported quality of life, 4) TRE did not alter actigraphy measured physical activity or sleep measures relative to baseline or the non-TRE. Therefore, although TRE-associated benefits are conventionally believed to be due to matching eating with circadian rhythms, our data challenge this notion and suggest that **prolonged daily fasting** as the key determining factor.



We hypothesize that TRE with *ad libitum* intake presents a highly viable alternative to CR in reducing weight, fat mass and improving insulin sensitivity, as daily prolonged fasting reduces caloric intake and enhances metabolic flexibility—the adaptability of fuel selection given ambient fuel exposure. (**Fig 1:** Conceptual model). We will recruit obese, insulin-

resistant humans (n=72, 24/group) with an eating window ≥14 hours. Participants will be randomized to 1 of 3 groups: 1) TRE (8 hour eating window) with *ad libitum* intake, 2) CR with 15% reduction of caloric intake, 3) non-TRE with *ad libitum* intake for 12 weeks. Measures performed pre-post intervention will address the following Aims:

Aim#1: Evaluate the effect of TRE with *ad libitum* intake on weight and body composition. H 1.1: Individuals in the TRE and CR groups will have similar weight loss, which will be greater than weight loss achieved in the non-TRE group (primary outcome). H 1.2: TRE will result in greater loss of loss of total body fat (quantified by DXA) and greater loss of hepatic/visceral fat/ectopic fat (quantified by MRI) than CR.

Aim#2: Assess the effect of TRE with *ad libitum* intake on caloric balance. H 2.1: TRE will reduce caloric intake compared with non-TRE [gold-standard interviewer administered 24-hour dietary recall (primary outcome)] with similar reduction as with CR, H.2.2: TRE will not alter physical activity, but will increase fat oxidation compared with CR and non-TRE.

Aim#3: Assess the effect of TRE with *ad libitum* intake on metabolic flexibility. H 3.1: TRE will enhance metabolic flexibility compared with CR and non-TRE as measured by indirect calorimetry [RQ:Respiratory quotient before and during 2 step 6,6-$^2H_2$ hyperinsulinemic-euglycemic clamp: primary outcome]. H 3.2: TRE will improve insulin sensitivity compared with non-TRE and similar to CR. H 3.3: TRE will augment greater fasting lipolysis compared to CR and non-TRE as measured by D5glycerol and enhance lipolysis suppression during the 2 step 6,6-$^2H_2$ hyperinsulinemic-euglycemic clamp.

If our hypotheses are confirmed, this project has **significant impact**. First, it will advance our understanding of the mechanisms underpinning this **innovative** intervention. Second, TRE can be a practical means of implementing prolonged fasting on a large scale, thereby transforming the treatment of obesity.

2.0 Background

2.1 Significance of Research Question/Purpose:

Caloric restriction is the mainstay of obesity treatment, alternative options need to be considered Obesity is reaching epidemic proportions, affecting 36% of the adults in the United States,2 so there is intense interest in dietary management to treat obesity and its complications. The first line of treatment is caloric restriction (CR), although recidivism is common.3 For moderate CR, attrition rates of 20% are reported, with most participants returning to their baseline weight within 5 years of completing therapy. Therefore, sustainable weight loss options beyond CR are urgently needed.

Constant eating is unhealthy in animal models, yet humans constantly eat. Mice permitted *ad libitum* intake and continuous access to high fat/high sugar food experience adverse metabolic effects, such as obesity, hyperinsulinemia, hepatic steatosis, and inflammation.[1,2] Yet, when these mice were allowed *ad libitum* intake only during a restricted eating window (TRE: ~8-9 hours window during the dark phase), marked reductions in adiposity, liver steatosis, hyperlipidemia, insulin resistance and glucose tolerance were observed despite similar energy intake compared with their unrestricted counterparts. The

metabolic benefits associated with TRE were proportional to the fasting duration[1] and suggest that adverse effects of diet can be disassociated from diet composition and caloric intake.

Similarly, humans in modern America have constant access to food. Not surprisingly, humans are perpetually eating. Dr. Panda (Co-I) who developed myCircadianClock (mCC), a smartphone app, to track food intake.[3] In a recent study (n=156 for 3 weeks, all food intake documented by the mCC app), he reported that humans have frequent and erratic eating patterns, with more than half of adults eating ≥15 hours per day and only stopping to eat when asleep (<1% of eating events between 1-6 am).[3]

As constant eating, rather than diet composition, drove the metabolic complications in mice,[1,2] constant eating, in the form of snacking, likely contributes to adverse metabolic complications in humans. In adults, snacking has increased in terms of number of snackers (1977-1978:60% of the population, 2009-2012: 86% of the population) and higher caloric intake from snacks (1977-1978:~200 kcal/day, 2009-2012: ~ 500 kcal/day).[4]

Time restricted eating studies in humans suggest metabolic benefits Time restricted eating (TRE) presents an alternative approach to CR. TRE imposes a consistent daily eating window (~8-10 hours) with *ad libitum* intake while preserving a daily 14-16 hour fasting period. **By focusing on restricting time rather than restricting calories, TRE is a practical means of implementing prolonged fasting on a large scale in the population.**

Not surprising, human TRE studies are promising[3,5,6] but preliminary, with limitations including lack of randomized control[3,6] or restriction to men in an environment where all food was provided.[5]

Generally, the TRE studies with *ad libitum* intake have shown TRE facilitates weight loss relative to baseline.[3,6] TRE studies with isocaloric intake maintain weight, with consistent fat mass loss,[7,8] and variable effects on fasting glucose,[decrease[7] no change[8]] fasting insulin[decrease[5,7]], fasting triglycerides [decrease[7] no change[8] increase[5]], insulin sensitivity [increase[5]] and β cell responsiveness [increase[5]].

To date, there are no published randomized, trials using TRE as an intervention to both sexes in the community setting. Hence, we performed a pilot study **(Preliminary data, submitted for publication),** a randomized clinical trial examining the effect of TRE vs non-TRE for 12 weeks on body composition, lipids and glycemic measures in overweight/obese humans without diabetes. Our key findings include: 1) Compared to non-TRE, TRE decreased weight, total fat mass, lean mass and visceral fat (all p≤0.05), Compared to pre-intervention, TRE reduced weight, fat mass, lean mass, visceral fat, fasting glucose, and fasting triglyceride levels. 2) Greater restriction of the eating window was associated with greater fat mass/visceral fat loss.

TRE appears to be sustainable. One study[3] (n=8) enrolled humans with ≥14 hour eating window for a 16 week TRE intervention (10-12 hours eating window). Post-intervention, the participants reduced their eating window by ~4.5 hours and lost weight (~ 3.3 kg). Afterwards, all participants continued with TRE for 36 more weeks. At study conclusion (Week 52), these participants maintained their weight loss (~ 3.3 kg) and reported improved energy, and sleep compared with baseline. Our preliminary data also reports TRE improving quality of life relative to baseline and the non-TRE group **(Section 3.3.5)**. To date, there are no published

studies comparing sustainability of TRE to CR. Yet, CR is associated with attrition rates of 20-30%.[9-11] With pilot studies demonstrating TRE improving self-reported quality of life, TRE may be a viable option to CR.

Mechanisms driving TRE need to move beyond matching to circadian rhythm The concept of TRE arose within the context of circadian rhythms. Circadian rhythms are daily ~ 24 hour rhythms observed in organisms. These rhythms are at the central level (suprachiasmatic nucleus-regulated by the light/dark cycle) as well as at the peripheral level (tissue specific-regulated by food intake).[12] In mice, restricting the feeding window to the dark cycle, where mice are most active, provides metabolic benefits compared with mice fed *ad libitum* despite similar diet composition and energy caloric intake.[1,2]

In humans, there is much interest in the clinical significance of TRE timing. Specifically, eating earlier in the day is believed to synchronize the central/peripheral clocks to improve metabolic outcomes.[12] Cross-sectional studies have shown that eating earlier in the day is associated with lower body fat than eating later in the day.[13,14]Clinical trials have demonstrated that higher caloric intake at breakfast (n=96, diet=1400 kcal/day * 12 weeks with 50% of calories consumed at breakfast or dinner) [15] or lunch (n=80, diet=2000 kcal/day * 12 weeks with 50% of calories consumed at lunch or dinner)[16] results in greater weight loss (1.3-2.5 fold) than higher calorie intake at dinner despite similar daily caloric intake.

Early eating may also improve glucose metabolism, as glucose metabolism appears to be under circadian regulation.[17] Oral glucose tolerance testing (OGTT) performed at either 7 AM or 7 PM demonstrated worse glucose tolerance (70% higher glucose) at 7 PM.[18] In men with prediabetes (n=13, 6 hour TRE with all food consumed before 2 pm vs 12 hour intake), early TRE with isocaloric intake did not alter OGTT glucose levels but reduced OGTT insulin levels by 50% compared with isocaloric intake over 12 hours.[5] Insulin sensitivity (improved by 15%) and glucose tolerance (improved by 7%), are improved when the majority of calories are consumed during breakfast than supper.[19] Yet, in obese, insulin resistant men, the timing of the meal [n=23, 4 week with hypocaloric diet (50% of caloric need) with 50% of daily energy consumed either at breakfast or dinner] was unrelated to weight loss, hepatic and peripheral insulin sensitivity (hyperinsulinemic-euglycemic clamp), intrahepatic triglyceride content, or resting energy expenditure.[20] Therefore, although early meal timing may be more advantageous over later meal timing, the differences are not dramatic.

Moreover, Dr. Panda (Co-I) recently showed that TRE protected circadian mutant mice from the consequences of a high fat diet (weight gain, fat accumulation, lipid profile, glucose intolerance, insulin resistance) similar to WT mice,[21] supporting the concept that **TRE can disassociated from the circadian clock in providing metabolic benefits.** As the evening meal has a significant social component, its inclusion in any dietary intervention will promote sustainability. In our preliminary data, participants self-selected their 8 hour eating window to intentionally include the evening meal. Therefore, timing of meals relative to the circadian rhythm is desirable, it is not imperative and may be difficult to sustain in practice.

Metabolic benefits are seen with fasting In animals and humans, fasting interventions are associated with metabolic benefits, including reduced weight, fat mass and insulin resistance.[12] In humans, fasting interventions with benefits include intermittent fasting (low

calorie intake 1-2 days/week, ad libitum intake 5 days/week),[22] fasting mimicking diets (5 day regimen which mimic water-only fasting with a micronutrient content which maximizes nourishment- performed once per month)[23] and most recently TRE.[3] Prolonged fasting mobilizes triglycerides from the adipose tissue, with 60% of the increase in lipid kinetics occurring between 12-24 hours of fasting.[24] In contrast, CR can be disassociated from fasting. CR in the form of constant eating, even in small amounts, raises serum insulin levels to inhibit lipolysis, promotes glucose utilization, and reduce lipid oxidation.[25]

This project's **objective** is to examine the role of prolonged fasting in TRE-associated metabolic changes. The **scientific premise** is that prolonged fasting associated with TRE reduces caloric intake and enhances metabolic flexibility—the adaptability of fuel selection given ambient fuel exposure. Therefore, TRE will enhance fat oxidation in the fasting state and carbohydrate oxidation in the fed state, to be more metabolically advantageous than CR without prolonged fasting. The **significance** is establishing TRE as a viable alternative to CR, potentially transform the treatment of obesity and its associated complications

2.2 Preliminary Data:

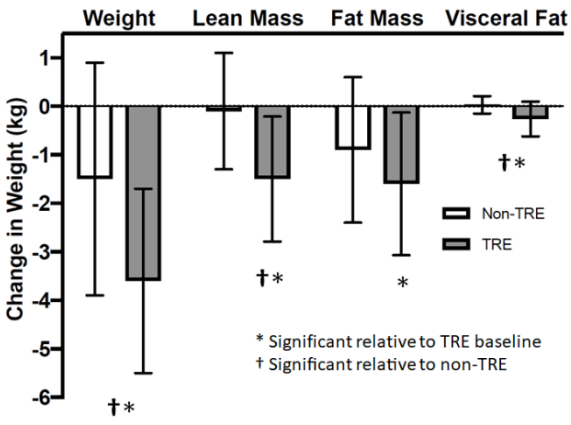
We performed a pilot study (October 2017-December 2018) randomizing overweight participants without self-reported diabetes and a prolonged eating window (≥14 hours) to either TRE with *ad libitum* intake (n=11: 8 hour eating window) or unrestricted eating (n=9: non-TRE) for 12 weeks. A baseline period [mean (SD): 28.5 days (8.8)] was need to establish the eating window [15.4 hours (0.9)] and to obtain CGM and actigraph data (up to 2 weeks) All oral intake was logged using a mobile phone app (MCC).[3] Adherence/logging issues were assessed weekly and participants contacted if issues were identified. TRE did not alter actigraphy-measured physical activity (% time in sedentary, light, moderate or vigorous activity) or sleep measures (Pittsburgh sleep quality index, Epworth sleepiness scale, sleep duration, sleep efficiency, wake or sleep time) compared to baseline or non-TRE. Key findings include the following:

Preliminary data: TRE alters body composition relative to non-TRE

Rationale: We examined the effects of TRE on weight compared with non-TRE, as well as the effects of TRE on body composition (determined by DXA) compared with non-TRE.

Results: Compared to the non-TRE group, TRE lost weight, visceral fat, and lean mass (all p< 0.05: **Fig 2**). The TRE group significantly reduced weight [Mean (SD): -3.7% (1.8)], fat mass [-4% (2.9)], lean mass [-3.0% (2.7)], visceral fat [11.1% (13.4)], fasting glucose [-7.7% (6.9)] and fasting triglyceride levels [-

Figure 2: TRE Alters Body Composition



23.6% (21.7)] compared to baseline. Given the observed weight loss of 1.5 kg (1.3) lean mass, 1.6 kg (1.5) fat mass and 0.26 kg (0.4) visceral fat over 12 weeks, consumption of ~270 calories less per day may explain the weight loss. Lean mass loss was inversely associated with higher baseline body fat mass and insulin resistance.

Relevance: TRE favorably alters body composition relative to non-TRE. The next steps include 1) Comparison of TRE vs CR effects on body composition (Aim 1), 2) Using MRI to evaluate TRE effects on region-specific fat changes (hepatic, visceral, ectopic) (Aim 1), 3) Detailed evaluation of TRE with *ad libitum* intake on caloric intake using multiple objective measures (Aim 2).

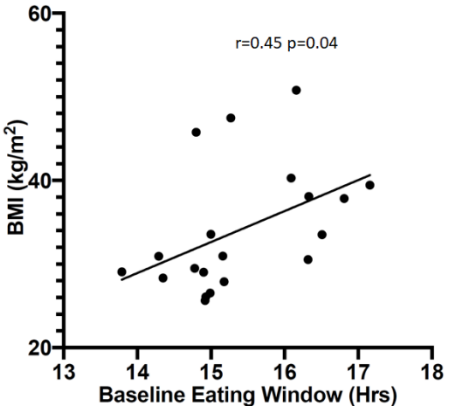
Preliminary data: Greater restriction of the TRE eating window is associated with greater improvement in body composition

Rationale: The metabolic effects of TRE likely depends on compliance. Therefore, we were interested in the relationship between eating window restriction and weight/body composition pre-post intervention.

Results: Prior to the intervention, we found that BMI was positively associated with eating window duration **(Fig 3).** In the setting of TRE, greater restriction of the eating window was moderately associated with greater loss of fat mass **(Fig 4, Panel A)** and significantly associated with greater loss of visceral fat **(Fig 4, Panel B).**

Relevance: Given no change in actigraphy-measured sleep measures, these results suggest that restricting the eating window, which prolongs the daily fasting period, may drive TRE associated changes in body composition.

Figure 3: Baseline Eating Window Correlates with BMI

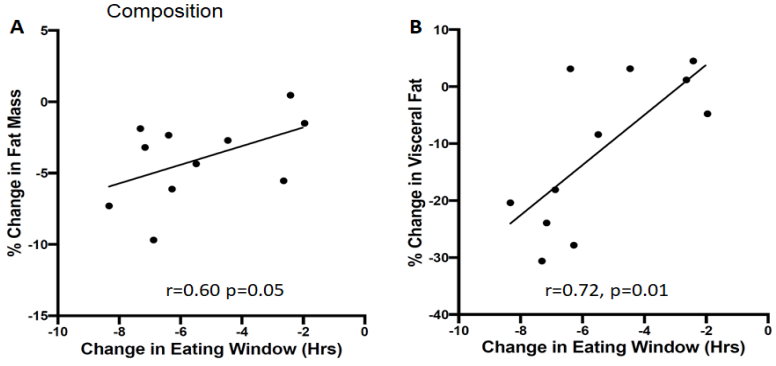


Preliminary data: TRE improves metabolic outcomes

Rationale: Evaluate the effects of TRE on metabolic measures in overweight/obese humans without self-reported diabetes and an eating window≥ 14 hours.

Results: Compared to baseline, the TRE group reduced fasting glucose [-7.7% (6.9)] and fasting triglyceride levels [(-23.6% (21.7)] (all p≤0.05). HOMA-IR, Matsuda index, HbA1c did not change. The non-TRE group did not change.

Figure 4: Eating Window Restriction Affects TRE Related Changes in Body Composition



Panel A: % change in fat mass positively correlates with reduction in eating window
Panel B: % change in visceral fat positively correlates with reduction in eating window

Relevance: Metabolic benefits are observed with TRE in obese humans without self-reported diabetes. TRE may benefit participants with insulin resistance or prediabetes.

Preliminary data: Metabolic effects of TRE are observed despite "real-world" compliance

Rationale: Using prospective documentation of food intake during the baseline and intervention by the mCC app, we characterized logging and TRE/non-TRE adherence.
Results: The baseline eating window was 15.4 hours (0.9). All participants logged adequately, as demonstrated by logging two meals ≥ 5 hours apart on a given day, during the 12 week study [86.6% (11.8) of days]. The TRE group narrowed their eating window to 9.9 hours (2.0), a significant ($p<0.001$) decline of 5.4 hours (2.2) from baseline and compared with non-TRE [decline of 0.4 hours (1.0)]. The average TRE eating window was from 10:40 AM(54 min) to 6:40 PM (54 min). The TRE group were ±15 minutes of their designated eating window for 55.5% (22.4) of days and ±1 hour of their eating window for 66.3% (20.7) of days.
Relevance: Participants were able to successfully log their food intake using the mCC app for 12 weeks. Despite the instruction of limiting TRE to 8 hours, the TRE participants realistically reduced their eating window to 10 hours. TRE is still efficacious despite "real-world" compliance.

Preliminary data: TRE is an acceptable intervention

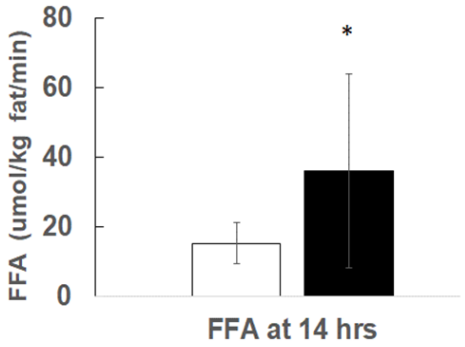
Rationale: Evaluate the impact of TRE on participant self-reported quality of life (QOL) using the short form health form questionnaire (SF-36)
Results: In the TRE group limitations due to Emotional Health improved from baseline ($p=0.02$) and relative to the non-TRE Group ($p=0.02$). In the TRE group, perception of change in health within the last year also improved relative to baseline ($p=0.01$) and the non-TRE group ($p=0.005$).
Relevance: TRE improves QOL and may be a viable alternative option to CR.

Metabolic flexibility from training enhances fasting lipolysis

Rationale: Use stable isotopes of palmitate ($U^{13}C$ palmitate, D^9palmitate) to measure lipolysis (Dr. Chow's R01) with prolonged fasting (14 hours fasting, 20 hours fasting) in lean, trained [VO_2 max:56 ml/kg/min (3.2] subjects and obese, sedentary [VO_2max: 27 ml/kg/min (4)], insulin resistant subjects (**Fig 5).**
Results: While fasting, lipolysis is higher in trained participants than obese, insulin resistant participants.

Relevance: This data shows several key points: 1) feasibility in measuring lipolysis with stable isotopes, 2) During prolonged fasting, trained

Figure 5: Trained participants have higher fasting lipolysis than sedentary, overweight, insulin resistant participants



*P<0.05 c/w sedentary group, s/p 14 hr fast
Sedentary: White bar; Trained: Black bar

participants have higher lipolysis rates, reflecting higher fat utilization, than overweight, sedentary, insulin resistant participants.

2.3 Existing Literature: This is extensively discussed in Section 2.1

3.0 Study Endpoints/Events/Outcomes

3.1 Primary Endpoint/Event/Outcome:

- The primary outcome for the study is the primary outcome in Aim 1: weight loss from baseline to 12 weeks. This was used to determine sample size.

3.2 Secondary Endpoint(s)/Event(s)/Outcome(s):

- The secondary outcome for Aim 1 is change in body composition from baseline to week 12.
- The primary outcome for Aim 2 is change in caloric intake from baseline to week 12.

The primary outcome for Aim 3 is metabolic flexibility from baseline to week 12.

4.0 Study Intervention(s)/Investigational Agent(s)

4.1 Description:

Intervention:

To equalize the intervention exposure, the TRE, CR, and non-TRE groups will receive an initial telephone counseling session with the study dietitian followed by telephone counseling sessions (including mCC logging) weekly*4 weeks, then every 2 weeks. The TRE and CR groups will receive counseling on eating window+mCC loggin (TRE) or dietary changes+mCC logging (CR). The non-TRE group will receive only the initial counseling which will address themCC logging. Separately, weekly feedback/counseling on the participants' mCC logging will be given via email or text message (participant preference) to all participants in the TRE, CR, and non-TRE groups.

Time Restricted Eating (TRE)

For the TRE group, we will restrict the eating window to 8 hours, where they will eat *ad libitum*. This is the same interval established by Dr. Panda[3] and by our preliminary data. This interval will be entered into the mCC app and participants will be asked to adhere to this eating window during the intervention. All eating occasions will be logged using the mCC app. Only water and medications will be allowed outside of the eating window.

Caloric Restriction (CR)

Participants randomized to CR will meet with the study dietitian prior to the intervention and be counseled on options to reduce their caloric intake by 15%, while maintaining their eating window. The 15% reduction was selected as our preliminary data and recent literature[6] suggest that TRE with *ad libitum* intake reduces caloric intake by ~270 to 300 cal/day. The 15% CR is similar to the 11.9% CR achieved by the CALERIE-2 study, which is a 2 year study of CR.[26] All eating occasions will be logged using the mCC app. The weekly dietitian review of the mCC information will include maintenance of the eating window and examination of dietary intake to determine compliance with the 15% CR.

Unrestricted Eating (non-TRE)

For the unrestricted eating (non-TRE) group, participants will eat *ad libitum* per their usual habits. They will receive initial counseling about mCC logging. All eating occasions will be logged using the mCC app.

4.2 IND exemption

An IND exemption is requested from the FDA as we will be using stable isotopes of glucose and fatty acids to trace glucose and lipid metabolism respectively in the body.

4.3 Drug/Device Handling:

Labeled D5Glycerol and 6,6,2H2 glucose (sterility and pyrogen-free certified) will be used for infusions during the clamp procedures and prepared by the Fairview Investigational Drug Services (IDS) pharmacy. They will be purchased from Cambridge Isotopes (Tewksbury, MA), an established vendor for stable isotopes. The D5Glycerol measures lipid kinetics using tracer doses. The 6,6,2H2 glucose measures metabolism using tracer doses and glucose disposal. We will employ MS/MS approaches to verify the purity of the isotope after purchase.

4.4 Biosafety: N/A

4.5 Stem Cells: N/A

4.6 Fetal Tissue: N/A

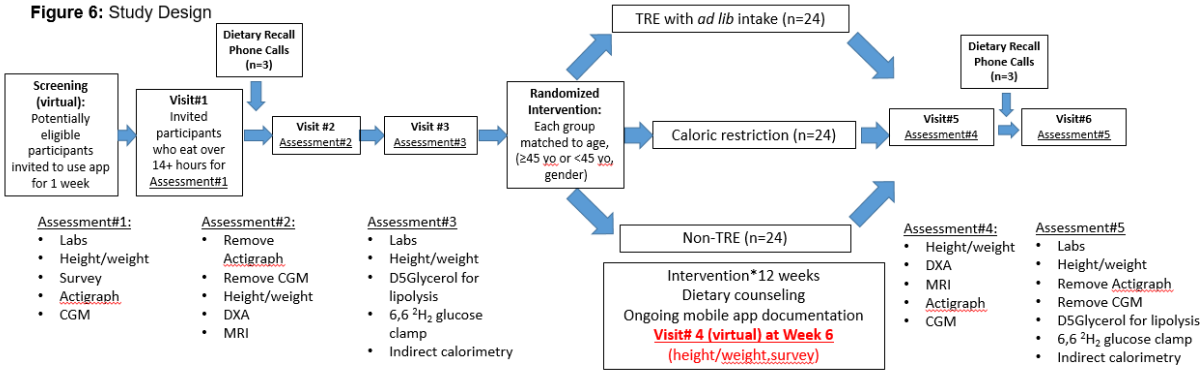
5.0 Procedures Involved

5.1 Study Design:

Fig 6 shows the study design. Our strategy is to test the metabolic effects of 12 week TRE (8 hour feeding window), CR (at 15% intake) and unrestricted eating (non-TRE). We will enroll adults with BMI ≥30 kg/m^2 who eat over ≥12 hours per day. Pre and post intervention, we will examine the effect of feeding window restriction on weight, caloric balance (caloric intake, activity), body composition

[DXA,MRI] and metabolic flexibility [indirect calorimetry, D5Glycerol to measure lipolysis, 6,6 $^{2}H_{2}$ hyperinsulinemic-euglycemic clamp].

Figure 6: Study Design



After the baseline assessment, the participants will be randomized to one of 3 dietary interventions; 1) TRE (8 hour feeding window), 2) CR (at 15% intake) and unrestricted eating (non-TRE). All oral intake will be logged using the mCC phone application and reviewed weekly with the participants. **We hypothesize that TRE with *ad libitum* intake presents a highly viable alternative to CR in reducing weight, fat mass and improving insulin sensitivity, as daily prolonged fasting reduces caloric intake and enhances metabolic flexibility—the adaptability of fuel selection given ambient fuel exposure.**

The study design is shown in **Fig 6**. Visits 1-3 will be completed within 3-5 weeks. Visits 5 and 6 will be completed within 2-3 weeks post-intervention. Assessment details are noted under each Aim.

5.2 Study Procedures:

Timeline

Each participant will be in the study for about 16 weeks with 6 phone visits, 2 remote/virtual visits (visits 0 and 4) and 5 in-person visits at the University of Minnesota (visits 1, 2, 3, 5, 6). Zoom (HIPAA-secure communciations platform used by the University of Minnesota) or Doximity (a HIPAA-secure communication platform used by healthcare professionals) will be used to conduct the remote/virtual visits. Econsent and surveys will be delivered via REDCap to the participant during the remote/virtual visits. Two of the 5 in-person visits (visit 3, visit 6) will be prolonged (~ 8 hours) due to the hyperinsulinemic-euglycemic clamp.

Intervention:

Randomized by computer generated code to 1 of 3 groups for a 12 week intervention. A dietician will provide the initial dietary telephone counseling to the participants in all 3 groups after Visit 3. Only, the TRE and CR groups will receive dietary counseling weekly for 1st 4 weeks

and every 2 weeks thereafter. Participants in all 3 groups will receive mCC logging feedback involving weekly email/text (participant preference) on mCC logging starting after Visit 1.

- **Time Restricted Eating (TRE)**
  For the TRE group, we will restrict the eating window to 8 hours, where they are allowed unrestricted eating within this window. This interval will be entered into the mCC app and participants will be asked to adhere to this eating window during the intervention. All eating occasions will be logged using the mCC app. Only water and medications will be allowed outside of the eating window.

- **Caloric Restriction (CR)**
  Participants randomized to CR will meet with the study dietitian prior to the intervention and be counseled on options to reduce their caloric intake by 15%, while maintaining their eating window. All eating occasions will be logged using the mCC app. The weekly dietitian review of the mCC information will include maintenance of the eating window and examination of dietary intake to determine compliance with the 15% CR.

- **Unrestricted Eating (non-TRE)**
  For the non-TRE group, participants will eat per their usual habits. They will receive initial counseling about mCC logging with routine feedback . All eating occasions will be logged with mCC app.

Procedures

Height/Weight at Clinical Research Unit (CRU) /Delaware Clinical Research Unit (DCRU)

Height and weight (primary outcome) will be measured at Visit 0 (self-report), Visit 1-3, Visit 4 (self-report), Visit 5-6.

Surveys

A 5 question screen will be conducted at the telephone visit to screen for eating disorders (see attached sheet).[27] Additional Surveys will be conducted at Visits 1,4,5 to assess participant's perception of appetite and at Visits 3 and 6 to assess a participant's quality of life.[28] A follow up survey (optional for participant) will be sent by email to Redcap line (1 month, 3 months, and 6 months) after the intervention to assess ongoing participation in either TRE or CR.

myCircadianClock (mCC) application

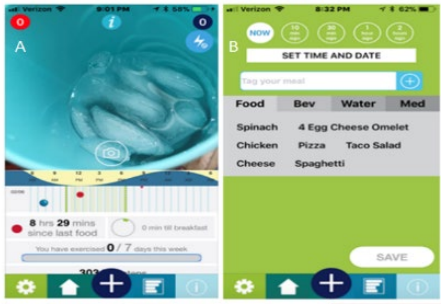
My Circadian Clock **(Fig 7)** : This smartphone app was designed and is currently maintained by Dr. Panda's group. The app is freely available to download for the iPhone or Android platform and allows a participant to use the smartphone camera to take a picture of the specific food or beverage prior to eating. The time stamp and the location are noted transferred de-identified to a data server, which

is linked back to the individual by the study team. Participants are also reminded at random times (1-2 times per day) to input recent food intake. The mCC data will be used to determine study eligibility between Visit 0 and Visit 1. Participants who are eligible for the study will continue to use the mCC app for the remainder of the study.

Sample collection at Clinical Research Unit (CRU)

Blood drawn at Visit 1 will be outside the clamp study visits and will be drawn in a fasting state (at least 8 hours from last-reported non-water intake). Blood at Visits 3 and 6 will be drawn in conjunction with the clamp procedure.

Figure 7 : MyCircadianClock application



Freely available on iTunes & Google Play
7A: Typical image for documentation
7B: Logging screen for food intake

Visit 1: Blood will be drawn to measure glucose and insulin and for complete metabolic panel, hemoglobin (Hgb), thyroid function, lipid panel and HbA1c as well as whole blood (5 ml) saved serum (5 ml) and saved plasma (5 ml) to determine eligibility. Anticipated total amount of blood drawn at 45 ml or 3 tablespoons. Participants will be provided the results of these tests.

Visit 3: Blood will be drawn to determine fatty acid lipolysis and insulin sensitivity. Baseline blood samples will be taken to measure glucose, FFA, insulin, glucose enrichment, and D5glycerol enrichment. Starting at time 0, each participant will receive a continuous infusion of D5glycerol (0.1 to 0.4 , will be generally ~ 0.28 mg/kg Bolus x over1-5 min (generally around 2 minutes) and then 0.01 to 0.04 mg/kg/min will be generally ~ 0.0195 mg/kg/min)[29] and 6,6$^2H_2$ glucose (initial bolus 3.5 mg/kg * over 1-10 min (generally around 7 minutes) , then continuous 0.04 mg/kg/min infusion).[30] Prior to initiation of the insulin clamp (time 120 minutes), blood will be sampled (q5-q10 minutes * 3) for a saved sample of plasma (5 ml) and serum (5 ml), glucose, insulin, and glucose enrichment. Next, a 2 hour low-dose hyperinsulinemic-euglycemic clamp (insulin: 10 mU/m^2/min, KPO_4 at 50 ml/hr, 20% Dextrose enriched with 1.5% of 6,6$^2H_2$ glucose)[31,32] will be initiated and the dextrose titrated for a target glucose of 90 mg/dl. Serum blood glucose will be measured every 10 minutes (Analox, UK) and insulin will be measured every 30 minutes. At the end of the 2 hour low-dose clamp, a 2 hour high-dose hyperinsulinemic-euglycemic clamp (insulin: 40 mU/m^2/min) will be initiated (time 240 min) with repeat indirect calorimetry (440-470 min) and blood sampling will be repeated (q5-q10 minutes * 3). We may also collect up to 3 urine samples (from the commode or urinal) over the course of the visit if the participant happens to void.

Anticipated blood obtained during this visit will be about 105 ml (7 tablespoons).

Visit 6: This will be similar to Visit 3. We will collect blood for glucose, insulin, HbA1c, lipid, clamp associated analysis and saved blood. We will also collect urine at this time if the participant voids. Anticipated blood obtained during this visit will be about 135 ml (9 tablespoons).

Dietary Recall via phone call

Preparing participants for the dietary recall

Prior to receiving calls from NCC, the participants will be attending a clinic visit (Visit 1 and Visit 5) for the study, at which time they will be prepared for the dietary recall. The study coordinator will do the following:

- Explain the dietary recall process to the participant by letting them know that: (see telephone script)
  - NCC Interviewers will call to ask about everything they had to eat and drink the day prior.
  - Participants do not need to keep track of their diet in anticipation of the call. Dietary interviewers will help the participant think through their day and remember what they had.
  - Calls average around 20-30 minutes.
- Provide the participant with the Food Amounts Booklet (FAB) that the dietary interviewer will refer to during the phone recall.
- Ask the participant to provide windows of time when they are generally available to receive a phone call. These should not be specific times on specific dates, but rather time frames of at least 2-3 hours. A good example would be "Mondays anytime, Tuesdays unavailable, Wednesdays any time after 4pm, etc…."

Three interviewer-administered 24-hour dietary recalls will be collected from each participant during each of measurement periods: 1) Weeks 1-2 of baseline; 2) Weeks 11-12 of the intervention **(total of 6 recalls/participant).** The recalls will be conducted over the telephone and will be unannounced (unscheduled within the measurement period) to minimize measurement reactivity. The Nutrition Data System for Research (NDSR)[33], a dietary analysis software program developed and maintained by the University of Minnesota Nutrition Coordinating Center (NCC), will be used to collect the dietary recalls. When collecting recalls using NDSR, we will use the multiple-pass interview technique to prompt for complete food and beverage recall and descriptions.[34] The time of each eating occasion will be also be recorded. A food amount booklet adapted from Van Horn et al[35] will be provided to participants for use in estimating food and beverage amounts. The dietary recalls will be carried out by staff at NCC who are trained and certified in telephone-based collection of dietary recalls using NDSR. Interview staff will be blinded to participant intervention assignment. NCC has a long history of performing dietary assessment services to researchers.

Using 24-hour dietary recall data, average energy intake at each measurement period will be calculated for each participant and used to compare pre-post change in energy intake between interventions. To evaluate change in diet quality a Healthy Eating Index 2015 (HEI-2015) score will be estimated for each participant at each measurement period. The HEI-2015 is a tool developed by the United States Department of Agriculture and the National Cancer Institute to evaluate the extent to which diets are consistent with the Dietary Guidelines for Americans[36,37] Possible total index points range from 0-100, with a higher score indicating greater consistency of the diet with the Dietary Guidelines for Americans. Scores for each of the 13 components of the index (e.g. total vegetables, whole grains, added sugars) will also be calculated.

DXA scan at Laboratory of Integrative Human Physiology (LIHP)

The Laboratory of Integrative Human Physiology (LIHP) has a Body Composition Testing Laboratory which contains equipment for the determination of body composition including bone mass, fat mass and lean body mass. At baseline (visit 2) and 10 weeks (visit 5), all subjects will have their height and weight determined using a wall-mounted stadiometer and an electronic scale. Body mass index (BMI) will be calculated as the body weight in kg/height^2 (m^2). Percent body fat and lean muscle mass will be determined by Hologic Horizon A (Hologic Inc., Marlborough, MA) dual X-ray absorptiometer (DXA). The DXA will provide bone densities and calculations of total body fat, lean body mass and bone mass. The Hologic Horizon A DXA can accommodate individuals who weigh up to 500 lbs and will provide calculations of total body fat, regional fat, lean body mass, and bone mass. The Hologic Horizon A can provide adipose measures of the android and gynoid regions as well as estimation of visceral adipose tissue.[38] Prior to daily testing the Hologic Horizon A DXA is calibrated using a phantom of known composition. The Hologic Horizon A DXA undergoes a yearly preventative diagnostic evaluation.

Lean mass, bone mineral content and fat mass (total, visceral, and subcutaneous) will be measured by DXA (Hologic Horizon A) pre and post intervention. The Hologic Horizon A will allow regional measurement of trunk, android, gynoid, abdominal subcutaneous and abdominal visceral adipose tissue.

If the Hologic DXA scan is not available, we will use the GE-LUNAR DXA scan from Dr. Kelly's group.

Actigraph Sensor placed at Clinical Research Unit (CRU)

We will use actigraphy (ie Actigraph Link or Phillips Spectrum) to quantify physical activity, sleep duration, and sleep quality. These accelerometry-based sensor has been used extensively in clinical research to objectively quantify physical activity. The sensors will be worn up to 14 days. This will be placed at Visit 1 and Visit 5.

Glucose Sensor placed at Clinical Research Unit (CRU)

We will use a blinded CGM (Freestyle Libre Pro, or DEXCOM G6) to evaluate the dietary intervention on the daily glucose profile**.** The CGM captures interstital glucose every 15 minutes to allow characterization of overnight glucose levels, average glucose levels and glycemic variability. The sensors will be worn up to 14 days. It will be placed at Visit 1 and Visit 5.

MRI at Center for Magnetic Resonance Research (CMRR)

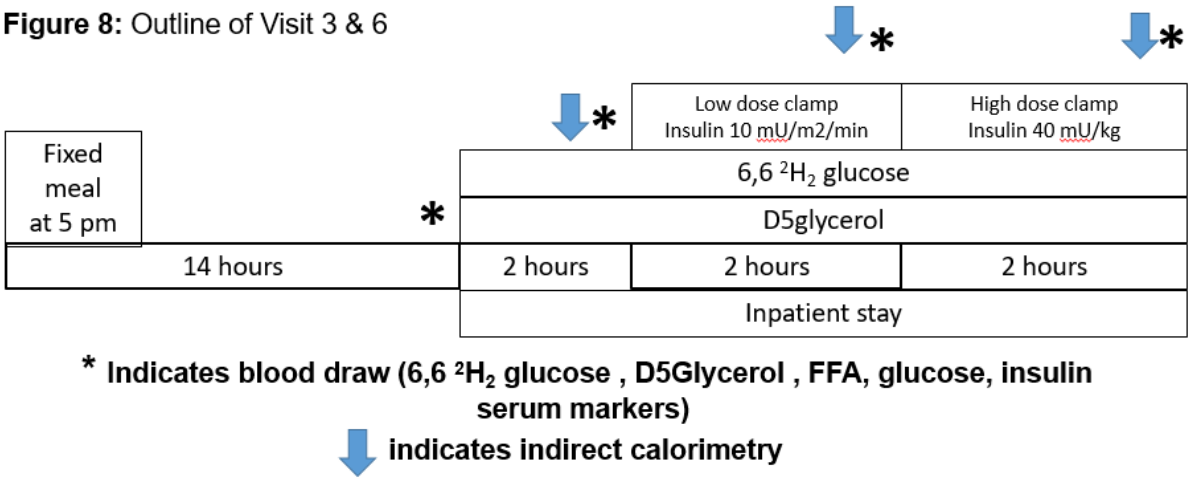
MRI will be used to measure abdominal fat in the liver, visceral fat, and ectopic fat in the pancreas and heart

All MRI scans will be performed on a 3T Prisma MR System (Siemens, Erlangen, Germany) located at the UMN Center for Magnetic Resonance Research (CMRR) / Center for Clinical Imaging Research (CCIR) at Visit 2 and Visit 5. Hepatic fat (HFF), pancreatic fat, visceral fat, and cardiac fat content will be measured using magnetic resonance spectroscopy (MRS).[39] Anticipated scan time ~ 1 hour.

Glucose clamp at Clinical Research Unit (CRU)

For Visit 3 and 6, participants will be provided a fixed meal to eat as dinner the night before the study, which they will eat at home. They will be instructed to consume this meal around 5-6 pm. They will then remain fasting after eating this meal (water and medications are allowed) and will report the next morning around 7 am to the CRU to perform their clamp (Figure 8). At 7 am the next day, participants will receive D5glycerol and 6,6 $^{2}H_{2}$ glucose for 2 hours prior to the 2 step hyperinsulinemic-euglycemic clamp. With this design, baseline lipolysis and endogenous glucose production will be measured after a 16 hour fast, which is the same duration as the TRE fasting period.

Figure 8: Outline of Visit 3 & 6



*** Indicates blood draw (6,6 $^2H_2$ glucose , D5Glycerol , FFA, glucose, insulin serum markers)**

indicates indirect calorimetry

Baseline blood samples will be taken to measure glucose, FFA, insulin, glucose enrichment, and D5Glycerol enrichment. Starting at time 0, each participant will receive a continuous infusion of D5glycerol (0.1 to 0.4 , will be generally ~ 0.28 mg/kg Bolus over 1-5 min (generally around 2 minutes) and then 0.01 to 0.04 mg/kg/min, will be generally ~ 0.0195 mg/kg/min)[29] and 6,6$^2H_2$ glucose (initial bolus 3.5 mg/kg over1-10 minute (generally around 7 minutes), then continuous 0.04 mg/kg/min infusion).[30] Prior to initiation of the insulin clamp (120 min), indirect calorimetry (80-110 min: ParvoMedics True One 2400 metabolic cart with a canopy hood) and blood will be sampled (time 110, 115, 120 min) for resting glucose, insulin, and glucose enrichment. Next (120 min), a 3 hour low-dose hyperinsulinemic-euglycemic clamp (insulin: 10 mU/m^2/min, KPO_4 at 50 ml/hr, 20% Dextrose enriched with 1.5% of 6,6$^2H_2$ glucose)[31,32] will be initiated and the dextrose titrated for a target glucose of 90 mg/dl. Serum blood glucose will be measured every 10 minutes (Analox, UK) and insulin will be measured every 30 minutes. At the end of the 3 hour low-dose clamp, indirect calorimetry (260-290 min) and blood sampling will be repeated (290, 295, 300 min). Finally, a 3 hour high-dose hyperinsulinemic-euglycemic clamp (insulin: 40 mU/m^2/min) will be initiated (300 min) with repeat indirect calorimetry (440-470 min) and blood sampling will be repeated (470, 475, 480 min). To account for the tracer enrichment in the dextrose solution during the insulin clamp, equations described by Finegood[40] will be used to calculate Ra and Rd. Hepatic glucose output, hepatic insulin sensitivity, oxidative glucose disposal, and nonoxidative glucose disposal will be calculated similar to published literature.[41,42] Metabolic flexibility will be calculated as RQ_{clamp}-RQ_{fast}.[43,44] Glucose enrichment will be measured by Dr. Bryan Bergman's laboratory; Dr Bergman directs the Molecular and Cellular Analytical Core Facility as part of the Colorado NORC (P30DK48520) and has an extensive history performing stable isotopes analyses.[42,45]

A key feature of reduced metabolic flexibility is impaired suppression of lipolysis in response to insulin.[46] After the evening meal, the participant will remain fasting until study conclusion. A continuous infusion of D5Glycerol will be started 2 hours before the clamp and continued through the clamp. Blood samples will be collected for plasma D5 glycerol to calculate lipolysis. This will be measured at baseline (16 hours from the last meal, 2 hours after starting the D5Glycerol infusion) **(Fig 8),** at the end of the low-dose clamp and at the end of the high-dose clamp. Calculation of lipolysis will be performed as previously described.[29] As another measure of adipose tissue sensitivity, we will also examine the difference between FFA_{clamp} vs FFA_{fast}. Dr Bergman's laboratory will measure the FFA concentration and enrichment.

We will also collect up to 3 urine samples (from the commode or urinal) over the course of the visit when the participant happens to void, or use the restroom.

Blood will be drawn here for stable isotope analysis. The total amount of blood taken will be about 8-10 tablespoons during the course of the clamp.

Indirect calorimetry at Clinical Research Unit (CRU)

This will be performed during the CRU stay. This test will be done to look at the oxygen and carbon dioxide from the expired breath. The participant will lie down in a bed at the CRU with a special facemask for 30 minutes at rest. We will then capture the expired oxygen and carbon dioxide for another 30 minutes. This will be repeated 2 more times during the clamp, for a total of 3 times with each clamp.

Aim Specific Outcome Measures are noted in the respective sections below

Aim#1: Evaluate the effect of TRE with *ad libitum* intake on weight and body composition

Rationale: TRE is associated with prolonged daily fasting. Prolonged fasting mobilizes adipose tissue triglycerides, with 60% of the increase in lipid kinetics occurring between 12-24 hours of fasting.[24] Although we reported that TRE reduces weight compared with non-TRE **(Fig 2)** it remains unknown:1) How TRE weight-loss may compare with CR weight loss, 2) Whether weight loss associated TRE (prolonged daily fasting) or CR (reduced caloric intake over a prolonged eating window) may differentially alter body composition.

Hypothesis : H 1.1: The TRE and CR groups will have similar weight loss, which will be greater than weight loss achieved in the non-TRE group (primary outcome). H 1.2: TRE will result in greater loss of loss of total body fat (quantified by DXA) and greater loss of hepatic/visceral fat/ectopic fat (quantified by MRI) than CR.

Outcome measure: Weight and body composition [Dual X-ray absorptiometry (DXA)]
Weight (primary outcome) will be measured at baseline, Week 6 and Week 12. Lean mass, bone mineral content and fat mass (total, visceral, and subcutaneous) will be measured by DXA (Hologic Horizon A) pre and post intervention. The Hologic Horizon A will allow regional measurement of trunk, android, gynoid, abdominal subcutaneous and abdominal visceral adipose tissue.[38,47]

Outcome measure: Magnetic Resonance Imaging - Liver Fat
Measurement of Liver and Pancreatic Fat Content via MRI
Each participant will have Hepatic Fat Fraction (HFF) measured by MRI via ^{1}H- magnetic resonance spectroscopy (MRS) at the Magnetic Resonance Research Institute at the University of Minnesota. HFF will be measured with single-voxel ^{1}H-MRS on a 3.0 T Prisma whole body MRI scanner (Siemens Medical, Erlangen, Germany) using the software package provided by the vendor (the HISTO sequence from Siemens' LiverLab). After localizer and anatomical images, a single voxel (3x3x3 cm) will be positioned in the right lobe of the liver while avoiding hepatic and portal vessels. A single volume stimulated echo acquisition mode (STEAM) measurement (TR/TM = 3000/10 ms) without water or lipid suppression will be acquired at 5 TE values (TE=12, 24, 36, 48, 72 ms) over a single breath-hold to enable measurement and correction of T2 effects. The HISTO program fits the water and lipid peaks and calculate a liver fat percentage. The MRS measurement will be performed 2 times to assess repeatability of HFF concentration. In our preliminary work, the coefficient of variation between

measurements was 0.78% with an inter-class correlation coefficient of 0.936, showing high repeatability and reproducibility. The pancreatic fat fraction will be measured using a similar MRS method, but with a smaller voxel size (2x2x2 cm) and two averages to improve signal-to-noise. Additionally, a proton density fat fraction image will be acquired using a chemical-shift encoded (CSE-MRI) method (LiverLab's "qdixon", 3D gradient echo, TR=9ms, flip angle=4°, TE=1.15, 2.46, 3.69, 4.92, 6.15, 7.38 ms, 2x2x3.5mm resolution, one 17s breath-hold) to evaluate spatial distribution of fat in both liver and pancreas. The MRS measurements of hepatic and pancreatic fat will be used as the primary metric. CSE-MRI has shown promise as a measure of steatosis staging compared to liver biopsy.[48] A HFF of ≥ 5.5% will be used to diagnose NAFLD, which has been previously shown to be sensitive and specific for detecting NAFLD in adults[49] and in children versus liver biopsy.[50]

Outcome Measure: Magnetic Resonance Imaging – Visceral Fat

In addition to DXA, Visceral fat will also be measured by MRI.[51,52] The volume of visceral adipose tissue will also be measured using a CSE-MRI method optimized for full abdominal coverage.[15] The CSE-MRI acquisition (qdixon, sagittal 3D gradient echo, TR=9ms, flip angle=4°, TE=1.15, 2.46, 3.69, 4.92, 6.15, 7.38 ms, 3.2x3.2x3 mm resolution, one 22s breath-hold) will produce a 3D isotropic fat fraction map covering from the liver dome to pelvic floor. The images will be reformatted in the axial plan and processed using ITK-SNAP.[16] The fat fraction image will be automatically thresholded to remove signal from background and air cavities. Manual processing will be used to exclude arms and breast tissue, and to delineate the visceral, subcutaneous, and vertebral/intramuscular compartments. From these the visceral (VAT), subcutaneous (SAT), and total adipose tissue (TAT) volumes (mL) will be extracted.

Exploratory Outcome: Magnetic Resonance Imaging - Ectopic fat in the pancreas and heart

Pancreatic fat quantification

The pancreatic fat fraction will be measured using a similar MRS method, but with a smaller voxel size (2x2x2 cm) and two averages to improve signal-to-noise. Additionally, a proton density fat fraction image will be acquired using a chemical-shift encoded (CSE-MRI) method (LiverLab's "qdixon", 3D gradient echo, TR=9ms, flip angle=4°, TE=1.15, 2.46, 3.69, 4.92, 6.15, 7.38 ms, 2x2x3.5mm resolution, one 17s breath-hold) to evaluate spatial distribution of fat in both liver and pancreas. The MRS measurements of hepatic and pancreatic fat will be used as the primary metric. CSE-MRI has shown promise as a measure of steatosis staging compared to liver biopsy.[48] A HFF of ≥ 5.5% will be used to diagnose NAFLD, which has been previously shown to be sensitive and specific for detecting NAFLD in adults[49] and in children versus liver biopsy.[50]

Cardiac (Ectopic) Fat Quantification via MRI

1H magnetic resonance spectroscopy (MRS) is non-invasive in vivo measurement of steatosis applied in youth.[45]It permits precise and reproducible quantitation of intracellular triglyceride content in the cytosol of non-adipose cells. As previously described, a spectroscopic volume of interest (a single voxel, 0.8×1.8×2.4cm3) will be positioned over the

interventricular septum using end-systolic cardiac cine images in two cardiac planes (short axis and semi 4 chamber axis), collected at end-expiration.[53] During acquisition, participants breathe freely, with data acquisition triggered simultaneously at end systole (via ECG gating) and end expiration (via a respiratory navigator PACE). Spectra are collected without water suppression using the following parameters: repetition time was ~4s depending on HR; echo time, 35ms; 1,024 data points over a 2,000-Hz spectral width; and 32 acquisitions. Spectroscopy data will be processed by Dr. Bolan using commercial software (NUTS, Acorn NMR, Fremont, CA) as previously described.[49] Final calculation of fat and water signal intensities will account for fat and water signal decay due to spin-spin relaxation.[49,54] Myocardial TG will be expressed as a percentage of tissue water content. Imaging extracellular fat (pericardial fat) content will be performed using a Turbo FLASH sequence with a multi-slice 2D transverse acquisition and single-shot per slice, as previously described.[55] We will acquire 13 slices in total, 6-mm thickness with 6-mm slice gap, FOV=400×400mm2, matrix=144×192, spatial resolution=2.78×2.08×6mm3, flip angle=10, TR/TE=3.2/1.27ms, receiver bandwidth = 651 Hz/pixel. Pericardial fat quantification will be performed using commercially-available ORS Visual Imaging Software (Object Research Systems, Inc., Montreal, Canada), in the following ways: 1) as volume (cm3) in a 6 mm slab at the level of the left main origin; 2) as volume (cm3) in a 6 mm slab at the level of the right ventricular (RV) free wall; and 3) as thickness (mm) of the pericardial fat at RV free wall in the same slice Tracing of the fat border will be manually performed by an experienced reader, blinded to all participant characteristics.

Aim#2: Assess the effect of TRE with *ad libitum* intake on caloric balance.

Rationale: By prolonging the fasting period, TRE reduces the available eating window and therefore may reduce caloric intake. This was suggested by previous literature [estimated energy intake declined by 341±53 kcal/day][6] and our preliminary data.It remains unknown whether reducing the eating window may alter nutritional density of ingested foods or alters other non-eating behaviors in compensation.

Hypothesis: H 2.1:TRE reduces caloric intake [gold-standard interviewer administered 24-hour dietary recall compared with non-TRE and similar to CR, H.2.2: TRE will not alter physical activity and will increase fat oxidation compared with CR or non-TRE.

Outcome Measure: Caloric intake as measured by 24-hour dietary recall (primary outcome)

Three interviewer-administered 24-hour dietary recalls will be collected from each participant during each of measurement periods: 1) Weeks 1-2 of baseline; and 2) Weeks 11-12 of the intervention (total of 6 recalls/participant). The recalls will be conducted over the telephone and will be unannounced (unscheduled within the measurement period) to minimize measurement reactivity. The Nutrition Data System for Research (NDSR)[33], a dietary analysis software program developed and maintained by the University of Minnesota Nutrition Coordinating Center (NCC), will be used to collect the dietary recalls. When collecting recalls using NDSR, we will use the multiple-pass interview technique to prompt for complete food and beverage recall and descriptions.[34] The time of each eating occasion will be also be

recorded. A food amount booklet adapted from Van Horn et al[35] will be provided to participants for use in estimating food and beverage amounts. The dietary recalls will be carried out by staff at NCC who are trained and certified in telephone-based collection of dietary recalls using NDSR. Interview staff will be blinded to participant intervention assignment. NCC has a long history of performing dietary assessment services to researchers.

Using 24-hour dietary recall data, average energy intake at each measurement period will be calculated for each participant and used to compare pre-post change in energy intake between interventions. To evaluate change in diet quality a Healthy Eating Index 2015 (HEI-2015) score will be estimated for each participant at each measurement period. The HEI-2015 is a tool developed by the United States Department of Agriculture and the National Cancer Institute to evaluate the extent to which diets are consistent with the Dietary Guidelines for Americans[36,37] Possible total index points range from 0-100, with a higher score indicating greater consistency of the diet with the Dietary Guidelines for Americans. Scores for each of the 13 components of the index (e.g. total vegetables, whole grains, added sugars) will also be calculated.

Outcome Measures: Fat oxidation/Basal Metabolic rate
This will be measured by indirect calorimetry (Described in Aim 3).

Outcome Measures: Physical activity.
All participants will wear the Actigraph Link (up to 2 weeks) pre and post-intervention

Aim#3: Determine the impact of TRE with *ad libitum* intake on metabolic flexibility

Rationale: Metabolic flexibility is the adaptability of fuel selection given ambient fuel exposure. Adverse health, such as obesity, insulin resistance, type 2 diabetes and sedentary behavior, are associated with metabolic inflexibility.[56,57] In contrast, exercise and CR induced weight loss enhances metabolic flexibility.[57] In animals, restricting the eating window improves metabolic flexibility by enhancing fat oxidation while fasting and augmenting carbohydrate oxidation while feeding.[1] In humans, higher fat oxidation stabilizes weight gain in response to chronic overfeeding. [58] A longitudinal study in Pima Indians reported that participants who were low fat oxidizers [90%tile of 24-hr RQ] were at 2.5 times higher risk of gaining ≥5 kg body weight (25 months follow up±11 months) than those who were high fat oxidizers [10%tile of 24-hr RQ].[59] Similar findings have been observed in Caucasian males; non-obese men who are low fat oxidizers (fasting RQ ≥ 0.85) were 2.42 times (95% CI 1.10-5.32) more likely to gain ≥5 kg over 10 years than non-obese men who are high fat oxidizers (fasting RER < 0.76).[60] This Aim has the following conceptual model (**Fig 1**): TRE results in daily prolonged fasting. Chronic daily prolonged fasting enhances metabolic flexibility, with enhanced lipolysis and fat oxidation while fasting and enhanced glucose oxidation with glucose exposure (i.e. hyperinsulinemic-euglycemic clamp setting). Augmenting metabolic flexibility will reduce systemic fat depots (Aim 1) and improve insulin sensitivity (Aim 3).

Hypothesis: H 3.1: TRE will enhance metabolic flexibility compared with CR and non-TRE as measured by indirect calorimetry at rest [before and during 2 step 6,6-$^2H_2$ hyperinsulinemic-euglycemic clamp: primary outcome.H 3.2: TRE will improve insulin sensitivity compared with non-TRE and similar to CR. H 3.3: TRE will augment fasting lipolysis as measured by D5Glycerol and enhance lipolysis

Outcome Measure: Metabolic flexibility – RQ response to hyperinsulinemic-euglycemic clamp

Each participant will receive a standardized mixed meal at 5 pm to take home. After the mixed meal, the participant will fast (water permitted) overnight (**Fig 8**). At 7 am the next day, participants will receive D5Glycerol and 6,6 $^2H_2$ glucose for 2 hours prior to the 2 step hyperinsulinemic-euglycemic clamp. With this design, baseline lipolysis and endogenous glucose production will be measured after a 16 hour fast.

Baseline blood samples will be taken to measure glucose, FFA, insulin, glucose enrichment, and glycerol enrichment. Starting at time 0, each participant will receive a continuous infusion of D5glycerol (0.1 to 0.4 , will be generally ~ 0.28 mg/kg Bolus over 1-5 min (generally around 2 minutes) and then 0.01 to 0.04 mg/kg/min, will be generally ~ 0.0195 mg/kg/min)[29] and 6,6$^2H_2$ glucose (initial bolus 3.5 mg/kg *over 1-10 minutes (generally around 7 minutes) , then continuous 0.04mg/kg /min infusion).[30] Prior to initiation of the insulin clamp (120 min), indirect calorimetry (80-110 min: ParvoMedics True One 2400 metabolic cart with a canopy hood) and blood will be sampled (time 110, 115, 120 min) for resting glucose, insulin, and glucose enrichment. Next (120 min), a 3 hour low-dose hyperinsulinemic-euglycemic clamp (insulin: 10 mU/m^2/min, KPO_4 at 50 ml/hr, 20% Dextrose enriched with 1.5% of 6,6$^2H_2$ glucose)[31,32] will be initiated and the dextrose titrated for a target glucose of 90 mg/dl. Serum blood glucose will be measured every 10 minutes (Analox, UK) and insulin will be measured every 30 minutes. At the end of the 2 hour low-dose clamp, indirect calorimetry (200-230 min) and blood sampling will be repeated (230,235,240 min). Finally, a 2 hour high-dose hyperinsulinemic-euglycemic clamp (insulin: 40 mU/m^2/min) will be initiated (240 min) with repeat indirect calorimetry (320-350 min) and blood sampling will be repeated (350,355,360 min). To account for the tracer enrichment in the dextrose solution during the insulin clamp, equations described by Finegood[40] will be used to calculate Ra and Rd. Hepatic glucose output, hepatic insulin sensitivity, oxidative glucose disposal, and nonoxidative glucose disposal will be calculated similar to published literature.[41,42] Metabolic flexibility will be calculated as RQ_{clamp}-RQ_{fast}.[43,44] Glucose enrichment will be measured by Dr. Bryan Bergman's laboratory; Dr Bergman directs the Molecular and Cellular Analytical Core Facility as part of the Colorado NORC (P30DK48520) and has an extensive history performing stable isotopes work.[42,45]

Outcome Measure: Lipolysis alterations in response to hyperinsulinemic-euglycemic clamp

A key feature of reduced metabolic flexibility is impaired suppression of lipolysis in response to insulin.[46] After the evening meal, the participant will remain fasting until study conclusion. A continuous infusion of albumin-bound D5Glycerol, will be started 2 hours before the clamp

and continued through the clamp. Blood samples will be collected for plasma D5Glycerol to calculate lipolysis. This will be measured at baseline (16 hours from the last meal, 2 hours after starting the D5Glycerol infusion) **(Fig 8),** at the end of the low-dose clamp and at the end of the high-dose clamp. Calculation of lipolysis will be performed as previously described.[29] As another measure of adipose tissue sensitivity, we will also examine the difference between FFA_{clamp} vs FFA_{fast}.Dr Bergman's laboratory will measure the FFA concentration and enrichment.

Schedule of Events

	Visit 0 (Virtual)	**Visit 1 (In Person-CRU)**	**Phone Call Visits 1**	**Visit 2 (In Person – Mariucci & CMRR)**	**Visit 3 (In Person-CRU)** ***Meal picked up night before***	**Visit 4 (Virtual)**	**Visit 5 (In Person – Mariucci & CMRR)**	**Phone Call Visits 2**	**Visit 6 (In Person-CRU)** ***Meal picked up night before***
	Screening	Baseline Visit 1	3 Dietary Recall Phone Calls	Baseline Visit 2	Baseline Visit 3 ***(Randomization)***	Mid Intervention Visit	End Study Visit 1	3 Dietary Recall Phone Calls	End Study Visit 2
	Pre Intervention	Pre Intervention	Pre Intervention	Pre Intervention	Week 1 of Intervention	Week 6 of Intervention	Week 10 of Intervention	Weeks 11&12 of Intervention	Week 12 of Intervention
Time Point	10-14 days prior to Visit 1	Day 1	2 week period between Visits 1 & 2	2-3 weeks after Visit 1	1-7 days after Visit 2	6 weeks after Visit 3	4 weeks after Visit 4	2 week period between Visits 5 & 6	2-3 weeks after Visit 5
Participant Stipend	$10	$ 15	$25	$50	$100	$25	$50	$25	$100
Consent	X								
Survey	X *(eating disorder)*	X *(appetite sensation)*			X *(SF-36)*	X *(appetite sensation)*	X *(appetite sensation)*		X *(SF-36)*
Height/Weight	X *(self-report)*	X		X	X	X *(self-report)*	X		X
mCC app downloaded	X								
Ongoing mCC monitoring		X	X	X	X	X	X	X	X
Blood draw *(fasting)*		*X1,X2*			X3				X1,3,4
Urine sample					X				X
Dietary recall			X					X	
DEXA Scan *(fasting)*				X			X		
Wearable sensor distributed (CGM)		X					X *(after MRI)*		
Wearable sensor removed (CGM)				X *(before MRI)*					X
Wearable sensor distributed (Actigraph)		X					X *(after MRI)*		
Wearable sensor removed(Actigraph)				X *(before MRI)*					X
MRI (1 hour scan)				X			X		
Dietician counseling: Initial dietary telephone counseling to all 3 groups after Visit 3. Dietician to call TRE and CR groups for dietary counseling weekly for 1st 4 weeks and every 2 weeks thereafter. mCC logging feedback: Weekly email/text (participant preference) on mCC logging to all groups starting after Visit 1.									

Inpatient CRU stay for clamp/fat breakdown measurements (7am)					X				X
Indirect calorimetry					X				X
X1 Fasting glucose, insulin, whole blood for saved serum & plasma									
X2 CMP, HbA1c, TSH, Hgb, Lipid									
X3 Clamp procedure, saved samples of serum, plasma, and whole blood									
X4 HbA1c, Lipid									

5.3 Study Duration: The anticipated duration for

- Each subject (screening visit to study completion): 6 months
- Screening of subjects (4 years)
- Completion of study visits (4 years)

5.4 Use of radiation*:* Yes, we will use DXA to measure body composition before and after the intervention.

5.5 Use of Center for Magnetic Resonance Research*:* Yes, we will use CMRR to measure ectopic fat by 3T MRI before and after the intervention. The research team will utilize the CMRR Center's screening tools and adhere to the screening SOP during enrollment of all research participants in this protocol. The CMRR Center's screening tools and SOP are IRB approved under the CMRR Center Grant (HSC# 1406M51205) and information regarding screening procedures is publically available on the CMRR website (CMRR Policies / Procedures).

6.0 Data and Specimen Banking

6.1 Storage and Access: The data will be stored electronically in the CTSI REDCap database as well as a secured AHC server designated for research purposes. Access to these databases will be through AHC computers, which are password protected in a locked office area. Hard copies of the data will be stored in a locked cabinet in a secured area in Endocrinology. De-identified data will be uploaded to the cloud (smartphone app data, wearables data, and CGMS data) and then downloaded by study staff. After the study is completed, the de-identified, archived data will be transmitted to and stored at the Salk Institute and University of Minnesota, for use by other researchers including those outside of the study.

Paper data will be stored in the locked office of the PI. REDCap is an electronic system that is accessed via a secure web interface.

The data will be stored electronically in the CTSI REDCap database as well as a secured AHC server designated for research purposes. Access to these databases will be through AHC computers which are password protected in a locked office area. Hard copies of the data will be stored in a locked cabinet in a secured area in Endocrinology.

With the participant's approval and as approved by the UMN IRB, de-identified biological samples will be stored at Dr. Mashek's laboratory or AHC Biorepository.

The samples will be stored using a code-link that will allow linking the biological specimens with the clinical/phenotypic data from each participant, maintaining the masking of the identity of the participant. The sample and associated data will be stored indefinitely. Only authorized study/repository staff will have access to the specimens and data. Genetic testing will not be performed.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage will not be possible after the study is completed.

The samples may also be analyzed by the study team's collaborators (internal or external) for additional analysis. If the samples are sent externally, a material transfer agreement will be signed. All samples will be sent deidentified. The samples may be analyzed for various metabolites/chemicals but will not be analyzed for genetic testing.

6.2 Data:

- Phone screening: Screening interview with personal information
  - This will include name, birthdate, email address, phone number, home address, and medical record number
- Visit 1-6: History and Measurement data. This will include information about glucose profiles(up to 14 days), physical activity, body composition (By DXA and MRI), plasma samples, details about dietary intake (24 hour recall)
- Dietary program: Documentation of compliance with dietary program through the MyCircadianClock application

6.3 Release/Sharing:

We attest that we will register this clinical trial in ClinicalTrials.gov prior to any subject enrollment. Applicable results will be posted upon completion of the project and specified timeline as required by the NIH policy on the dissemination of NIH-funded clinical trials information. The informed consent documents for this study will include a specific statement regarding the posting of the clinical trial information at ClinicalTrials.gov. The University of Minnesota has an institutional policy ensuring that clinical trials registration and results reporting occur in compliance with policy requirements.

This data will also be available for future use by other investigators if proper IRB approval and material transfer agreements are obtained. The data will be made available after the main findings from the final research data set have been accepted for publication. This shared data will be provided de-identified for analysis.

7.0 Sharing of Results with Participants

7.1 Each participant's individual results will be provided to the specific individual. The results will otherwise be analyzed using de-identified data for presentation at national meetings and publication in relevant journals

7.2 Sharing of genetic testing: N/A

8.0 Study Population

8.1 Inclusion Criteria:

- Age 18-65,
- BMI ≥30 and ≤ 55 kg/m2
- Own a smartphone compatible with the myCircadianClock phone application.
- Self-reported habitual wakening between 5-9 am,
- Self reported sleep duration of 6-9 hours
- Weight must be stable [+/- 5 pounds] for at least 3 months prior to the study.
- Eating window (time between 1st food intake and last food take) ≥12 hours using mCC
- Able to understanding English

8.2 Exclusion Criteria:

- Beta blockers or medications known to affect weight such as TZD, insulin, GLP-1 agonists, phentermine, or sibutamine.
- Shift work (working from 11pm to 7 am)
- Clinically significant medical issues (diabetes, cardiovascular disease, uncontrolled pulmonary disease),
- A history of abnormal laboratory results, such as hematologic (platelets < 100), hepatic (LFTs > 2X nl), renal (Cr > 1.5)
- MRI contraindication (metal in body, claustrophobia).
- Eating window < 12 hours
- Unable to consistently document food intake using the myCC app (need at least 2 eating occasions> 6 hours apart on a given day for at least 50% of days)
- Pregnancy and illiteracy
- Concern for active eating disorder per screening questionnaire
- Self-reported eating disorder or history of eating disorder

8.3 Screening:

The screening phone call will introduce the study (see phone script), review study eligibility (including a 5 question survey to screen for disordered eating) and include a verbal consent for fasting.[27]

We will request a waiver of documentation of consent during the phone screening.

The Screening Visit (Visit 0) will be a virtual visit using Zoom or Doximity. Informed consent will be obtained by the study team. The entire consent document will be reviewed, including all study procedures and expectations, risks, benefits, and what volunteering means. Candidates will be given time to read the consent, ask questions, and to take the consent home to review if requested. Height and weight will be self-reported. An eConsent/HIPAA form will be developed in REDCap and provided to potential participants for esignature. Paper copies will be used dependent on participant preference.

The current UMN consent/HIPAA form will include Salk approved consent language for the mCC app usage. The signed consent/HIPAA form will be applicable for both UMN and Salk procedures. Once the participant has signed the consent/HIPAA form, the mCC app will be downloaded from the iTune Appstore or Google Play appstore. After activation of the app and device ID authentication, the study code cannot be concurrently used with another device. A unique random alphanumeric character string will be used to encode the user and only that identifier will be used to transmit encrypted data between the device and a HIPAA compliant cloud server.

Once consented using the site consent form and the online app consent form, the subject will be scheduled for their baseline appointment (Visit 1).

9.0 Vulnerable Populations

9.1 Vulnerable Populations:

Population / Group	Identify whether any of the following populations will be targeted, included (not necessarily targeted) or excluded from

	participation in the study. Children greater than age 18 will be allowed to participate.
Children	Excluded from Participation
Pregnant women/fetuses/neonates	Excluded from Participation
Prisoners	Excluded from Participation
Adults lacking capacity to consent and/or adults with diminished capacity to consent, including, but not limited to, those with acute medical conditions, psychiatric disorders, neurologic disorders, developmental disorders, and behavioral disorders	Excluded from Participation
Non-English speakers	Excluded from Participation
Those unable to read (illiterate)	Excluded from Participation
Employees of the researcher	Excluded from Participation
Students of the researcher	Excluded from Participation
Undervalued or disenfranchised social group	Included/Allowed to Participate
Active members of the military (service members), DoD personnel (including civilian employees)	Included/Allowed to Participate
Individual or group that is approached for participation in research during a stressful situation such as emergency room setting, childbirth (labor), etc.	Excluded from Participation
Individual or group that is disadvantaged in the distribution of social goods and services such as income, housing, or healthcare.	Included/Allowed to Participate

Individual or group with a serious health condition for which there are no satisfactory standard treatments.	Excluded from Participation
Individual or group with a fear of negative consequences for not participating in the research (e.g. institutionalization, deportation, disclosure of stigmatizing behavior).	Excluded from Participation
Any other circumstance/dynamic that could increase vulnerability to coercion or exploitation that might influence consent to research or decision to continue in research.	Excluded from Participation

9.2 Additional Safeguards:

Undervalued/disenfranchised/Active members of the military and DoD employees will not be identified and information will not be collected as to a patient's status. This research does not add risk to this group. We will not specifically recruit from this population but will not exclude a participant from this population if they volunteer and are eligible for the study.

10.0 Local Number of Participants

10.1 Local Number of Participants to be Consented: We anticipate screening and consenting ~250 people in person. We anticipate recruiting ~ 90 participants for which 72 will complete the study.

11.0 Local Recruitment Methods

11.1 Recruitment Process: The success in recruitment lies in a multimedia approach, including recruitment through the electronic health record, online advertisement, newspaper, television and radio public interest stories; public service announcements; solicitation of health care provider referrals; direct mailings to potential participants; advertisements; small print media, registration in StudyFinder and Clinicaltrials.gov and creation of a study website similar to previous clinical trials at the University of Minnesota. Based on our success with our current R01 recruitment, we will rely heavily on using the electronic health record (EHR:EPIC) for recruitment (BPIC based)

Potentially, individuals who are a patient of the Endocrinology Clinic and have an upcoming visit will be approached at that visit. Otherwise, we may also have their primary endocrinologist sign a letter supporting their eligibility which we will mail

to the patients and then follow up with a phone call. We will only call participants who are part of the group practice for the Endocrinology division to follow up on the mailed letters.

If the individual is not part of the Endocrinology clinic, the general recruitment will use BPIC to identify the patients and Fairview Research Services/Addressing and Mailing to mail study information to potentially eligible patients explaining the study and asking them to contact research staff.

This study will also be listed at ResearchMatch.org. ResearchMatch is an electronic volunteer recruitment registry that allows people from anywhere in the country to self-register and express an interest in being prospectively considered for participation in research studies. It was created through the Clinical & Translational Science Awards Consortium in 2009. This registry provides information about those volunteers to researchers who are looking for people to participate in studies, while protecting the privacy of the volunteers.

11.2 Identification of Potential Participants: The University of Minnesota is a partner of the Fairview Health System which has used EPIC since 2011. As of January 2019, the Fairview EHR has ~2.8 million living patients. Of these patients, 1,700 had prediabetes diagnosed either as fasting glucose between 100-125 (inclusive) or HbA1c between 5.7-6.4 (inclusive) within the last 3 months (Data from February 2019 – May 2019) and have given consent for participation. Using the recruitment infrastructure in place with R01, we anticipate a 10% response rate (n=170) every 3 months. BPIC will identify potential participants by applying the eligibility criteria for the study to the EHR record. The first screen will encompass the most recent 6 months review of the EHR. Subsequent screens will include only the past month review of eligible participants. Patients who have opted out of research in their Epic EHR will be excluded from consideration for participation.

11.3 Recruitment Materials:

Participants may self-refer if they become aware of the study through recruitment materials or word of mouth referrals. [A letter about the study may be sent to potential participants, asking them to contact the study coordinator if they are interested.] Individuals who respond to the recruitment materials will be taken through a phone-screening questionnaire. If following the phone screen the potential participant is eligible and interested, the study coordinator will schedule the screening study visit.

The study will utilize social media accounts such as (Facebook, Twitter, Instagram, independent website, etc.) to create an informative space about research opportunities and information for patients with obesity or prediabetes.

11.4 Strategies to be implemented in the event of enrollment shortfalls:

Recruitment rates will be reviewed monthly. If recruitment is not proceeding as planned, we will recruit from additional health plans in the Twin Cities that have previously worked with the University of Minnesota on clinical trials, including Hennepin County Medical Center, Health Partners, and the Allina Health System. These health systems also have electronic health records in place to target recruitment to potentially eligible participants. We will also engage electronic media to raise the study profile within the community, including television and radio. Other options will include online advertisement, newspaper, television and radio public interest stories; public service announcements; solicitation of health care provider referrals; direct mailings to potential participants; advertisements; small print media, registration in StudyFinder and Clinicaltrials.gov and creation of a study website similar to previous clinical trials at the University of Minnesota.

11.5 Participant stipend: Subject compensation will be tied with retention. For the screening visit, the subjects will be paid $25. If they are eligible for the study and complete the baseline visits (Visit 3), they will be paid $125. The will receive $50 for the midpoint visit (Visit 4) and $200 for completing the entire study (Visit 7). If the subject successfully completes the entire study, monetary compensation will be $400. Additional retention techniques will include the following: 1) Upfront notice during phone screening and screening visit about time needed for study commitment, 2) Prorated compensation, 3) Tailoring dietary interventions to improve palatability while remaining within the parameters. Compensation will be provided by a check mailed to the participant. To increase retention in the non-TRE (control) group, we will offer a complimentary dietary session after the intervention and Visit 6 have been completed to point out dietary changes the participant may wish to consider to support good nutrition and health body weight. A resource book may also be provided.

12.0 Withdrawal of Participants

12.1 Withdrawal Circumstances:

- Stopping rules met
- Participant request
- Per judgement of PI

12.2 Withdrawal Procedures: If the participants want to discontinue their dietary intervention, we will offer the following options in a sequential fashion: 1) Address any limiting factors that may prevent program completion, 2) Move the final assessment sooner to be performed at the time of dropping out, 3) Continue data collection as intensively as possible given the participant's preferences. We will also collect reasons for drop out (i.e. time/duration, transportation, side effects, perceptions of efficacy, interaction with staff) to use as potential covariates in the statistical model. In terms of missing data, results from the last observation carried forward as well as multiple imputation analysis[61] For the final analysis, intention to treat analysis and per-protocol analysis (analysis in those who completed the protocol) will be performed.

12.3 Termination Procedures: This will be the same as the withdrawal procedures as previous described.

13.0 Risks to Participants

13.1 Foreseeable Risks: Foreseeable Risks:

There will be potential physical risks associated with the study as described in next section. The seriousness of these risks is low. The risks to the subjects arise from using established laboratory techniques (Venipuncture, hyperinsulinemic, euglycemic clamp, stable isotope tracers, DXA, CGMs, MRI). The infusions used in the study are naturally occurring (i.e. insulin, glucose, potassium, D5Glycerol, 6,6 2H2 glucose) and have a history of being successfully used previously in human studies.

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

• Related – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.

• Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established

Study Procedure	Risk	Likelihood/seriousness/mitigation
Phone screening	Breach of confidentiality	Low/Low. We will ask questions related to health, fitness, and eligibility for the study. Data will be stored on password protected computer in locked area.
Periods of fasting	Hunger, lightheadedness	Low/Low. This will be kept to less than 24 hrs. Adequate fluids (ie water) will be given to minimize dehydration.

History data	Incidental findings, breach of confidentially	Low/Low. We will exclude subjects if history is clinically relevant.
Venipuncture lab tests	Incidental findings, Transient pain/bleeding/bruising;	High/Low. This is a standard blood draw and total amount of withdrawn blood will be held to IRB guidelines.
MRI	Claustrophobia, disruption/heating of metal implants	Low/Low. Screening questionnaire will be performed prior to at the phone screen and repeated just prior to MRI. Any participants who have a contraindication to MRI will be excluded from imaging.
DXA	Radiation exposure	High/Low. This is less than one day from the natural background radiation in Minnesota.
CGM	Rash on Skin, irritation to skin from adhesive pads, incidental findings	Low/Low. The CGM results will be blinded to the participant. The goal is to monitor glycemic fluctuations with exercise. If significant hyperglycemia or hypoglycemia is noted after unblinding, the patient and their primary provider will be notified for further evaluation and management.
Actigraphy derived sleep and physical activity data	Rash on Skin	Low/Low. Noninvasive measurement of sleep and activity data by wearing Actigraph Link on wrist for up to 14 days
Dietary Recall	Breach of confidentiality	Low/Low. Study staff will call participants and ask about dietary intake for the last 24 hours. The actual call will be unannounced but within a specified 2 week window. Participant will be aware of the 2 week window. This will occur 6 times during the study.

Study Procedure	**Risk**	**Likelihood/seriousness/mitigation**
Hyperinsulinemic-euglycemic clamp	May cause low blood sugar, resulting in hunger, sweating, or weakness.	High/Low. Blood glucose levels will be monitored every ten minutes. The participant will be kept supine for the visit with bathroom privileges (bedside commode/urinal). Glucose will be administered by IV at the same time as insulin. After the clamp is completed, the participant will be fed a meal and kept at the CRU until glucose level meets criteria for discharge.
6,6 $^{2}H_2$ glucose infusion	Possible nausea and discomfort associated with infusion.	Low/Low. This will be prepared by the Fairview Investigational Drug Pharmacy. This is an established technique for measuring glucose

		disposal. Subjects will be monitored during administration.
D5Glycerol	Possible nausea and discomfort associated with infusion.	Low/Low. This will be prepared by the Fairview Investigational Drug Pharmacy. This is an established technique for measuring glucose disposal. Subjects will be monitored during administration.
Documentation of eating events using mCC	Breach of confidentiality	Low/Low. All documented eating events (time, image) will be stored and sent deidentified as per Salk Institute protocols.
Indirect calorimetry	Possible discomfort associated with canopy hood	Low/Low. A special facemask will be used to noninvasively collect breath samples for CO2 and O2 to calculate fat and glucose oxidation (RQ). This will be for ½ hour for each measurement and the participant will be supervised by study staff and CRU nursing during this time.

Protection against Risk

We will plan the following to minimize risk:

1) Clinical procedures will be performed by properly trained personnel who are qualified by training and licensure to perform the procedures.

2) Investigator has completed required training re: human subject protections. All personnel will comply with all related regulations and laws.

3) Subjects will be rigorously screened against inclusion/exclusion criteria to ensure that their participation is safe.

4) AEs and SAEs will be assessed and followed throughout study; Subjects will have contact information to enable them to contact study personnel easily and quickly.

5) Study data and information will be kept confidential and managed in accordance with requirements of HIPAA. All data will be stored in locked offices and not released without subject permission.

6) Subjects may discontinue participation at any time, for any reason. Any subject observed to have unacceptable responses to research procedures, or to be unable to safely tolerate participation in the study will be withdrawn.

7) A data safety monitoring plan will be in place (see appropriate section).

8) IRB approval and if needed, IND approval will be obtained (to use stable isotopes in healthy humans).

9) Monitoring will be conducted by the research team, IRB and independently (i.e., at a minimum of annually) by qualified staff of the University of Minnesota's

Clinical and Translational Science Institute (CTSI) in accordance with the established monitoring plan.

10) Dr. Chow will allocate adequate time for such monitoring activities. Dr. Chow will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

13.2 Reproduction Risks: N/A

13.3 Risks to Others: N/A

14.0 Potential Benefits to Participants

14.1 Potential Benefits: There are no guarantee of benefits. However, there is potential benefit to the human subjects would be weight-loss with the dietary intervention. Since we are deliberately enrolling obese, insulin resistant humans, their physical health may benefit from any observed weight loss. The subjects are allowed to withdraw from the study at any time per their request or per the judgment of the investigator.

15.0 Statistical Considerations

15.1 Data Analysis Plan:

Statistical Analysis Plan

Participants' demographic and characteristics at baseline will be summarized and compared between groups using Chi-square test or Fisher's exact test for categorical variables and analysis of variance (ANOVA) F test for continuous variables. Baseline characteristics that are significantly different between groups will be considered as adjusters in primary analyses. Pre-post changes in weight and body composition (Aim 1); caloric intake, diet quality, physical activity, sleep quality and duration, and fat oxidation (Aim 2); and metabolic flexibility, insulin sensitivity, and lipolysis (Aim 3) will be compared between groups using ANOVA F test. If the overall F test is significant, pairwise comparisons will be conducted with Tukey's method to adjust for multiple comparisons. In addition, because weight and diet quality are measured at three time points we will also investigate temporal trends using linear mixed effects models with subject-specific random intercepts. Data distributions will be checked before conducting statistical tests. Transformation will be done if the normality assumption is not met. We will investigate how sex and other clinical factors, such as age, influence change in weight and body composition, caloric balance, and metabolic flexibility by including relevant clinical factors into our models. Secondary analysis will be conducted to examine the association between achieved fasting duration and pre-post change in outcomes, using multivariate linear regression models. Models will include effects of achieved fasting duration, treatment group, and the

interaction between both effects. Analyses will be performed in R or SAS (Version 9.4, SAS Institute Inc., Cary, NC). We will also analyze by intention to treat and per protocol.

Missing data management:

If the participants want to discontinue their dietary intervention, we will offer the following options in a sequential fashion: 1) Address any limiting factors that may prevent program completion, 2) Move the final assessment sooner to be performed at the time of dropping out, 3) Continue data collection as intensively as possible given the participant's preferences. We will also collect reasons for drop out (i.e. time/duration, transportation, side effects, perceptions of efficacy, interaction with staff) to use as potential covariates in the statistical model. In terms of missing data, results from the last observation carried forward as well as multiple imputation analysis32 For the final analysis, intention to treat analysis and per-protocol analysis (analysis in those who completed the protocol) will be performed.

15.2 Power Analysis:

Sample size determination is based on the primary outcome in Aim 1: weight loss from baseline to 12 weeks. Based on the preliminary data and the literature,100 we anticipate weight loss of 3.6 kg (SD of 1.9) in the TRE group, 1.5 kg (SD of 2.4) in the non-TRE group, and 4 kg (SD of 2.5) in the CR group. With n=24 per group and alpha = 0.05/3 to adjust for multiple comparisons, we will have 80% power to detect the described difference in weight loss between the TRE group vs. the non-TRE group, 85% power to detect the described difference in weight loss between the CR group vs. the non-TRE group, and 83% power to detect a difference of 1 SD between groups in pre-post change in other outcomes including body composition (Aim 1), caloric intake (Aim 2), and metabolic flexibility (Aim 3). Based on the preliminary data, the SD is 1.5, 1.4, and 0.3 kg for change in fat mass, lean mass, and visceral fat, respectively. The SD for change in energy intake is estimated to be 254 kcal/d and the SD for metabolic flexibility is estimated to be 0.05.7, 101 Conservatively accounting for a 20% dropout rate, we will enroll 90 subjects (30/group).

15.3 Statistical Analysis:

Participants' demographic and characteristics at baseline will be summarized and compared between groups using Chi-square test or Fisher's exact test for categorical variables and analysis of variance (ANOVA) F test for continuous variables. Baseline characteristics that are significantly different between groups will be considered as adjusters in primary analyses. Pre-post changes in weight and body composition (Aim 1); caloric intake, diet quality, physical activity, sleep quality and duration, and fat oxidation (Aim 2); and metabolic flexibility, insulin sensitivity, and lipolysis (Aim 3) will be compared between groups using ANOVA F

test. If the overall F test is significant, pairwise comparisons will be conducted with Tukey's method to adjust for multiple comparisons. In addition, because weight and diet quality are measured at three time points we will also investigate temporal trends using linear mixed effects models with subject-specific random intercepts. Data distributions will be checked before conducting statistical tests. Transformation will be done if the normality assumption is not met. We will investigate how sex and other clinical factors, such as age, influence change in weight and body composition, caloric balance, and metabolic flexibility by including relevant clinical factors into our models. Secondary analysis will be conducted to examine the association between achieved fasting duration and pre-post change in outcomes, using multivariate linear regression models. Models will include effects of achieved fasting duration, treatment group, and the interaction between both effects. Analyses will be performed in R or SAS (Version 9.4, SAS Institute Inc., Cary, NC)

15.4 Data Integrity:

1. Rigor: Recruitment of participants will be performed using the defined inclusion/exclusion criteria, with particular efforts devoted towards even distributions of men/women and inclusion of minority populations. Data collected at the initial and final study visits will be entered by research staff into Research Electronic Data Capture (REDCap), which uses a MySQL database via a secure web interface with data checks used during data entry to ensure data quality. Data summaries and quality control checks will be run routinely.

2. Transparency: All data obtained in this project will be de-identified with each study subject receiving a unique study ID number. The de-identified data will be routinely shared among team members and discussed at regular meetings (Table 1) to evaluate recruitment status and the next step of experiments, analyses, and interpretation of the findings. Results of our studies will be submitted for publication in peer reviewed journals, and NIH requirements for access of manuscripts through PubMed Central will be fulfilled.

16.0 Health Information and Privacy Compliance

16.1 Select which of the following is applicable to your research:

☐ My research does not require access to individual health information and therefore assert HIPAA does not apply.

☒ I am requesting that all research participants sign a HIPCO approved HIPAA Disclosure Authorization to participate in the research (either the standalone form or the combined consent and HIPAA Authorization).

☐ I am requesting the IRB to approve a Waiver or an alteration of research participant authorization to participate in the research.

Appropriate Use for Research:

☐ An external IRB (e.g. Advarra) is reviewing and we are requesting use of the authorization language embedded in the template consent form in lieu of the U of M stand-alone HIPAA Authorization. Note: External IRB must be serving as the privacy board for this option.

16.2 Identify the source of Private Health Information you will be using for your research (Check all that apply)

☒ I will use the Informatics Consulting Services (ICS) available through CTSI (also referred to as the University's Information Exchange (IE) or data shelter) to pull records for me

☒ I will collect information directly from research participants.

☐ I will use University services to access and retrieve records from the Bone Marrow Transplant (BMPT) database, also known as the HSCT (Hematopoietic Stem Cell Transplant) database.

☐ I will pull records directly from EPIC.

☐ I will retrieve record directly from axiUm / MiPACS

☐ I will receive data from the Center for Medicare/Medicaid Services

☐ I will receive a limited data set from another institution

☐ Other. Describe:

16.3 Only patients who have authorized their participation in research in EPIC and have not opted out of research will be provided by BPIC for us to review for our study recruitment.

16.4 Approximate number of records required for review:

Up to 2.8 million (EPIC EHR records). This will be done by BPIC. Only patients who have authorized their participation in research in EPIC will be reviewed. We anticipate that ~ 2000 records will be referred to us for potential contact for study recruitment.

16.5 Please describe how you will communicate with research participants during the course of this research. Check all applicable boxes

☐ This research involves record review only. There will be no communication with research participants.

☐ Communication with research participants will take place in the course of treatment, through MyChart, or other similar forms of communication used with patients receiving treatment.

☒ Communication with research participants will take place outside of treatment settings. If this box is selected, please describe the type of communication and how it will be received by participants.

This communication will include the following: 1) phone calls, 2) letters, 3) texting and 4) Email. We will communicate with participants via telephone (using a study-specific phone number) for pre-screening, appointment scheduling, appointment reminders, etc. We will also communicate through text message or unsecure email if the participant agrees in writing by signing the GUIDELINES AND CONSENT FOR TEXT MESSAGE CORRESPONDENCE FOR RESEARCH PARTICIPANTS and/or GUIDELINES AND CONSENT FOR UNSECURED EMAIL CORRESPONDENCE FOR RESEARCH PARTICIPANTS.

16.6 Explain how the research team has legitimate access to patients/potential participants: The research team will be permitted to access sources of private information because all participants will be required to sign a HIPAA waiver at the time of informed consent.

16.7 Location(s) of storage, sharing and analysis of research data, including any links to research data (check all that apply).

☒ In the data shelter of the Information Exchange (IE)

☒ Store ☒ Analyze ☐ Share

☐ In the Bone Marrow Transplant (BMT) database, also known as the HSCT (Hematopoietic Stem Cell Transplant) Database

☐ Store ☐ Analyze ☐ Share

☒ In REDCap (recap.ahc.umn.edu)

☒ Store ☒ Analyze ☐ Share

☐ In Qualtrics (qualtrics.umn.edu)

☐ Store ☐ Analyze ☐ Share

☒ In OnCore (oncore.umn.edu)

☒ Store ☒ Analyze ☒ Share

☒ In the University's Box Secure Storage (box.umn.edu)

☒ Store ☒ Analyze ☒ Share

☒ In an AHC-IS supported server. Provide folder path, location of server and IT Support Contact:

MedDerm(\\med.ahc.umn.edu\med)(N:)\Chow Group

☒ Store ☒ Analyze ☒ Share

☒ In an AHC-IS supported desktop or laptop.

Provide UMN device numbers of all devices: 20181216

☒ Store ☒ Analyze ☒ Share

☒ Other. Describe:

Indicate if data will be collected, downloaded, accessed, shared or stored using a server, desktop, laptop, external drive or mobile device (including a tablet computer such as an iPad or a smartform (iPhone or Android devices) that you have not already identified in the preceding questions

☐I will use a server not previously listed to collect/download research data

☐I will use a desktop or laptop not previously listed

☐I will use an external hard drive or USB drive ("flash" or "thumb" drives) not previously listed

☒I will use a mobile device such as an tablet or smartphone not previously listed: AHC tablet to input survey data (20181716)

16.8 Consultants. Vendors. Third Parties.

Electronic data will be shared with our external collaborators using the UMN Box account. We will also use the UMN Box account to store results from our external collaborators. All samples sent to the external collaborators (i.e. electronic information to Dr. Panda, blood samples to Dr. Bergman) will be sent de-identified. These samples will be analyzed and stored in their laboratories as per their institutional protocol. Results from our external collaborators will be placed into the UMN Box account (as per above) for our analysis.

16.9 Links to identifiable data: There is be a participant unique study ID established in REDCap, which will link participants to the data. This will be used to identify the data with the REDCap data. All shared data will be deidentified.

16.10 Sharing of Data with Research Team Members. Data between study members at the University of Minnesota will be shared by phone, voice mail, U of MN email, BOX, centralized shared drive, and REDCap.

16.11 Storage and Disposal of Paper Documents: In accordance with NIH policy, all study documents will be maintained for at least 3 years ater the study ends and for a longer time if required by University of Minnesota policy. All signed and dated HIPAA authorizations and consent documents that include HIPAA authorizations wil be maintained for at least 6 years after completion of the study. After this time period, all research records will be destroyed. Computer files will be deleted and hard copy materials will be discarded in accordance with University of Minnesota policy.

17.0 Confidentiality

17.1 Data Security:

- Training: All study staff will be appropriately trained in data security.
- Authorization of access: Only designated IRB-approved staff will have access to the data.
- Password protection/encryption/physical controls: All data will be stored in REDCap.
- Certificates of confidentiality: Since this study will be funded by an NIH RO1 Grant, we will apply for a NIH Certificate of Confidentiality. All entities that are part of this study will be subject to the requirements of this Certificate.
- Separation of Identifiers: Study staff will keep the mapping of identification code to the identity of the participant in a database protected by two-levels of password protection stored separately from the data on Box.
- A Certificate of Confidentiality is automatically issued by the NIH For this project.

18.0 Provisions to Monitor the Data to Ensure the Safety of Participants

18.1 Data Integrity Monitoring. Monitoring will be conducted by study staff, IRB and independently (i.e. at least annually, as a minimum) by qualified staff of the University of Minnesota's Clinical and Translational Science Institute (CTSI) in accordance with the established monitoring plan. Monitoring events will include the following: Study recruitment, subject compliance with visits, subject accrual, adverse events, stopping rules with regard to enrollment/drop out/missing data/adverse events.

The data for each subject will be entered into a REDCap database. The REDCap database uses a MySQL database via a secure web interface with data checks used during data entry to ensure data quality. REDCap includes a complete suite of features to support HIPAA compliance, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL

database and the web server will both be housed on secure servers operated by the University of Minnesota Academic Health Center's Information Systems group (AHC-IS). The servers are in a physically secure location on campus and are backed up nightly, with the backups stored in accordance with the AHC-IS retention schedule of daily, weekly, and monthly tapes retained for 1 month, 3 months, and 6 months, respectively. Weekly backup tapes are stored offsite. The AHC-IS servers provide a stable, secure, well-maintained, and high-capacity data storage environment, and both REDCap and MySQL are widely-used, powerful, reliable, well-supported systems. Access to the study's data in REDCap will be restricted to the members of the study team by username and password.

The clinical data will be reviewed weekly by the study team to ensure progress, timely data entry and addressing missing data. The clinical data will also be reviewed monthly by Dr. Chow to double-check proper data entry and missing data.

Frequency of Monitoring	
Data Type	Frequency of Review
Study recruitment	Monthly
Subject compliance with visits	As event occurs and weekly
Subject accrual (adherence to protocol regarding demographics, inclusion/exclusion)	Quarterly
Achievement of study milestones	Quarterly
Adverse event rates	As event occurs and quarterly
Stopping rules with regards to enrollment/drop out/missing data	Yearly
Patient safety officer	As event occurs and quarterly

18.2 Data Safety Monitoring

Recording of Adverse Event:

At each contact with the subject, the investigator and study staff must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study participation is not the cause. Serious adverse events that are still

ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study participation should be recorded and reported immediately.

Reporting of Serious Adverse Events

A serious adverse event is any adverse event that is: 1) Fatal 2) Life Threatening 3) Requires or prolongs a hospital stay 4) Results in persistent or significant disability or incapacity or 5) A congenital anomaly or birth defect. Of note, important medical events are events that may not be immediately life-threatening, but are clearly of major clinical significance and may be a serious adverse event. They may jeopardize the subject, and may require intervention to prevent one or the other serious outcomes noted above.

Adverse Event Reporting Plan:

Reporting of adverse events will follow University of Minnesota IRB requirements. Within 48 hrs of any serious adverse event, a verbal or email report will be made to IRB followed by a detailed written report within 10 business days.

Patient safety officer:

We will have Dr. Amir Moheet serve as the patient safety officer for the study. He will review any adverse events related to the study, independent of the IRB and the clinical trials monitor. His report within be submitted to the IRB. His focus will be to provide independent medical opinion on study associated adverse events.

IRB Notification by Investigators

Reports of all serious adverse events (including follow-up information) must be submitted to the IRB within 10 working days if it falls under the UPIRTSO guidelines. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder

Unanticipated Problems Involving Risk To Subjects or Others (UPIRTSO) Events

Investigators are required to submit a report of UPIRTSO events to the IRB within 10 working days of first learning of the event.

Responsible Individual(s) or Group

Monitoring will be conducted by the PI, IRB and independently (i.e. at least annually as a minimum) by qualified staff of the University of Minnesota's Clinical and Translational Science Institute (CTSI, supported by the CTSA grant awarded in 2018) in accordance with the established monitoring plan.

Frequency of Monitoring	
Data Type	Frequency of Review
Study recruitment	Monthly
Subject compliance with visits	As event occurs and weekly

Subject accrual (adherence to protocol regarding demographics, inclusion/exclusion)	Quarterly
Achievement of study milestones	Quarterly
Adverse event rates	As event occurs and quarterly
Stopping rules with regards to enrollment/drop out/missing data	Yearly
Patient safety officer	As event occurs and quarterly

19.0 Provisions to Protect the Privacy Interests of Participants

19.1 Protecting Privacy:

The study consent form will describe in detail any intrusive, uncomfortable, or unfamiliar questions, procedures, or interactions with researchers or study personnel that the participant will be asked to complete. Furthermore, the study consent form will communicate that it is the participant's right to opt-out of any study procedures or the study as a whole or withdraw from the study at any time and this information will be reiterated and revisited periodically throughout the study in advance of intrusive, uncomfortable, or unfamiliar questions procedures or interactions. Participants will not be compelled or pressured to provide information or specimens or study data that they do not wish to provide.

19.2 Access to Participants*:*

Participants have been fully informed of the ways in which their data will/may be used during the informed consent process. The research team has been trained in conducting these conversations and the participants are also assessed for their understanding of consent prior to signing the consent form or initiating any study procedures.

20.0 Compensation for Research-Related Injury

20.1 Compensation for Research-Related Injury:

In the event that research-related activities result in an injury, treatment will be provided to the participant (e.g., first aid, emergency treatment, and follow-up care as needed). Care for such injuries will be billed in the ordinary manner to the participant or the participant's insurance company

20.2 Contract Language: N/A

21.0 Consent Process

21.1 Consent Process (when consent will be obtained):

Consent forms describing in detail the study, study procedures, and risks are given to the participant and documentation of informed consent is required prior

to any research procedures via written signatures or esignatures using REDCap. The following consent materials are submitted with this protocol:

Consent will take place via a virtual visit using Zoom or Doximity. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants will be given the opportunity to review the consent with family and caregivers and return on a subsequent day to sign the consent and enroll in the study.

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. We will use the teach-back method to assess the participant's understanding of the study.

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with others or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

21.2 Waiver or Alteration of Consent Process (when consent will not be obtained): We will obtain verbal consent over the phone for subject to be fasting for the first visit.

21.3 Waiver of Written/Signed Documentation of Consent (when written/signed consent will not be obtained):

We will ask the IRB to approve a Waiver of Signed Documentation of Consent for the phone screening to determine eligibility and to explain to the potential participant what will happen during the virtual Visit 0 Screening Visit.

21.4 Non-English Speaking Participants: N/A

21.5 Participants Who Are Not Yet Adults (infants, children, teenagers under 18 years of age): N/A

21.6 Cognitively Impaired Adults, or adults with fluctuating or diminished capacity to consent: N/A

21.7 Adults Unable to Consent: N/A

22.0 Setting

22.1 Research Sites:

Fairview Health System Electronic Health Record (EHR):

The University of Minnesota is a partner of the Fairview Health System which uses a modern enterprise level EHR (EPIC) since 2011. As of January 2019 the Fairview EHR has ~2.8 million living patients. Of these patients, 1,700 had prediabetes diagnosed either as fasting glucose between 100-125 (inclusive) or HbA1c between 5.7-6.4 (inclusive) within the last 3 months (since February 2019) and have given consent for participation.

UMMC Central Lab

Blood work, other than saved serum/ plasma samples, will be processed at the UMMC main lab and results faxed to the PI.

Clinical and Translational Science Institute (CTSI), University of Minnesota

The Clinical and Translational Science Institute (CTSI) at the University of Minnesota offers comprehensive research support for clinical investigators, from concept through publication. CTSI supports all stages of the research process, providing research and regulatory support, specialized facilities, and analytical services. Concept and pre-study services include assistance with study design (biostatistical support), protocol and budget development, study population definition (i2b2/SHRINE resources), bioinformatics support, investigational new drug (IND) or investigational device exemption (IDE) application assistance, contract negotiations, institutional committee's approval assistance (IRB, Radiation Safety, Institutional Biosafety, etc.), and clinical trial registration with ClinicalTrials.gov. CTSI also offers educational and training opportunities for new investigators and study coordinators. Strong relationships across the University's Academic Health Center provide access to extensive expertise and collaborative opportunities. Biological specimen handling, storage and retrieval facilities and software (caTissue) are available within the University.

During the clinical study, both inpatient and outpatient adult clinical spaces are available in several facilities. Dr. Chow will be doing most of her clinical research

work at the Clinical Research Unit (CRU), a 16,250 square foot inpatient facility including 10 inpatient beds, 4 outpatient beds, rooms dedicated to glucose clamp studies, rooms for human performance studies with specialized equipment for exercise testing, non-invasive vascular investigation and body composition assessments. The CRU is directly connected to the University of Minnesota Medical Center, Fairview and the office building (Phillips Wangensteen Building) where Dr. Chow has her office. The Delaware Clinical Research Unit (DCRU), a 36,750 square foot outpatient facility including separate adult and pediatric oriented facilities: Adult facilities include: 10 exam rooms, 4 consultation rooms, 5 specialized rooms, a metabolic kitchen, sample acquisition room and on-site laboratory. The facility provides 23 free parking spaces for research participants. The DCRU is within 2 blocks of the CRU.

- Implementation staffing (research project managers, research nurses and certified medical assistants, clinical research coordinators, clinical trial monitors, a registered dietitian, laboratory technician, and other specialized technical services) can be provided to the extent needed by the investigator. A state of the art iDXA for bone density and body composition is available on site. Trial monitoring, and ongoing regulatory reporting and support are provided. The CTSI provides comprehensive clinical research facilities and support across specialty areas, tailored to the needs of the investigator, to facilitate effective and efficient use of resources without unnecessary duplication.

LIHP Facilities

The LIHP, directed by Dr. Dengel, occupies 4500 square feet in Room 141 of Mariucci Arena on the University of Minnesota campus. A University of Minnesota parking lot is located adjacent to the LIHP and provides easy parking for participants. The LIHP houses the Hologic Horizon A iDXA, where the body composition will be measured. There is a separate male and female restroom located next to the LIHP.

Nutrition Coordinating Center:

The Nutrition Coordinating Center (NCC) is located in the Division of Epidemiology and Community Health, and occupies about 6,000 square feet of office space. Dr. Lisa Harnack (Co-I) is the Director of the NCC since 2007. The NCC includes office space for staff, telephone calling stations, resource area, training room, and conference rooms. The NCC has developed and maintains a research-quality food and nutrient database linked to computerized interactive interview software, the Nutrition Data System for Research (NDSR). The NDSR is designed primarily for clinical research and epidemiological studies investigating relationships between diet and health. The NDSR dietary data collection and nutrient analysis tool is well recognized in the nutrition research community and has been licensed for

use in hundreds of research studies. Clients may also choose to enlist the services of NCC to accomplish some or all of their dietary data collection and processing objectives. The NCC Service Center offers training and certification of dietary interviewers, collection of dietary intake by telephone interview, processing of food records, analysis of menu and recipe data, development of dietary data collection protocols, and customized support services for additional related research needs. The Service Center has provided the aforementioned types of services to hundreds of studies since 1974, including carrying out entry and nutrient analysis of food records obtained via meal observation procedures similar to those to be utilized in the proposed study.

Center for Magnetic Resonance Research

Dr. Ryder will also utilize the Center for Magnetic Resonance Research (CMRR for measurement of liver fat content via MRI collected during the study. The CMRR is fully furnished and equipped for modern translational and clinical research. The facility house multiple ultrahigh magnetic fields (7 Tesla and above) but also contains three 3 Tesla (Siemens, Berlin, Germany) scanners which will be used in this study. The 3 Tesla Trio (Siemens) system is a standard system similar to those used in hospitals for clinical diagnosis. This system can be used for standard imaging or spectroscopy measurements of liver fat. The CMRR is equipped with extensive computation resources, including onsite file servers and compute servers are located in a modern secured server room with diesel-backed UPS power and fully redundant cooling. The center also includes subject rooms for consenting participants, or other participant needs. The center provides MR Technologists to run the 3T scanners, assist with data collection, and maintain equipment and troubleshoot scanner issues.

Salk Institute:

Environment. The Salk Institute offers an excellent intellectual environment for the scientific success of its faculty. Dr. Panda's lab is part of the Regulatory Biology Laboratories and is housed in the same floor as the Laboratory of Genetics. The open lab structure of the institute offers constant scientific exchange among researchers and PIs. Open lab design of the Salk Institute fosters persistent interactions among these lab members. Panda lab has ongoing collaborations with several Salk Institute faculty in the field of metabolism, endocrinology, and data science. They include Ron Evans, Marc Montminy, Reuben Shaw, Saket Navlakha, Alan Saghatelian with whom Panda lab has several active collaboration projects. These colleagues and their scientific staffs offer a productive intellectual environment relevant to the success of this project.

Laboratory. Laboratory space for 10 full time researchers furnished with standard laboratory equipment (thermocyclers, benchtop centrifuges, dissecting microscopes, DNA/RNA, protein gel electrophoresis apparatus, heat blocks, water baths, freezers, refrigerators, tissue culture hoods, tissue culture incubators, Q-PCR, FPLC) is available within the department of Regulatory biology at Salk institute.

Clinical. The lab has developed, validated, and deployed the myCircadianClock app for monitoring and intervening human eating pattern. The app is hosted on secured HIPAA compliant Amazon Web Server (AWS). The database and the backend processes communicate with the user's smartphone using double encryption.

Computer. The laboratory has 12 recent model PCs, and 3 Macintosh computers. In addition, shared facilities at Salk include high-end personal computers for image processing, database searches, and modeling. Salk institute's central Research Computing department maintains an extensive data communications network available to all laboratories.

Office. The PI has a 100 sq. ft. office located adjacent to the laboratory; desk with computer are available for staff associated with the project.

Data analysis only facilities – This will be using deidentified data

Dr. Mashek's laboratory

Dr. Mashek's lab is approximately 1600 ft2 plus two adjacent 125 ft2 rooms for tissue culture and radioisotope studies. The laboratory is equipped with a tissue culture hood, two fume hoods, an autoclave and a walk-in cold room. The following equipment are either in Dr. Mashek's laboratory or is readily accessible: Class 2, Type A2 biological safety cabinet, NuAire, Inc.; water jacketed CO2 incubator, NuAire, Inc.; Nucleofector II, Amaxa Biosystems, Inc.; Synergy HT multi-detection microplate reader, BIO-TEK Instruments, Inc.; FLUOstar OPTIMA microplate reader, BMG Labtech; Mini-Protean 3 Cell and Trans-Blot electrophoretic transfer Cell, BIO-RAD; PowerPac HC Power Supply, BIO-RAD; CKX41SF2 inverted microscope, Olympus; IX70 inverted microscope with fluorescence and imaging capabilities, Olympus; Labphoto 2 microscope, Nikon; Saturn 5 GC/MS, Varian; PTC-200 DNA Engine Cycler, BIO-RAD; ABI StepOne Plus Real-Time PCR system, Applied Biosystems; SMARTSPEC Plus, BIO-RAD; Spinchron

R refrigerated centrifuge, Beckman Instruments; RT6000B refrigerated centrifuge, Sorvall; J2-21 centrifuge, Beckman; Orion Star Series pH meter, Thermo Electron; L8-70 Ultracentrifuge, Beckman; Ultralow temperature freezer, Sanyo; AQT series top loading balance, Adams Equipment; A30 analytical balance, Mettler, Inc.; G24 environmental incubator shaker, New Brunswick Scientific Co., Inc; Gyrotory water bath shaker-model G76, New Brunswick Scientific Co., Inc.; general purpose water bath, Precision, Seahorse XF-96, Seahorse Bioscience.

Coordinating Centers for Biometric Research (CCBR) and Biostatistical Design & Analysis Center (BDAC)

Several SPARC servers (Solaris operating system) and Intel servers (Linux and FreeBSD operating systems) provide application service to the CCBR and the Division of Biostatistics. Access to these applications is provided by more than 100 thin-client (X-servers) desktops. All servers are connected to the Internet. The CCBR machines have more than 20 terabytes of disk storage available. Statistical analysis and data management are the primary applications provided by the servers. Statistical analyses are performed using SAS, SAS/GRAPH, SAS/IML, and R. Oracle is the primary relational database management system. Various SQL clients are used including SAS, Perl, SQL*Plus, and PHP.

Bryan Bergman's laboratory

The facilities and other resources available to the research team include everything needed to complete the proposed studies. Dr. Bergman has been performing human research studies similar to the one proposed for 18 years at this institution, so the infrastructure and collaborations are ready to successfully complete this project. In addition to Dr. Bergman's laboratory, there is an NIH funded Clinical Translational Research Center (CTRC) at the University of Colorado Anschutz Medical campus (UCAMC) for all human work proposed. The intellectual environment is filled with other NIH funded investigators who are performing work complimentary to what is proposed, and there is a strong history of human clinical research and funding at our institution. Together, these facilities available to the research team provide a scientific environment that strongly supports the proposed research and, therefore, collectively contributes to the success of the project.

Dr. Bergman has approximately 1000 sq. ft. of wet laboratory space located in the School of Medicine to support the proposed research. He has all the necessary equipment and expertise to perform muscle dissection, western analyses, cell culture experiments, and tissue incubation required for these analyses as they are standard procedures in the laboratory. Specifically, he has a fume hood, an Organomation 100 position multivap nitrogen evaporator, Beckman clinical centrifuge, a Sanyo -80C freezer, 2 stand up freezers, 2

refrigerators, Mettler-Toledo analytical balance, pH meter, gel rocker, BioRad power supplies and western equipment. Additionally, Dr. Bergman has half of a 120 sq. ft cold room, and a 200sq. ft. cell culture laboratory across the hall from his laboratory containing two incubators, an inverted microscope, water bath, refrigerator, centrifuge, and a cell culture hood. Dr. Bergman's GC/MS and LC/MS instruments are on the 6th floor of the building containing his laboratory, in a space designated as core space for the Colorado Nutrition Obesity Research Center. Importantly, he has all the necessary internal standards and bone fide standards required to identify and quantify neutral lipids including diacylglycerol isomers, polar lipids such as acyl-carnitines, as well as deoxysphingolipids via LC/MS. These analyses are routine in the Bergman laboratory. o Local scientific and ethical review structure.

22.2 International Research: N/A

23.0 Multi-Site Research

N/A

24.0 Coordinating Center Research

N/A

25.0 Resources Available

25.1 Resources Available:

Division of Endocrinology, Department of Medicine, University of Minnesota (Primary location Dr. Lisa Chow, PI, Contact PI)

Office:

The Division of Endocrinology has adequate office space for this project. Each faculty member has an individual office and computer assigned for their use. Modern office equipment such as facsimile machines, photocopiers, postage machine, and mailroom services will be available to this project.

Computer Services:

The Division of Endocrinology is part of the Academic Health Center (AHC) and has AHC support for computer maintenance and centralized backup of computer files.

Clinical and Translational Science Institute (CTSI), University of Minnesota

The Clinical and Translational Science Institute (CTSI) at the University of Minnesota offers comprehensive research support for clinical investigators, from concept through publication. CTSI supports all stages of the research process, providing research and regulatory support, specialized facilities, and analytical services. Concept and pre-study services include assistance with study design (biostatistical support), protocol and budget development, study population definition (i2b2/SHRINE resources),

bioinformatics support, investigational new drug (IND) or investigational device exemption (IDE) application assistance, contract negotiations, institutional committee's approval assistance (IRB, Radiation Safety, Institutional Biosafety, etc.), and clinical trial registration with ClinicalTrials.gov. CTSI also offers educational and training opportunities for new investigators and study coordinators. Strong relationships across the University's Academic Health Center provide access to extensive expertise and collaborative opportunities. Biological specimen handling, storage and retrieval facilities and software (caTissue) are available within the University.

During the clinical study, both inpatient and outpatient adult clinical spaces are available in several facilities. Dr. Chow will be doing most of her clinical research work at the Clinical Research Unit (CRU), a 16,250 square foot inpatient facility including 10 inpatient beds, 4 outpatient beds, rooms dedicated to glucose clamp studies, rooms for human performance studies with specialized equipment for exercise testing, non-invasive vascular investigation and body composition assessments. The CRU is directly connected to the University of Minnesota Medical Center, Fairview and the office building (Phillips Wangensteen Building) where Dr. Chow has her office. The Delaware Clinical Research Unit (DCRU), a 36,750 square foot outpatient facility including separate adult and pediatric oriented facilities: Adult facilities include: 10 exam rooms, 4 consultation rooms, 5 specialized rooms, a metabolic kitchen, sample acquisition room and on-site laboratory. The facility provides 23 free parking spaces for research participants. The DCRU is within 2 blocks of the CRU.

Implementation staffing (research project managers, research nurses and certified medical assistants, clinical research coordinators, clinical trial monitors, a registered dietitian, laboratory technician, and other specialized technical services) can be provided to the extent needed by the investigator. A state of the art iDXA for bone density and body composition is available on site. Trial monitoring, and ongoing regulatory reporting and support are provided. The CTSI provides comprehensive clinical research facilities and support across specialty areas, tailored to the needs of the investigator, to facilitate effective and efficient use of resources without unnecessary duplication.

Data for this study will be entered into a REDCap database. The REDCap database uses a MySQL database via a secure web interface with data checks used during data entry to ensure data quality. REDCap includes a complete suite of features to support HIPAA compliance, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL database and the web server will both be housed on secure servers operated by the University of Minnesota Academic Health Center's Information Systems group (AHC-IS). The servers are in a physically secure location on campus and are backed up nightly, with the backups stored in accordance with the AHC-IS retention schedule of daily, weekly, and monthly tapes retained for 1 month, 3 months, and 6 months, respectively. Weekly backup tapes are stored offsite. The AHC-IS servers provide a stable, secure, well- maintained, and high-capacity data storage

environment, and both REDCap and MySQL are widely-used, powerful, reliable, well-supported systems. Access to the study's data in REDCap will be restricted to the members of the study team by username and password.

Fairview Health System Electronic Health Record (EHR):

The University of Minnesota is a partner of the Fairview Health System which uses a modern enterprise level EHR (EPIC) since 2011. As of January 2019 the Fairview EHR has ~2.8 million living patients. Of these patients, 1,700 had prediabetes diagnosed either as fasting glucose between 100-125 (inclusive) or HbA1c between 5.7-6.4 (inclusive) within the last 3 months (since February 2019) and have given consent for participation. Data analysis of the EHR will be through BPIC.

Department of Kinesiology

Dr. Don Dengel (Co-I)

Office:

The School of Kinesiology has adequate office space for this project. Each faculty member has an individual office and computer assigned for their use. Modern office equipment such as facsimile machines, photocopiers, postage machine, and mailroom services will be available to this project. The School of Kinesiology is part of the College of Education and Human Development (CEHD) and has CEHD support for computer maintenance and centralized, secure backup of computer files.

Relevant resources available to Dr. Dengel: Laboratory of Integrative Human Physiology (LIHP):

LIHP staff

Currently, Dr. Dengel directs the LIHP, which provides vascular, exercise and body composition testing. His research group consists of one laboratory coordinator and 6 graduate students. If this proposal is funded, he will use current facilities, equipment and infrastructure to facilitate implementation of the indirect calorimetry and DXA analysis. Dr. Dengel's laboratory coordinator will perform the DXAs and indirect calorimetry measurements for this study.

LIHP Facilities

The LIHP, directed by Dr. Dengel, occupies 4500 square feet in Room 141 of Mariucci Arena on the University of Minnesota campus. A University of Minnesota parking lot is located adjacent to the LIHP and provides easy parking for participants. The LIHP

houses the Hologic Horizon A DXA where the body composition will be measured. There is a separate male and female restroom located next to the LIHP.

Assessments performed at LIHP

Body Composition.

The LIHP has a Body Composition Testing Laboratory which contains equipment for the determination of body composition including bone mass, fat mass and lean body mass. At baseline and 12 weeks, all subjects will have their height and weight will be determined using a wall-mounted stadiometer and an electronic scale. Body mass index (BMI) will be calculated as the body weight in kg/height2 (m2). Percent body fat and lean muscle mass will be determined by Hologic Horizon A (Hologic Inc., Marlborough, MA) dual X-ray absorptiometer (DXA). The DXA will provide bone densities and calculations of total body fat, lean body mass and bone mass. The Hologic Horizon A DXA can accommodate individuals who weigh up to 500 lbs and will provide calculations of total body fat, regional fat, lean body mass, and bone mass. The Hologic Horizon A can provide adipose measures of the android and gynoid regions as well as estimation of visceral adipose tissue.1 Prior to daily testing the Hologic Horizon A DXA is calibrated using a phantom of known composition. The Hologic Horizon A DXA undergoes a yearly preventative diagnostic evaluation.

Database for Indirect Calorimetry and DXA test results

The indirect calorimetry and DXA data for each subject will be entered into a REDCap database by laboratory coordinator immediately after each test. The REDCap database uses a MySQL database via a secure web interface with data checks used during data entry to ensure data quality. REDCap includes a complete suite of features to support HIPAA compliance, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL database and the web server will both be housed on secure servers operated by the University of Minnesota Academic Health Center's Information Systems group (AHC-IS). The servers are in a physically secure location on campus and are backed up nightly, with the backups stored in accordance with the AHC-IS retention schedule of daily, weekly, and monthly tapes retained for 1 month, 8 weeks, and 16 weeks, respectively. Weekly backup tapes are stored offsite. The AHC-IS servers provide a stable, secure, well-maintained, and high-capacity data storage environment, and both REDCap and MySQL are widely-used, powerful, reliable, well-supported systems. Access to the study's data in REDCap will be restricted to the members of the study team by username and password.

Division of Epidemiology and Community Health, School of Public Health, University of Minnesota

(Primary location Dr. Lisa Harnack, Co-I)

Nutrition Coordinating Center:

The Nutrition Coordinating Center (NCC) is located in the Division of Epidemiology and Community Health, and occupies about 6,000 square feet of office space. Dr. Lisa Harnack (Co-I) is the Director of the NCC since 2007. The NCC includes office space for staff, telephone calling stations, resource area, training room, and conference rooms. The NCC has developed and maintains a research-quality food and nutrient database linked to computerized interactive interview software, the Nutrition Data System for Research (NDSR). The NDSR is designed primarily for clinical research and epidemiological studies investigating relationships between diet and health. The NDSR dietary data collection and nutrient analysis tool is well recognized in the nutrition research community and has been licensed for use in hundreds of research studies. Clients may also choose to enlist the services of NCC to accomplish some or all of their dietary data collection and processing objectives. The NCC Service Center offers training and certification of dietary interviewers, collection of dietary intake by telephone interview, processing of food records, analysis of menu and recipe data, development of dietary data collection protocols, and customized support services for additional related research needs. The Service Center has provided the aforementioned types of services to hundreds of studies since 1974, including carrying out entry and nutrient analysis of food records obtained via meal observation procedures similar to those to be utilized in the proposed study.

The Nutrition Coordinating Center was established in 1974, and continues to address the ongoing needs of the nutrition research community. The Center is located on West Bank of the University of Minnesota campus and is a 10 minute drive from Dr. Chow's office.

Department of Biochemistry, Molecular Biology and Biophysics.

(Dr. Doug Mashek, Co-I)

Office:

Dr. Mashek's laboratory is located on the University of Minnesota Molecular Cell Biology Building which is connect by walkway to Dr. Chow's office and the CRU (5 minutes walk). Dr. Mashek's office is 125 ft2 and the laboratory has office space within the laboratory for 9 people.

Computer:

Dr. Mashek has a new iMac that is equipped with all the needed software (Microsoft Office, Photoshop, etc.). Additionally, he also has a Macbook Air laptop and the laboratory has 7 Dell desktops.

Laboratory:

Dr. Mashek's lab is approximately 1600 ft2 plus two adjacent 125 ft2 rooms for tissue culture and radioisotope studies. The laboratory is equipped with a tissue culture hood, two fume hoods, an autoclave and a walk-in cold room. The following equipment are either in Dr. Mashek's laboratory or is readily accessible: Class 2, Type A2 biological safety cabinet, NuAire, Inc.; water jacketed CO2 incubator, NuAire, Inc.; Nucleofector II, Amaxa Biosystems, Inc.; Synergy HT multi-detection microplate reader, BIO-TEK Instruments, Inc.; FLUOstar OPTIMA microplate reader, BMG Labtech; Mini-Protean 3 Cell and Trans-Blot electrophoretic transfer Cell, BIO-RAD; PowerPac HC Power Supply, BIO-RAD; CKX41SF2 inverted microscope, Olympus; IX70 inverted microscope with fluorescence and imaging capabilities, Olympus; Labphoto 2 microscope, Nikon; Saturn 5 GC/MS, Varian; PTC-200 DNA Engine Cycler, BIO-RAD; ABI StepOne Plus Real-Time PCR system, Applied Biosystems; SMARTSPEC Plus, BIO-RAD; Spinchron R refrigerated centrifuge, Beckman Instruments; RT6000B refrigerated centrifuge, Sorvall; J2-21 centrifuge, Beckman; Orion Star Series pH meter, Thermo Electron; L8-70 Ultracentrifuge, Beckman; Ultralow temperature freezer, Sanyo; AQT series top loading balance, Adams Equipment; A30 analytical balance, Mettler, Inc.; G24 environmental incubator shaker, New Brunswick Scientific Co., Inc; Gyrotory water bath shaker-model G76, New Brunswick Scientific Co., Inc.; general purpose water bath, Precision, Seahorse XF-96, Seahorse Bioscience.

Relevant Resources Available to Dr. Mashek

Center for Mass Spectrometry and Proteomics (CMSP)

This facility contains diverse instrumentation capable of generating different data types for workflow development, including newly installed Thermo Fisher Fusion Orbitrap mass spectrometry for high sensitivity proteomics analysis, along with a Proxeon nanoLC system; A newly installed AB Sciex 5600+, along with Eksigent nanoLC for targeted, SWATH analyses and other proteomic operating modes; A newly installed Q-Exactive instrument (Thermo) equipped with Dionex LC capable of both nanocapillary and high flow is a main instrument for high resolution metabolomics analysis. Complementing this instrumentation is a Thermo LTQ-Velos mass spectrometer equipped with Eksigent nanoLC and ETD ionization and a Sciex/AB Qtrap 5500 both equipped with fully automated nanoflow LC systems. In addition to this instrumentation, the facility also houses ten additional mass spectrometers offering full services for small (metabolites) and large (proteins) molecule biological mass spectrometry applications. The facility also has a Leco GC-GC TOF-MS instrument for small molecule/metabolite studies, and also a Waters LCT Premiere TOF-MS for metabolomic profiling.

Department of Pediatrics

(Dr. Justin Ryder Co-I).

Computer and Information Technology Resources

Dr. Ryder has a personal desktop computer and a laptop computer including necessary software (Microsoft Office, Reference Manager, SPSS, SAS, R, GraphPad, Adobe Acrobat Pro), to use for analysis of data, manuscript preparation, and any other activities associated with the conduct of the study. Each member of Dr. Ryder's study team has a personal computer capable of performing all necessary activities associated with the study. Dr. Ryder has full access to the wide-array of computer services offered at the University of Minnesota. These include network infrastructure, web-access, email, etc. The Department of Pediatrics is supported by the Academic Health Center Information Services, which provides comprehensive support for computer, hardware, and software. Dr. Ryder's office is a 5 min walk from Dr. Chow's office.

Office Facilities and Supplies

Fully equipped office facilities and supplies are available to Dr. Ryder and his staff within the Department of Pediatrics and the CTSI. The Division of Epidemiology and Clinical Research has approximately 5,000 square feet of office space located in the Moos Health Sciences Tower, which is located in close proximity to the CTSI and hospital. These facilities include desk space, filing cabinets, telephones, network copiers, scanners, fax machines, printers, and a postage meter.

Relevant Resources Available to Dr. Ryder

Center for Magnetic Resonance Research

Dr. Ryder will also utilize the Center for Magnetic Resonance Research (CMRR for measurement of liver fat content via MRI collected during the study. The CMRR is fully furnished and equipped for modern translational and clinical research. The facility house multiple ultrahigh magnetic fields (7 Tesla and above) but also contains three 3 Tesla (Siemens, Berlin, Germany) scanners which will be used in this study. The 3 Tesla Trio (Siemens) system is a standard system similar to those used in hospitals for clinical diagnosis. This system can be used for standard imaging or spectroscopy measurements of liver fat. The CMRR is equipped with extensive computation resources, including onsite file servers and compute servers are located in a modern secured server room with diesel-backed UPS power and fully redundant cooling. The center also includes subject rooms for consenting participants, or other participant needs. The center provides MR Technologists to run the 3T scanners, assist with data collection, and maintain equipment and troubleshoot scanner issues. This location is a 10 minute walk from Dr. Chow's office and the CRU.

Coordinating Centers for Biometric Research (CCBR) and Biostatistical Design & Analysis Center (BDAC)

(Dr. Erika Helgeson, Co-I, Qi Wang)

Communications, Photocopying, Faxing, Electronic Images

Dr. Helgeson's office is located in the CCBR, which is part of the Division of Biostatistics in the School of Public Health at the University of Minnesota. The CCBR is connected to the internet via fiber-optic cable to the University network backbone which provides internet access for all staff members. The CCBR also has 8 fax modems for receiving images of case report forms, surveys, and other hard-copy documents. Case report forms received this way are automatically filed into patient folders. In addition, the CCBR also has two high-speed copying machines capable of collating, 2-sided copying, reduction and stapling. These two multi-function machines also serve as high-speed printers and as high-speed scanners for electronic imaging needs.

Computer Servers

Several SPARC servers (Solaris operating system) and Intel servers (Linux and FreeBSD operating systems) provide application service to the CCBR and the Division of Biostatistics. Access to these applications is provided by more than 100 thin-client (X-servers) desktops. All servers are connected to the Internet. The CCBR machines have more than 20 terabytes of disk storage available. Statistical analysis and data management are the primary applications provided by the servers. Statistical analyses are performed using SAS, SAS/GRAPH, SAS/IML, and R. Oracle is the primary relational database management system. Various SQL clients are used including SAS, Perl, SQL*Plus, and PHP.

Ms. Wang has an office at BDAC, which is a sub-unit of the University's Clinical and Translational Science Institute (CTSI). She has a current Windows machine with access to the internet through the University's Academic Health Center's IS system, with all standard software (e.g., the Microsoft Office Suite) as well as SAS and R.

Salk Institute

The Salk Institute for Biological Studies

Environment. The Salk Institute offers an excellent intellectual environment for the scientific success of its faculty. Dr. Panda's lab is part of the Regulatory Biology Laboratories and is housed in the same floor as the Laboratory of Genetics. The open

lab structure of the institute offers constant scientific exchange among researchers and PIs. Open lab design of the Salk Institute fosters persistent interactions among these lab members. Panda lab has ongoing collaborations with several Salk Institute faculty in the field of metabolism, endocrinology, and data science. They include Ron Evans, Marc Montminy, Reuben Shaw, Saket Navlakha, Alan Saghatelian with whom Panda lab has several active collaboration projects. These colleagues and their scientific staffs offer a productive intellectual environment relevant to the success of this project.

Laboratory. Laboratory space for 10 full time researchers furnished with standard laboratory equipment (thermocyclers, benchtop centrifuges, dissecting microscopes, DNA/RNA, protein gel electrophoresis apparatus, heat blocks, water baths, freezers, refrigerators, tissue culture hoods, tissue culture incubators, Q-PCR, FPLC) is available within the department of Regulatory biology at Salk institute.

Clinical. The lab has developed, validated, and deployed the myCircadianClock app for monitoring and intervening human eating pattern. The app is hosted on secured HIPAA compliant Amazon Web Server (AWS). The database and the backend processes communicate with the user's smartphone using double encryption.

Computer. The laboratory has 12 recent model PCs, and 3 Macintosh computers. In addition, shared facilities at Salk include high-end personal computers for image processing, database searches, and modeling. Salk institute's central Research Computing department maintains an extensive data communications network available to all laboratories.

Office. The PI has a 100 sq. ft. office located adjacent to the laboratory; desk with computer are available for staff associated with the project.

Other. The Salk Institute has several service cores including microarray, transgenic, machine shop, metabolic, rodent behavior, microscopy, antibody production, peptide synthesis, and deep-sequencing cores. These cores are run by expert technicians and directors who constantly help and train lab personnel in carrying out their experiments.

Equipment – The Salk Institute for Biological Studies

Panda lab occupies ~2000 sqft space in the regulatory biology section of the Salk institute. The lab has wetlab are and dry lab area. This collaboration relates to the dry lab area of Panda lab.

Dry-lab.

The lab has a dedicated ~550 sqft space for dry lab computation area that houses personnel, computer and equipment for data collected through digital devices and clinical measurements. The dry-lab area contains 5 desktop computers with updated software for analyses of diet, physical activity, sleep, and survey data collected through relevant wearable devices, smartphones, paper- or computer-generated questionnaire.

The lab has ~120 wrist-worn activity monitors for use in human studies for longitudinal monitoring of sleep, activity and light exposure.

The dry-lab area has secure physical storage space for storing back-up hard drives, paper copies of informed consent documents, clinical lab reports, and other human subject related information.

Through Salk Institute's high-speed internet access line the lab has access to HIPAA compliant cloud server.

The lab has a Tanita body composition scale, and other necessary tools for collecting anthropometric measurements from human subjects.

Facilities: Colorado

Dr. Bergman

The facilities and other resources available to the research team include everything needed to complete the proposed studies. Dr. Bergman has been performing human research studies similar to the one proposed for 18 years at this institution, so the infrastructure and collaborations are ready to successfully complete this project. In addition to Dr. Bergman's laboratory, there is an NIH funded Clinical Translational Research Center (CTRC) at the University of Colorado Anschutz Medical campus (UCAMC) for all human work proposed. The intellectual environment is filled with other NIH funded investigators who are performing work complimentary to what is proposed, and there is a strong history of human clinical research and funding at our institution. Together, these facilities available to the research team provide a scientific environment that strongly supports the proposed research and, therefore, collectively contributes to the success of the project.

Dr. Bergman has approximately 1000 sq. ft. of wet laboratory space located in the School of Medicine to support the proposed research. He has all the necessary equipment and expertise to perform muscle dissection, western analyses, cell culture experiments, and tissue incubation required for these analyses as they are standard procedures in the laboratory. Specifically, he has a fume hood, an Organomation 100 position multivap nitrogen evaporator, Beckman clinical centrifuge, a Sanyo -80C freezer, 2 stand up freezers, 2 refrigerators, Mettler-Toledo analytical balance, pH meter, gel rocker, BioRad power supplies and western equipment. Additionally, Dr.

Bergman has half of a 120 sq. ft cold room, and a 200sq. ft. cell culture laboratory across the hall from his laboratory containing two incubators, an inverted microscope, water bath, refrigerator, centrifuge, and a cell culture hood. Dr. Bergman's GC/MS and LC/MS instruments are on the 6th floor of the building containing his laboratory, in a space designated as core space for the Colorado Nutrition Obesity Research Center. Importantly, he has all the necessary internal standards and bone fide standards required to identify and quantify neutral lipids including diacylglycerol isomers, polar lipids such as acyl-carnitines, as well as deoxysphingolipids via LC/MS. These analyses are routine in the Bergman laboratory.

HOW THE ENVIRONMENT WILL CONTRIBUTE TO SUCCESS

We are an integrated, interdisciplinary team with unique expertise to complete the proposed study. Dr. Chow has experience recruiting and conducting studies in overweight participants from her ongoing NIH grant (R01DK098203) which trained sedentary overweight/obese participants for 16 weeks. The proposed study will leverage this existing infrastructure for recruitment and implementation (screening, consent, metabolic assessment, training). Dr. Mashek (Co-I on current R01, 4 papers with Dr. Chow) will analyze the acquired serum, lend his expertise on hepatic fat metabolism/hepatic insulin resistance on the observed findings, and have regular discussions with Dr. Chow about interpreting the findings within the current scientific literature. Dr. Ryder (3 papers with Dr. Chow) will provide his expertise in measuring body fat using MRI, supported by Dr. Pat Bolan. Dr. Dengel has an established working relationship with Dr. Chow (Co-I on current R01, 8 joint publications) and will lend his expertise in on the indirect calorimetry and DXA measurements. Dr. Harnack is an internationally recognized nutritional epidemiologist and registered dietician. She will provide her expertise in nutritional assessment (1 submitted R01 with Dr. Chow as MPI) and will work with Dr. Chow in documenting the caloric intake and co-supervising the study dietician to ensure compliance with the intervention. Dr. Satchinananda Panda (Co-I with Dr. Chow on current HFHL grant) will lend his expertise in time restricted eating (TRE) and provide support for the myCircadianClock (mCC) app. Dr. Bergman (Director of Molecular and Cellular Analytical Core Facility [Colorado NORC:P30DK48520] will perform the stable isotope analysis from the plasma. Dr. Erika Helgeson (1 submitted R01 with Dr. Chow) and Qi Wang (5 papers with Dr. Chow) will provide the statistical support for the proposal.

Adequate personnel are in place to support the proposed study. For the study, Dr. Chow has her own research group (one physician assistant, one study coordinator) and can draw upon the research staff from the University of Minnesota Division of Diabetes and Endocrinology with clinical trial expertise (1 physician assistant, 3 research nurses and 3 study coordinators) if needed. Dr. Chow will work with Dr. Harnack to leverage the existing infrastructure of the NCC to develop and implement the meal observation protocol.

The team will have scheduled meetings to ensure study progression as shown Table 1.

Table1: Meeting Schedule during Study		
Personnel	**Role**	**Schedule (May meet more frequently if needed)**
Dr. Harnack and Research study staff	Study recruitment, flow and progress	Weekly in-person
Dr. Mashek	Review scientific literature relevant to study, measure serum inflammatory markers	Weekly in-person (Dr. Chow already attends)
Dr. Panda's team	Review participant compliance and adherence via review using the mCC app	Weekly by phone or email
Dr. Panda	Review study progress	Every 6 months by phone
Dr. Dengel and Dr. Ryder	Address issues related to indirect calorimetry measurement (Dr. Dengel) and MRI (Dr. Ryder)	Every 2 months in-person
Dr. Helgeson and Qi Wang	Organize study setup and data flow; review study progress; data analysis	Bi-weekly for 3 months in-person, then every 6 months while study is ongoing, bi-weekly for last 6 months of grant
Dr. Bergman	Analysis of blood samples for stable isotope (glucose, glycerol) enrichment	Monthly for 3 months by phone to set up analysis procedures, q6 months during sample acquisition phase, then q3 months during sample analysis phase
Dr. Moheet – Patient Safety Officer	Follow up adverse events	Every 6 months in-person and prn event occurrence

Of note, the University of Minnesota CTSI received a CTSA award in 2018 to support the clinical research. Adequate personnel and infrastructure are in place to support the proposed clinical study

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