PROTOCOL
Biomedical
Colorado State
University

Protocol # 15-5837H
Date Printed: 11/05/2018

Protocol Title:	Milk protein feeding after aerobic exercise in older adults with pre-diabetes taking the biguanide metformin
Protocol Type:	Biomedical
Date Submitted:	10/04/2018
Approval Period:	10/04/2018-05/13/2019
Important Note:	This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol. Questions that appear to not have been answered may not have been required for this submission. Please see the system application for more details.

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* * * Amendment * * *

Amendment (1. Complete this one-page form, 2. Update the sections of your protocol that you are requesting to amend, 3. Electronically "sign" your application by clicking the check box on the Obligations page, 4. Remember to click "Submit Form" so that the IRB administrators receive your request.)

1. Summarize the proposed changes to the protocol in lay terms.

   Change of principal investigator to Karyn Hamilton.

Proceed to the appropriate section(s) of the protocol and make your changes.

IMPORTANT NOTE ON AMENDING ATTACHED DOCUMENTS: If you are requesting to amend a file that has been previously attached and approved, you MUST: 1. Delete the file that is currently attached, 2. Browse on your computer, and 3. Upload the revised file that you are requesting to use.

2. Indicate level of risk involved with the changes proposed.

(If level of risk has changed, please update the section 'Risks' in the protocol information.)

No Change

Approval Includes

3. List of sections (and questions) that have been changed/modified

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* * * Personnel Information * * *

IMPORTANT NOTE: Mandatory Personnel on a protocol are: Principal Investigator and Department Head. Only the Principal Investigator can submit the protocol; although other personnel listed on the protocol can create the protocol. Human Subjects Protection Training is mandatory for Principal Investigator, Co-Principal Investigator, and Key Personnel (as defined by NIH). Training must be updated every three (3) years.

Principal Investigator Mandatory

Name of Principal Investigator	Degree	Title

(Faculty, Staff or Postdoc)

Hamilton, Karyn		Professor
Email	**Phone**	**Fax**
Karyn.Hamilton@ColoState.EDU	(970) 491-3961	
Department Name	**Campus Delivery Code**	
244		
Human Subjects Training Completed? PIs must complete training every three (3) years.		Y

Department Head Mandatory

Name of Department Head	**Degree**	**Title**
Braun, Barry	Ph.D.	Professor
Email	**Phone**	**Fax**
Barry.Braun@colostate.edu	(970)491-7875	
Department Name	**Campus Delivery Code**	
1582 Dept Hlth & Exer Sci	1582	
Human Subjects Training Completed?? Training is not required for Department Head. Select "No" if you do not know if your Department Head has completed training or not.		Y

Administrative Contact

Name of Administrative Contact, Project Director, or Lab Coordinator	**Degree:**	**Title**
Biela, Laurie	BS	Research Associate III
Email	**Phone**	**Fax**
Laurie.Biela@colostate.edu	970-491-2242	970-491-0445
Department Name	**Campus Delivery Code**	
1582 Dept Hlth & Exer Sci	1582	
Human Subjects Training Completed? Training is not required for Administrative Contacts		Y

No training data is available.

Other Researcher or Key Personnel

Name of Other Researcher (NOTE: Anyone listed in this role will have View Mode access only)	**Degree**	**Title**
Leach, Heather		Assistant Professor
Email	**Phone**	**Fax**
Heather.Leach@colostate.edu		
Department Name	**Campus Delivery Code (CSU) or off-campus mailing address**	
244		
Human Subjects Training Completed? Training is required for all Key Personnel on NIH grants. Training must be updated every three (3) years.		Y

No training data is available.

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* * * Subject Population * * *

Subject Population(s) Checklist

Â Select All That Apply - Note that this is your Targeted Population :

X Adult Volunteers
Decisionally Challenged
X Elderly
Employees
Fetuses
Long-Term Patients
Mentally Disabled
Minors (under 18)
Pregnant Women
Prisoners
Soldiers
X Students
Other (i.e., non-English Speaking or any population that is not specified above)

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* * * Study Location * * *

Study Location(s) Checklist

Select All That Apply - NOTE: Check "Other" and input text: 1.) If your study location is not listed, or 2.) If you would like to list details of your already-checked location (e.g., specific school within a school district)

Aims Community College
Colorado Department of Public Health & Environment
X Colorado State University
Colorado State University - Pueblo Campus
Denver Public Schools
Greeley/Evans School District
Poudre School District
University of Colorado Health - North (Formerly -Poudre Valley Health System - PVHS)
Rocky Mountain National Park
Thompson School District
University of Colorado - Boulder
University of Colorado - Colorado Springs
University of Colorado - Denver
University of Colorado Health Sciences Center
University of Northern Colorado
Other (In the box below, list your study location if not checked above. You may also list details of your already-checked location (e.g., specific school within a school district).

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* * * General Checklist * * *

General Checklist

Select All That Apply :

X Proposed Start Date (cannot be before IRB approval): July 1, 2015

X Sponsored Project (Check if you will be funded OR if you have or plan to submit a grant application in association with this protocol)

NSF Sponsored (Please upload mandatory Data Management Plan in the Attachment section)

X FDA or EPA-regulated research. Please contact the CSU Quality Assurance Manager, Cat Bens, at 970-491-5445 to determine if your study is under Good Laboratory, Good Clinical, or Good Manufacturing Practices (GLP, GCP, GMP).

Training Grant

X Clinical Trial. To register your trial on Clinicaltrials.gov, please contact Cat Bens, CSU Quality Assurance Manager and Clinical Trails Administrator at: 970-491-5445.

Project is associated with the Colorado School of Public Health - CSPH(faculty and/or student)

Cooperating/Collaborating Institution(s) Institution where recruitment will occur OR Institution where Collaborating PI will conduct associated research.

Interview

Questionnaire/Survey

X Subjects will be compensated for participation

Thesis or Dissertation Project

X ÃÂ Radioisotopes/radiation-producing machines, even if standard of care. Please contact Jim Abraham, Radiation Safety Officer for questions related to use of all radiation-producing machine: 970-491-3736; james.abraham@colostate.edu. Upload your radiation-use approval (if available) or your Radiation Safety Training certificate in the attachment section.

X Human blood, cells, tissues, or body fluids. You will need to obtain IBC approval if you check this box. For information regarding IBC approval, contact Christine Johnson, IBC Coordinator: christine.johnson@colostate.edu

Tissues to be stored for future research projects

Tissues to be sent out of this institution as part of a research agreement

Human Embryos. You will need to obtain IBC approval if you check this box. For information regarding IBC approval, contact Christine Johnson, IBC Coordinator: christine.johnson@colostate.edu

Human Embryonic Cells? Provide NIH Code Number(s) or state that no federal funding will be used to support this research. You may need to obtain IBC approval if you check this box. For information regarding IBC approval, contact Christine Johnson, IBC Coordinator: christine.johnson@colostate.edu

Use of Patient-related equipment? If Yes, specify what equipment is being used.

Medical equipment used for human patients/subjects also used on animals. For questions regarding animal use approval, contact Elaine Kim, IACUC Senior Coordinator: 491-0236

Protocol involves studying potentially addicting drugs. For questions regarding approval for possession of controlled substances, contact Chris Giglio, DRC Coordinator: 491-4830; Chris.Giglio@colostate.edu.

Investigational drugs, reagents, or chemicals (IND)

X Commercially available drugs, reagents, or other chemicals administered to subjects (even if they are not being studied)

X Investigational Device (IDE)

Cancer Subjects (e.g., clinical trials, behavior/prevention) or Cancer Tissues (e.g., blood, cells, body fluids). You may need to obtain IBC approval if you check this box. For information regarding IBC approval, contact Christine Johnson, IBC Coordinator: christine.johnson@colostate.edu

Other (clarify in text box to the right)

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* * * Funding * * *

Please complete this section if: 1. This protocol will be funded, 2. You have submitted or will submit a grant application associated with this protocol. Please be sure to input your PASS/SP1 number to assist Sponsored Programs in setting up an account for your funds.

If this protocol is funded by the NIH or NSF, or will lead to the regulatory involvement of the FDA or EPA, please be certain you are cognizant of any specific regulatory requirements for data acquisition, storage, retention and sharing, as well as research expenditure allowability, with regard to this IRB protocol.

Funding Checklist

NONE

NOTE: Applicable Federal Grant Application, including competing renewals, must be attached in the Attachment Section (#16). Applicable investigator's brochure and sponsor's protocol must also be attached in section #16 for all industry-sponsored clinical trials.

Funding - Grants/Contracts

Funding Administered By	UNIVERSITY
CSU PASS #	121860
Sponsor's ID # (If known)	
Funded By	Other
	Dairy Management Inc.
Principal Investigator	Benjamin Miller
Grant/Contract Title if different from Protocol Title	Milk Protein Feeding After Aerobic Exercise in Older Adults with Pre-Diabetes Taking the Biguanide Metformin
	For Federal projects, are contents of this protocol the same as described in Federal proposal application?
N	Is this an Umbrella protocol?
N	Is this protocol under an Umbrella protocol?

Funding Administered By	UNIVERSITY
CSU PASS #	
Sponsor's ID # (If known)	
Funded By	Other
	Dexcom Inc.
Principal Investigator	
Grant/Contract Title if different from Protocol Title	
	For Federal projects, are contents of this protocol the same as described in Federal proposal application?
	Is this an Umbrella protocol?
	Is this protocol under an Umbrella protocol?

Funding - Fellowships

Funding - Other

Gift Funding

Dept. Funding

Other Funding

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* * * Expedited Paragraphs * * *

PLEASE READ: This online application is for projects that will be reviewed by the IRB via the expedite or full-board review process. The criteria for expedited review are listed below. Review and check what expedite criteria is/are appropriate for your project. NOTE: If your research involves or may involve greater than minimal risk, an element of deception, or is FDA-regulated research, do NOT check any of the expedited criteria listed below. Your protocol will then be reviewed by the full-board at their next regularly scheduled meeting. If your project meets the exempt criteria, please submit your exempt application via email to: RICRO_IRB@mail.colostate.edu. Information regarding exempt applications can be found here: http://ricro.colostate.edu/IRB/ExemptReview.html

Expedite Criteria:

1. Clinical studies of drugs and medical devices only when condition (a) or (b) is met.

   a) Research on drugs for which an investigational new drug application (21 CFR Part 31,32) is not required. (Note: Research on marketed drugs that significantly increases the risks or decreases the acceptability of the risks associated with the use of the product is not eligible for expedited review.)

   b) Research on medical devices for which

      i) An investigational device exemption application (21 CFR Part 812) is not required; or
      ii) The medical device is cleared/approved for marketing and the medical device is being used in accordance with its cleared/approved labeling.

2. Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture as follows:

   a) From healthy, nonpregnant adults who weigh at least 110 pounds. For these subjects, the amounts drawn may not exceed 550 ml in an 8- week period and collection may not occur more frequently than 2 times per week; or

   b) From other adults and children, considering the age, weight, and health of the subjects, the collection procedure, the amount of blood to be collected, and the frequency with which it will be collected. For these subjects, the amount drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8-week period and collection may not occur more frequently than 2 times per week.

3. Prospective collection of biological specimens for research purposes by non-invasive means.

4. Collection of data through non-invasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving x-rays or microwaves. Where

medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for expedited review, including studies of cleared medical devices for new indications.)

Examples:

a) Physical sensors that are applied either to the surface of the body or at a distance and do not involve input of significant amounts of energy into the subject or an invasion of the subject's privacy;

b) Weighing or testing sensory acuity;

c) Magnetic resonance imaging;

d) Electrocardiography, electroencephalography, thermography, detection of naturally occurring radioactivity, electroretinography, ultrasound, diagnostic infrared imaging, doppler blood flow, and echocardiography;

e) Moderate exercise, muscular strength testing, body composition assessment, and flexibility testing where appropriate given the age, weight, and health of the individual.

5. Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis). (NOTE: Some research in this paragraph may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(4). This listing refers only to research that is not exempt.)

6. Collection of data from voice, video, digital, or image recordings made for research purposes.

7. Research on individual or group characteristics or behavior(including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies. (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(2) and (b)(3). This listing refers only to research that is not exempt.)

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* * * Purpose,Study Procedures,Background * * *

Original Protocol Number (e.g., 07-226H)

Title (Please indicate if the protocol title is different from the proposal title)

Milk protein feeding after aerobic exercise in older adults with pre-diabetes taking the biguanide metformin

Complete Sections 1 - 16. Specify N/A as appropriate. Do not leave any required sections blank.

1. Purpose of the study

a) Provide a brief lay summary of the project in <200 words. The lay summary should be readily understandable to the general public, and is, for example, what would be released to a newspaper if requested.

We propose that post-exercise milk protein feeding will enhance the mitochondrial protein synthesis (biogenesis) response to an exercise-training program. In addition, we propose that this stimulatory effect of protein feeding will overcome the potential blunting effect of metformin on exercise responses. We will investigate these outcomes over a 12-week exercise-training program in older adults with pre-diabetes with or without metformin treatment.

Sub-study:
1. CGM in Individuals with prediabetes undergoing an exercise-training program with or without Metformin.
2. CGM in healthy control individuals to compare to individuals with prediabetes.

b) **What does the Investigator(s) hope to learn from the study?**

We hope to:
a) provide the first evidence that post-exercise milk protein supplementation is effective in diseased population (i.e., older adults with pre-diabetes) and those on medications (i.e., metformin).
b) demonstrate that the potential inhibitory effects of metformin on aerobic exercise adaptations can be opposed by milk protein feeding.

Sub-study
1. Glucose Profiles via CGM in pre diabetic individuals before, during and after the intervention (i.e., aerobic exercise training with or without metformin with post-exercise carbohydrate or carbohydrate+protein feeding as listed below in 2a).
2: Glucose profiles via CGM in healthy individuals. Healthy individuals will complete baseline procedures but will not complete an intervention.

c) **Proposed Start Date (may not precede IRB approval date):**

August 1, 2015

2.

Study Procedures (If this is a student project, the methods section of the thesis or dissertation proposal must be attached in section #16 - Attachment section.)

a) **In lay language, describe all the procedures, from screening through end-of-study, that the human subject must undergo in the research project, including study visits, drug treatments, randomization and the procedures that are part of standard of care. Please note: Do NOT respond "See Attachment Section." If you would like to add tables, charts, etc., attach those files in the Attachment section (#16).**

Study Overview:

Pre-diabetic and healthy participants will first undergo a medical questionnaire, resting ECG, graded exercise test with ECG and assessment of VO2max, anthropometric measurement (height, weight and body composition by DEXA scanning), an oral glucose tolerance test (OGTT), and a muscle biopsy.

Prediabetic participants will be screened to eliminate those lactose intolerant, medications known to affect the primary outcomes, medications or recent procedures contraindicated with metformin or not pre-diabetic. Subjects who meet these criteria will be randomized so that subject characteristics are matched between groups, while the study team and participants will remain blinded to treatment (i.e., randomized, double-blind study design). Subjects will be assigned to exercise + carbohydrate + placebo, exercise + protein + placebo, exercise + carbohydrate + metformin, or exercise + protein + metformin.

A minimum of two days apart from the VO2max test, skeletal muscle will be sampled from pre-diabetic and healthy participants. During these visits, pre-diabetic subjects will have the option to participate in a study to test the impact of one counseling session on physical activity after completion of the exercise training and to identify individual, socio-cultural and environmental determinants of physical activity 12 weeks after the end of the exercise intervention. If the subjects wish to participate they will complete questionnaires to measure sociodemographic information, physical activity, self-efficacy, planning, self-regulation and outcome expectancy of exercise, and perceptions of neighborhood support at baseline, completion of exercise training and 12 weeks after the exercise training.

Pre diabetic and healthy participants will also have the option to participate in a study to wear a Dexcom generation 6 (G6) continuous glucose monitor (CGM) to measure glucose values every 5 minutes. Healthy participants will wear the device once for up to 10 consecutive days. Pre-diabetic participants will wear the device for up to 10-consecutive days at three different time points: before, during and after the 12-week exercise intervention for a total of 30 days wearing the CGM. The Dexcom G6 CGM is for investigational use only and is not FDA approved. The G6 is modeled after commercially available, FDA approved CGM devices. The differences between the G6 and commercially available devices are a reduced calibration scheme, meaning less finger stick blood glucose measurements for calibration; a longer wear for up to 10 days for the G6 vs. 7 days for commercially available product. Last, commercially available devices could give false high glucose readings if individuals took acetaminophen. The G6 sensor contains updated sensor technology aimed to block the interference of acetaminophen.

After randomization into placebo or metformin groups, the pre-diabetic subjects will begin a 12-wk exercise

intervention consisting of 3 days per week of aerobic exercise training with post-exercise consumption of a 20 gram protein beverage or isocaloric carbohydrate beverage. We have completed this supplementation protocol in previous studies and a currently ongoing investigation.
At the 8-wk time point, pre-diabetic subjects will begin the daily consumption of heavy water (D2O) for the assessment of mitochondrial and individual protein synthesis. At the completion of the training period, the pre-diabetic subjects will repeat all assessments performed prior to training including the DEXA, VO2max, OGTT and muscle sample. Muscle samples obtained pre- and post-training will be used to evaluate mitochondrial function using high-resolution respirometry, western blot analysis, and stable isotope incorporation (post only).

Healthy participants will complete the medical history questionnaire, resting ECG, graded exercise testing (ECG and VO2max), DEXA, OGTT, CGM, and muscle biopsy.

Detailed Procedures:

Oral glucose tolerance test (OGTT): An OGTT is the most common clinical procedure to determine impaired glucose tolerance and indices of insulin sensitivity. First, an intravenous catheter will placed in an arm or hand vein to obtain blood samples for analysis of plasma glucose and glucoregulatory hormones. Blood will be sampled before, 5, 10, 20, 30, 45, 60, 90, and 120 min after completing the consumption of 75 gram glucose beverage. The fasted blood sample will also be used for a blood chemistry panel that includes electrolytes, glucose, in addition to kidney and liver related variables (including creatinine, eGFRif AFR AMER, eGFR, Albumin, Bilirubin, Alk Phos, AST, Uric Acid, ALT, A/G ratio, etc.).Subjects will not be allowed to participate until a medical doctor has signed-off on the blood screening. A 6-week blood draw will be taken to confirm that kidney and liver variables have not changed. Additional blood draws may be obtained per physician discretion.

Body Composition: Height, weight will be recorded. Percent body-fat, fat-free mass, total and regional adipose tissue mass, and total bone mineral density will be determined using whole-body dual energy x-ray absorptiometry (DEXA) as we have previously used.

VO2Max: Subjects will undergo a 12-lead electrocardiogram (ECG) examination at rest and during a graded exercise test on a stationary bicycle until volitional fatigue, in order to determine maximal oxygen uptake (VO2max). ECG measurements will be made continuously, and blood pressure and ratings of perceived exertion will be recorded. These tests will be performed in the Human Performance/Clinical Research Laboratory (HPCRL) following standard procedures.

Skeletal Muscle Biopsy: Skeletal muscle biopsies will be performed using well-established IRB approved SOPs. The belly of the vastus lateralis will be anesthetized locally with 1% lidocaine without epinephrine. A small incision (~4-mm in length) will be made and a Bergstrom needle inserted. Negative pressure will be applied to the inner channel of the needle using a sterile 30-ml plastic syringe. Benjamin Miller, Ph.D., Adam Konopka, Ph.D., or Matthew Hickey Ph.D. will perform the skeletal muscle biopsies. Collectively, the investigative team has performed this procedure on over 1,000 subjects. A muscle sample will be obtained once before and after the 12-week exercise training (a total of 2 muscle biopsies).

Exercise Training: The exercise-training program will be performed within the facilities for the Noon Hour Fitness Program at CSU according to our previously published procedures in older individuals. Exercise will be performed on a treadmill, stationary bicycle, or elliptical and include warm-up (15 min at a heart rate corresponding to 60% of max) and workout stages (30 min, progression from 65 to 85% of max heart rate by wk 5). The participants will wear heart rate monitors to ensure proper heart rate ranges and the speed, grade or resistance of equipment will be changed to maintain heart rate at the specified percentage of max HR. A CPR trained technician will supervise each exercise session to monitor adherence to the exercise prescription and ensure subject safety. The drinks will be consumed immediately after exercise under supervision.

Nutritional Intervention: Subjects will consume either a protein-carbohydrate or carbohydrate drink with a consistency and temperature similar to a smoothie or milk shake immediately after each exercise bout. The protein-carbohydrate drink will be greek yogurt+fruit (~300 kcal, 63g carbohydrate, 20g protein, 1g fat) and the carbohydrate will be fruit (~300 kcal, 82g carbohydrate, 2g protein and 1g fat).

Metformin and Placebo: We use a titration dosing protocol in which the amount of metformin starts at 500 mg/day in week 1 and increases by 500 mg/day/week until reaching 2000 mg/day by week 4. 2000 mg/day is the standard clinical dose and used previously by the investigative team. The titration dosing scheme and taking metformin/placebo with meals will help reduce the most common side effects (i.e., gastrointestinal discomfort). If participants experience gastrointestinal discomfort, the dose will be lowered to 1500 mg/day. If participants have a body weight of 75 kg or less the maximal dose will be 1500 mg/day to decrease the risk of gastrointestinal discomfort.

Stable Isotope Labeling: The subjects will consume D2O (70%; Cambridge Isotope Laboratories, Andover

MA, USA) during the last four-week period of the 12 week intervention. D2O is a stable, non-radioactive isotope that is indistinguishable from normal water, A target of 1–2% enrichment is achieved during a one-week priming stage (50 ml of D2O 3x/d = 150 ml/d) and maintained for three weeks (50 ml of D2O 2x/d = 100 ml/d) as we have previously performed.

Optional Continous Glucose Monitoring (CGM): CGM is a commonly used device to continuously measure glucose values in healthy control and diabetic populations in both research and clinical applications. Only trained members of the research team will place and remove the CGM devices following a standard operating procedure and user guide. The sensor pod will be secured to the skin of the abdomen using medical grade adhesive. Once secure, a plunger will insert the sensor underneath the skin to record interstitial glucose values every 5 minutes. After careful removal of the applicator, a transmitter will be attached to the secured sensor pod. The transmitter sends data to a receiver. The receiver is smaller than the size of a cell phone to easily fit in a pocket or bag. The receiver needs to be within 20 feet of the device to continuously transmit data. The receiver will be set to blinded mode so the participant will not know their glucose values. The device can be worn up to 10 days. After each 10-day wear a trained member of the research team will remove the sensor. During the 10-day wear the device will prompt the participant to calibrate. After verbal and written instruction, participants will be shown how to perform a finger stick, measure blood glucose using the glucose meter provided and enter the glucose value into the receiver to calibrate the device. During the 10-day wear, participants will be provided a log to record the timing and amount of medications, physical activity, food and drink.

Optional Physical Activity Behavior Counseling: After completion of the 12-week exercise intervention, participants will be randomized to 1) receive one session of physical activity behavior counseling (PABC), or 2) no counselling control (CON).The counseling session will consist of strategies based on social-cognitive theory hypothesized to increase physical activity adherence. Session activities will include 1) discussion of the benefits of physical activity, 2) discussion of evidence based recommendations for frequency, intensity, time and type of physical activity for reducing the risk of type 2 diabetes and associated co-morbidities, 3) setting individual physical activity goals, 4) identifying and discussing barriers and facilitators for physical activity, and 5) identifying strategies to overcome barriers. The counseling session will be completed in person while the follow-up questionnaires 12 weeks after completing the exercise training can be done via phone or email.

Healthy participants will complete the medical history questionnaire, resting ECG, graded exercise testing (ECG and VO2max), DEXA, OGTT, CGM, and muscle biopsy.

b) Explain why human subjects must be used for this project.

We are interested in improving human health using recommendations by American Diabetes Association with dairy protein supplementation and therefore need to use human subjects.

c) Alternative Procedures. If the proposed study is a clinical trial of a drug, vaccine, device or treatment, describe alternative procedures, if any, that might be advantageous to the subject. Describe the important potential risks and benefits associated with the alternative procedure(s) or course(s) of treatment. Any standard treatment that is being withheld must be disclosed. This information must be included in the consent form.

No alternative procedures

d) If the proposed study is a clinical trial of a drug, vaccine, device or treatment, will it be possible to continue the more (most) appropriate therapy for the subject(s) after the conclusion of the study?

Clinical parameters obtained during the study will be available to the participants to share with their primary physician. Then the subject and primary physician can decide on the most appropriate therapy for the subject after the conclusion of the study.

e) Study Endpoint. If the proposed study is a clinical trial of a drug, vaccine, device or treatment, what are the guidelines or end points by which you can evaluate the alternative treatments during the study? If one treatment proves to be clearly more effective than another (or others) will the study be terminated before the projected total subject population has been enrolled? When will the study end if no important differences are detected?

No alternative treatments

f) State if deception will be used. If so, provide a rationale and describe debriefing procedures. Submit a

debriefing script in the Attachment Section (#16).

No deception will be used.

3. Background

a) Describe past experimental and/or clinical findings leading to the formulation of the study, if applicable.

Physical activity and the biguanide drug Metformin have been shown independently to reduce the incidence of T2D. Interestingly, these data also suggest in adults over the age of 60 metformin had marginal efficacy while physical activity lowered incidence of T2D by 70%. Currently, governing agencies recommend physical activity and metformin together for individuals with pre-diabetes of all ages to prevent the progression to overt T2D. Until recently, the interaction between exercise and metformin has been largely understudied. Members of our investigative team have previously showed that metformin does not enhance but may blunt the glucoregulatory and cardiovascular response to acute and chronic exercise, which is especially relevant to older adults. A purported mechanism of action is that metformin inhibits mitochondrial respiration, which likely limits VO2max and mitochondrial protein synthesis. We have previously demonstrated that post-aerobic exercise dairy feeding can improve VO2max to a greater extent than post-exercise carbohydrate beverage. Therefore, we feel dairy supplementation may override the potential inhibition of metformin on exercise adaptations and maximize the benefits for older adults. To date, real-time continuous glucose monitoring is limited in individuals at risk of T2D. Moreover, glucose profiles via CGM during or in response to exercise, metformin and/or post-exercise nutrition have yet to be fully characterized. Much clinical research is limited to measurements in the clinical research setting while real-time glucose monitoring performed in both the standardized clinical research setting and in free-living scenarios is novelty of the current study design that will close a gap in knowledge regarding glucoregluatory effects of exercise, metformin and/or standardized post-exercise nutrition.

b) Describe any animal experimentation and findings leading to the formulation of the study, if applicable.

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* * * Radioisotopes or Radiation Machines * * *

You selected NO for Radioisotopes in the General Checklist. If you would like to add Radioisotopes, change the selection to YES in general Checklist.

4.

Radioisotopes or Radiation Machines Please note: For projects requiring radiation procedures, please contact the CSU Radiation Control Office (RCO). For more information see: http://www.ehs.colostate.edu/WRad/Home.aspx :

a) If applicable, summarize in lay language the radiographic diagnostic and therapeutic procedures associated with this protocol.

Body composition will be measured by dual energy x-ray absorptiometry (DEXA) before and after the 12 weeks of exercise in pre diabetic participants. In healthy individuals, one DEXA scan will be performed.

b)

Are the radiation procedures being performed a normal part of the clinical management for the medical condition that is under study (Standard of Care) or are the procedures being performed because the research subject is participating in this project (extra CT scans, more fluoroscopy time, additional Nuclear Medicine Studies, etc.,) (Not Standard of Care)? If some procedures are Standard of Care and some are Not Standard of Care, check both boxes.

NOT STANDARD OF CARE If it is not standard of care, complete the rest of this section. Provide the CSU RCO approval information	X	STANDARD OF CARE If it is only standard of care, skip the rest of this section.

CSU Radiation Control Office approved protocol number:

CSU Radiation Control Office protocol approval date:

For more information, see the RCO website at:http://www.ehs.colostate.edu/Wrad/home.aspx or Contact: James Abraham, Radiation Safety Officer, at 970-491-3736.

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* * * Medical Equipment for Human Subjects and Laboratory Animals; Investigatiional Devices * * *

5. Medical Equipment for Human Subjects

If medical equipment is being used for human subjects/patients, describe this equipment and indicate if the use is normal practice for the population under study. You may have already described this equipment in the Study Procedures section. If you have already listed this information in the Study Procedures Section, please do not duplicate this information here. In the space below, input N/A if not applicable, indicate if this is already listed in the Study Procedures Section, or describe the equipment.

Continous Glucose Monitor (CGM) described in study procedures and in the attached investigator brochure and user guide. The Dexcom G6 CGM is for investigational use and is not FDA approved.

6. Investigational Devices

Please list in the space below all Investigational Devices to be used on Subjects.

Investigational Devices

Investigational Devices

Describe the device(s) to be used

DEXCOM G6 Continuous Glucose Monitoring (CGM) System. CGM can supplement, but does not replace, blood glucose monitoring. Previous versions of the device (Dexcom SEVEN PLUS, G4 PLATINUM, and G5 Mobile CGM) have been classified as a Class III device according to 21 CFR Part 814. The device's product code is MDS. Class III devices generally are those for which insufficient information exists to determine that general or special controls are sufficient to provide a reasonable assurance of safety and effectiveness.

Device Name DEXCOM G6 Continuous Glucose Monitoring (CGM) System

Manufacturer Dexcom, Inc.

Significant risk Y **Non-Significant risk**

IDE #

Rationale for Non-Significant Risk Device

Device is not exempt from requirements in 21 CFR 812. Device is intended as an implant? Device is purported or represented to be for use supporting or sustaining human life? Device used of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health? Device presents a potential for serious risks to the health, safety, or welfare of a subject? If yes to any, then considered SR device.

If a non-significant risk device study is indicated, provide rationale for the device being non-significant risk. Please state if you need IRB guidance on whether this is a significant or non-significant risk device.

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* * * Drugs, Reagents, or Chemicals * * *

7. Drugs, Reagents, or Chemicals

a) Please list in the space below all investigational drugs, reagents or chemicals to be administered to subjects during this study.

b) Please list in the space below all commercial drugs, reagents or chemicals to be administered to subjects during this study.

Drug Name	Metformin
Source (e.g., Pharmacy, Sponsor, etc.,)	Pharmacy
If not premixed, where will the material be mixed and by whom	
Manufacturer	
IND # (if available)	
Dosage	Maximum dose of 1500 mg/day
Administration Route	Oral
N	Are these new or different uses of these commercially available drugs, reagents, or chemicals?
N	IND Regulations

Please read the IND Statements

---

* * * Subject Population (a-g) * * *

8.

Subject Population - In the space below, please detail the participants that you are requesting to recruit (include requested participant number and description of each group requested). (Input N/A if not applicable)

a) Requested Participant Description (Include number of participants that you plan to study and description of each group requested, if applicable).

We plan to recruit 120 men and women with an estimation that 15-25% of participants will not qualify (i.e., screen out) and 15-25% of participants will not complete the study (i.e. drop out). We plan to complete the study in 60 men and women aged >55 years who are prediabetic defined as impaired fasting glucose (100 to 126 mg/dl), HbA1c (5.7-6.4%), family history of type 2 diabetes, and/or impaired glucose tolerance defined as 2 hour postprandial blood glucose of 140 to 200 mg/dl. Participants will be eligible if they meet any 1 of these criteria.

10 healthy individuals, 18-45 years old who have normal glucose values (fasting glucose (<100mg/dl), HbA1c (<5.7%), 2 hour postprandial blood glucose (<140mg/dl)) or no family history of type 2 diabetes will be recruited.

Sub-study: 20 pre diabetic participants and 10 healthy individuals will complete the study. Subpopulation of [xx] participants will be recruited for Physical Activity Behavior Counseling (previously approved in Amendment 3.

b) What is the rationale for studying the requested group(s) of participants?

Individuals at risk for T2D are the intended population for metformin; CGM; and Counseling studies. We will use a healthy control group to compare to individuals at risk for T2D.

c) **If applicable, state the rationale for involvement of potentially vulnerable subjects to be entered into the study, including minors, pregnant women, economically and educationally disadvantaged, or decisionally impaired subjects. Specify the measures being taken to minimize the risks and the chance of harm to the potentially vulnerable subjects.**

We will not enroll minors, pregnant women, economically and educationally disadvantaged, or decisionally impaired subjects.

d) **If women, minorities, or minors are not included, a clear compelling rationale must be provided. Examples for not including minors: disease does not occur in children; drug or device would interfere with normal growth and development; etc.**

Minors are not included because the cost-benefit ratio is not clear and therefore inappropriate for this study.

e) **State if any of the subjects are students, employees, or laboratory personnel. They should be presented with the same written informed consent. If compensation is allowed, they should also receive it.**

If any of the subjects are students, employees, or laboratory personnel they will be presented with the same written informed consent and compensation.

f) **Describe how potential subjects will be identified for recruitment (e.g., chart review, referral from individual's treating physician, those individuals answering an ad). How will potential participants learn about the research and how will they be recruited (e.g., flyer, email, web posting, telephone, etc.)? Attach recruitment materials in the Attachment Section (#16). Important to remember: potential subjects may not be contacted before IRB approval.**

Potential subjects will be recruited initially from the Colorado State and Fort Collins community via email, electronic, newspaper, radio, or bulletin advertisements. Colorado State employees, local physicians and interested participants may be emailed for recruitment and communication purposes. Physicians will be contacted so if they have prediabetic patients they can be informed of the research study which includes standard care treatment options (i.e., physical activity and metformin). Text for advertisements will be obtained from the attached flyer. Information for local physicians is also attached.

g) **If applicable, provide rationale for the inclusion of healthy volunteers in this study. Specify any risks to which these healthy volunteers may possibly be exposed. Specify the measures being taken to minimize the risks and the chance of harm to these volunteers.**

We will include healthy volunteers to compare to individuals at risk for T2D. No additional risks are known for healthy individuals compared to those at risk for T2D. Healthy individuals will not perform the intervention.

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* * * Subject Population (h-m) * * *

8. Subject Population (Input N/A if not applicable)

h) **Inclusion and Exclusion Criteria (e.g., Participants must have 20/20 vision, Participants must be 30-45 years of age, etc.)**

Identify inclusion criteria.

We plan to recruit 120 men and women with an estimation that 15-25% of participants will not qualify (i.e., screen out) and 15-25% of participants will not complete the study (i.e. drop out). We plan to complete the study in 60 men and women aged >55 years who are prediabetic defined as impaired fasting glucose (100 to 126 mg/dl), HbA1c (5.7-6.4%), family history of type 2 diabetes, and/or impaired glucose tolerance defined as 2 hour postprandial blood glucose of 140 to 200 mg/dl. Participants will be eligible if they meet any 1 of these criteria.

10 healthy participants will be recruited who are 18-45 years old who have normal glucose values (fasting glucose <100mg/dl, HbA1c <5.7%, 2 hour postprandial blood glucose <140 mg/dl) and no family history of T2D.

Identify exclusion criteria.

Medications contraindicated with Metformin (Dofetilide, Lamotrigine, Pegvisomant, Somatropin, Trimethoprim, Trospium, Gatifloxacin, Cephalexin, Cimetidine, Dalfampridine)
Recent (less than 6 weeks) or planned imaging that requires IV contrast
Renal failure, creatinine $\geq$ 1.3 mg/dL in men or $\geq$ 1.2 mg/dL in women
ALT levels exceed 52 IU/L
Heart, Kidney or Liver Disease
Type I or Type II Diabetes
Anti-coagulant therapy (warfarin/heparin)
Lung/respiratory dysfunction
Medications affecting primary outcomes
Lactose Intolerant
Tobacco Use
Heavy Alcohol Use
Cancer
Lidocaine Allergy
EKG that is nondiagnostic for ischemia, such as: Complete Left bundle branch block or nonspecific Intraventricular conduction delay with ST/T changes; LVH with significant ST changes; Wolff-Parkison-White syndrome; ventricular pacemaker; digoxin therapy with greater than 1 mm ST depression or
Arrythmias, such as: Uncontrolled rate in atrial fibrillation; nonsustained ventricular tachycardia.

i) **Describe your screening procedures. Attach your screening document(s) (e.g., health history questionnaire) in the Attachment Section (#16).**

Blood draw for chemistry panel, health questionnaire, medical history questionnaire, oral glucose tolerance test, resting and exercise EC.

j) **Describe how you will be cognizant of other protocols in which subjects might be participating. Please explain if subjects will be participating in more than one study.**

Subjects will be instructed to communicate with investigators should they consider or begin to participate in other studies. Subjects participating in studies that may confound the results of the present investigation will be asked to withdraw from the the present investigation.

k) **Compensation. Explain the amount and schedule of compensation, if any, that will be paid for participation in the study. Compensation includes food, gift cards, money, tokens, etc. Include provisions for prorating payment, if applicable. Compensation should be prorated if several activities are involved for different time periods (e.g., $10 for session #1, and $10 for session #2).**

Pre diabetic ubjects will be paid $500 at the completion of the study. Proration will occur at $20 per oral glucose tolerance test and $50 per muscle biopsy. Participants that complete the CGM portion of this study will receive additional compensation of $100 because of the additional time required for this study component.
Healthy participants will be compensated $50 for the time spent completing the oral glucose tolerance test (OGTT) and up to 10 days of CGM.

l) **Costs. Please explain any costs that will be charged to the subject.**

Subjects will not incur any costs.

m) **Estimate the probable duration of the entire study. This estimate should include the total time each subject is to be involved and the duration the data about the subject is to be collected (e.g., This is a 2-year study. Participants will be interviewed 3 times per year; each interview will last approximately 2 hours. Total approximate time commitment for participants is 12 hours). These times should be consistent with the time commitment listed on the consent document.**

After initial screening Pre-diabetic subjects will undergo a 12 week intervention followed by post-intervention assessments. Total time demand for each subject is estimated at 15 weeks with a total

commitment of approximately 46 hours over that 15 weeks. If participants chose to participate in the optional physical activity behavior counseling there will be additional time spent completing questionnaires (less than 1 hr) at baseline, immediately after completion of exercise training and 12 weeks after completion of exercise training but these can be completed via email or phone. Additionally, participants will be randomized to complete 1 session of in person physical activity behavior counseling (approximately 1 hour).
Pre-diabetic subjects who choose to participant in wearing the Dexcom G6 CGM will wear it for up to 10 days at a time on three separate occasions: before, during and after the 12-week intervention.

Healthy subjects who participant in wearing the Dexcom G6 CGM will wear it for up to 10 days on one occasion.

*** * * Risks * * ***

9. Risks (Input N/A if not applicable)

US Department of Health & Human Services (HHS) Regulations define a subject at risk as follows: "...any individual who may be exposed to the possibility of injury, including physical, psychological, or social injury, as a consequence of participation as a subject in any research, development, or related activity which departs from the application of those accepted methods necessary to meet his needs, or which increases the ordinary risks of daily life, including the recognized risks inherent in a chosen occupation or field of service."

a) PI's evaluation of the overall level of Risk. (Please check one: minimal or > minimal.)

Minimal (everyday living)
Y **> Minimal (greater than everyday living)**

b)

For the following categories include a scientific estimate of the frequency, severity, and reversibility of potential risks. Wherever possible, include statistical incidence of complications and the mortality rate of proposed procedures. Where there has been insufficient time to accumulate significant data ON risk, a statement to this effect should be included. (In describing these risks in the consent form to the subject, it is helpful to use comparisons which are meaningful to persons unfamiliar with medical terminology.) Address any risks related to:

1. **Use of investigational devices. Please include the clinical adverse events (AEs) associated with each of the devices with an indication of frequency, severity and reversibility. This information can often be found in the Investigator(s) brochure.**

Dexcom CGM G6 is not an FDA approved device. This is currently available for research and investigational purposes. Please see IB in attachments and notes in protocol notes. Wearing of device should not be placed through baggage x-ray machine or body scanner. Rarely, the sensor wire may break or detach from the sensor pod. These do not pose a significant medical risk, but participants should contact the Study Coordinator within 24 hours if experiencing a broken wire. Risks include infection, bleeding, pain or skin irritations (e.g., redness, swelling, bruising, itching, scarring or skin discoloration) from the sensor and wearing the adhesive patch.

2 **Use of investigational drugs. Please include the clinical AEs associated with each of the drugs with an indication of frequency, severity and reversibility. This information can often be found in the Investigator(s) brochure.**

n/a

3 **Use of commercially available drugs, reagents or chemicals. Please include the clinical AEs associated with each of the drugs with an indication of frequency, severity and reversibility. This information can often be found in the package insert provided by the manufacturer.**

Metformin has been commonly used as a diabetes medication for many years, both within and outside of the US. It is safely used by nearly 50 Million people on a daily basis in the US.
Like most medication there are possible side effects: From the MAYO CLINIC:
Risks:

More common

Abdominal or stomach discomfort
Cough or hoarseness
Decreased appetite
Diarrhea
Fast or shallow breathing
Fever or chills
General feeling of discomfort
Lower back or side pain
Muscle pain or cramping
Painful or difficult urination
Sleepiness

Less common

Anxiety
Blurred vision
Chest discomfort
Cold sweats
Coma
Confusion
Cool, pale skin
Depression
Difficult or labored breathing
Dizziness
Fast, irregular, pounding, or racing heartbeat or pulse
Feeling of warmth
Headache
Increased hunger
Increased sweating
Nausea
Nervousness
Nightmares
Redness of the face, neck, arms, and occasionally, upper chest
Seizures
Shakiness
Shortness of breath
Slurred speech
Tightness in the chest
Unusual tiredness or weakness
Wheezing

Rare

Behavior change similar to being drunk
Difficulty with concentrating
Drowsiness
Lack or loss of strength
Restless sleep
Unusual sleepiness

4 **When performing procedures, please include all investigational, non-investigational and non-invasive procedures (e.g., surgery, blood draws, treadmill tests).**

Catheter Placement for OGTT (beginning and end of study): Minor discomfort of having the

needle inserted and taped to an arm. In about 1 in 10 cases, a small amount of bleeding will occur under the skin that will cause a bruise. The risk of forming a blood clot in the vein is about 1 in 100, and the risk of significant blood loss is 1 in 1,000. This procedure is currently CSU-IRB approved and active.

Muscle biopsy (beginning and end of study): The risk of allergic reaction to lidocaine and lidocaine toxicity is extremely low (0 in the ~1500 cases witnessed by PI). There is a small risk of infection at the site of incision. There is a small risk of the incision reopening/bleeding after the subject leaves the lab. All subjects will have a small scar at the incision site. The rate and degree of healing varies considerably, but it is expected that scars will be difficult to see within 6-12 months after the procedure.

Consumption of Heavy Water: Brief periods (< 30 minutes) of dizziness have been reported in a small number of people (< 5%) after drinking special water. This is due to the special water being heavier than normal water. Heavier water can temporarily disrupt your inner ear ability to control balance, producing a sensation of dizziness.

Exercise and Exercise Testing (VO2max): There is a very small chance of an irregular heartbeat during exercise (< 1% of all subjects). Other rare risks of a stress test are heart attack (< 5 in 10,000) and death (<2 in 10,000). Wearing a mouthpiece and nose-clip during the graded exercise test can sometimes cause dryness in the mouth and mild discomfort.

CGM: The insertion of the CGM is a very quick procedure that causes no more discomfort than the blood sampling or finger prick. The sensor sits beneath the skin and the participant should not feel the senor once it is inserted. There is a small risk of infection, bleeding, pain or skin irritations (e.g., redness, swelling, bruising, itching, scarring or skin discoloration) after inserting the CGM sensor and wearing the adhesive patch. To minimize the risk of discomfort, researchers will clean the area of skin with sterile alcohol swabs. The sensors and applicator are shipped in sterile packaging and will be inserted within 5 minutes of opening package. Over 325 G6 CMG devices have been studied in 241 subjects across 9 independent clinical study sessions. There were 5 total adverse events reported in these studies that included reddening, irritation, infection and shooting pain at the site of the insertion or adhesive site that resolved within 24 hours. No serious adverse device effects or unanticipated adverse device effects were reported. A detailed report can be found in the investigational brochure in Section 4 Existing Clinical Data.

Fingersticks: Participants will likely experience brief (<30s) physical discomfort during the finger stick procedure when the lancet goes into your finger. In about 10% of the cases a small amount of bleeding under the skin will produce a bruise. A small scare may persist for several weeks. The risk of local infection is less than 1 in 1,000 and is minimized by using the alcohol (70% ethanol) swabs provided.

5 **Radioisotopes/radiation-producing machines(e.g., X-rays, CT scans, fluoroscopy).**

Body Composition by DEXA scanning -
A small amount of radiation exposure (0.05 mRem) associated with the DEXA that is less than 1/20 of a typical chest x-ray. No immediate short-term or long-term risk to adults from this radiation dose. This procedure is currently CSU-IRB approved and active.

c)

For the following categories, include an estimate of the potential risk, if applicable.

1. **Physical well-being.**

None anticipated

2. **Psychological well-being.**

None anticipated

3. **Economic well-being.**

None anticipated

4. **Social well-being.**

None anticipated

d) **In case of overseas research, or working with a specific race/ethnicity in the United States, provide background on what experience the Investigator(s) have with the proposed population. Describe qualifications/preparations that enable the Investigator(s) to evaluate cultural appropriateness and estimate/minimize risks to subjects.**

N/A

e) **Special Precautions. Describe the planned procedures for protecting against or minimizing potential risks. If appropriate, include the standards for termination of the participation of the individual subject. Discuss plans for ensuring necessary medical or professional intervention in the event of adverse effects to the subjects.**

The potential risks of the proposed studies will be minimized by: a) using only safe, well-established procedures; b) constant, personal monitoring of each experimental session by the investigators and their respective staffs; and c) the availability of emergency equipment and medicine. Students and other subordinate personnel on the research team will undergo the required human subjects training and will be very carefully monitored in their technique and interactions with subjects.

Venous catheter risks
Venous catheters will only be placed by experienced phlebotomists.

Muscle Biopsy
The skin will be disinfected with Betadine and all instruments will be sterilized to reduce the risk of infection.

Exercise
Qualified personel will supervise your initial resting and exercise ECG to determine if you are healthy enough to perform regular exercise. During the exercise intervention a CPR trained exercise physiologist will supervise every exercise session.

CGM
CGM will only be placed and removed by trained personnel following standard operating procedures. The skin will be disinfected using sterile alcohol swabs.

f)

Data Safety Monitoring

N **Is there a Data Safety Monitoring Board (DSMB)?**

If yes, describe its role and indicate who set up the Data Safety Monitoring Board (e.g., sponsor or Protocol Director).

Describe the data and safety monitoring plan developed to ensure the safety of participants and the validity and integrity of research data. Monitoring should be commensurate with risks and with the size and complexity of the trials.

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* * * Benefits, Procedures to Maintain Confidentiality * * *

10. Benefits (Input N/A if not applicable)

a) **Describe the potential benefit(s) to be gained by the subjects. If there is no direct benefit to the subjects describe how the results of the study may benefit society or a particular group.**

The pre diabetic and healthy individuals will learn about their physiology, body composition and general cardiovascular risk factors determined from blood samples and ECG. Although there are no guaranteed benefits from participating in this study, we are providing the first line of care for preventing the progression of prediabetes to overt Type 2 Diabetes. Therefore, the anticipated benefits of the research are a positive effect on glucose metabolism, aerobic fitness, quality of life, and performance of daily activities.

11. Procedures to Maintain Confidentiality

a) **Describe the procedures that protect the privacy of the subjects and maintain the confidentiality of the data. If a linked list is used, explain when the linked list will be destroyed. Provide a sample of the code that will be used, if applicable.**

Research records and data will be stored on file in locked cabinets in the investigators' office/laboratory, or digitally on a password protected central server. Only members of the research team will have access to these records. The materials will be archived for a minimum of three years after completion of the project. All data will be coded; e.g. XYZ123456

b) **If information derived from the study will be provided to the subject's personal physician, a government agency, or any other person or group (other than the research team), describe to whom the information will be given and the nature of the information, if applicable.**

Information derived from this study will be available for the subjects to provide to their personal physician.

c) **Specify where and under what conditions study data will be kept, how samples will be labeled, who has access to the data, and what will be available and to whom. Federal regulations require that study data and consent documents be kept for a minimum of three (3) years after the completion of the study by the PI. For longitudinal projects and federally regulated studies, the PI may be required to keep the data and documents for a longer time period.**

Research records and data will be stored on file in locked cabinets in the investigators' office/laboratory, or digitally on a password protected central server. Only members of the research team will have access to these records. The materials will be archived for a minimum of three years after completion of the project.

All data will be coded; e.g. XYZ123456. De-identfied data from subjects who participate in the CGM substudy will be shared with Dexcom, Inc.

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* * * Potential Conflict of Interest * * *

12. Potential Conflict of Interest

Although you have already submitted CSU's official Conflict of Interest form (FCOI/COI/COC) to the University, it is the IRB's responsibility to ensure that conflicting interests related to submitted protocols do not adversely affect the protection of participants or the credibility of the human research protection program at CSU. Please answer questions a-d below. Please note that if you indicate that you have a potential financial or professional conflict of interest in relation to this protocol, your CSU FCOI/COI/COC Reporting Form must reflect this potential conflict. Link to CSU's Conflict of Interest policy: :http://www.facultycouncil.colostate.edu/files/manual/sectiond.htm#D.7.7

a) N **In connection with this protocol, do you or any of the protocol investigators or their immediate family members (i.e., spouse and legal dependents, as determined by the IRS) have a potential financial or professional conflict of interest?**

b) N/A **If you do have a potential conflict of interest, is this reported in your current FCOI/COI/COC?**

c)	N/A	**If you do have a potential conflict of interest, is there a management plan in place to manage this potential conflict?**
d)	N/A	**If you do have a potential conflict of interest, is this potential conflict of interest included in your consent document (as required in the Management Plan)?**

If you have reported a possible conflict of interest, the IRB will forward the title of this protocol to your Research Associate Dean to complete your COI file.

For more information on CSU's policy on Conflict of Interest, please see the Colorado State University Academic Faculty and Administrative Professional Manual Sections D.7.6 & D.7.7. :http://www.facultycouncil.colostate.edu/files/manual/sectiond.htm#D.7.7

Link to CSU's Conflict of Interest Policy: http://www.provost.colostate.edu/index.asp?url=faculty_affairs.

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* * * Informed Consent * * *

13. Informed Consent

NOTE: In order to complete this protocol, you must upload either a Consent Form or an Alteration of Consent Form (i.e., Cover Letter or Verbal Script) OR (if neither of those apply to your project) you must complete the Waiver of Consent information.

In the space below, please provide consent process background information for each Consent Form(s), Alteration of Consent Form(s), or Waiver(s).

Informed Consent

Title	15-5837H CGM Consent 02242017	
Consent Information Type	Consent	
Sponsor's Consent Version Number: (if any)		
Consent Form Template	X Attachment	15-5837 CGM Informed Consent 24Feb2017 (1)

<a href='http://ricro.colostate.edu/IRB/ConsentAssentTemplates.html' target=_blank Consent Form Samples

Who is obtaining consent? The person obtaining consent must be knowledgeable about the study and authorized by the PI to consent human subjects.

How is consent being obtained?

What steps are you taking to determine that potential subjects are competent to participate in the decision-making process?

Title	15-5837H Main Study 24April2017 Stamped	
Consent Information Type	Consent	
Sponsor's Consent Version Number: (if any)		
Consent Form Template	X Attachment	15-5837H Informed Consent 24April2017_Main Study RICRO STAMPED (1)

<a href='http://ricro.colostate.edu/IRB/ConsentAssentTemplates.html' target=_blank Consent Form Samples

Who is obtaining consent? The person obtaining consent must be knowledgeable about the study and authorized by the PI to consent human subjects.

How is consent being obtained?

What steps are you taking to determine that potential subjects are competent to participate in the decision-making process?

Title	Healthy Individual Consent 11July2017	
Consent Information Type	Consent	
Sponsor's Consent Version Number: (if any)		
Consent Form Template	X Attachment	15-5837 Informed Consent Healthy Individuals (1)

<a href='http://ricro.colostate.edu/IRB/ConsentAssentTemplates.html' target=_blank Consent Form Samples

Who is obtaining consent? The person obtaining consent must be knowledgeable about the study and authorized by the PI to consent human subjects.

How is consent being obtained?

What steps are you taking to determine that potential subjects are competent to participate in the decision-making process?

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* * * Assent Background * * *

14. Assent Background (Complete if applicable)

All minors must provide an affirmative consent to participate by signing a simplified assent form, unless the Investigator(s) provides evidence to the IRB that the minor subjects are not capable of assenting because of age, maturity, psychological state, or other factors.

See sample consent/assent forms at http://ricro.colostate.edu/IRB/ConsentAssentTemplates.html

Provide assent process background information, in the space below, for each Assent Form, Alteration Form (i.e., Cover Letter or Verbal Script), and Waiver.

Assent Background

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* * * HIPAA * * *

15. HIPAA

Are you using PHI*? (See definition below)

N

Colorado State University is a hybrid entity and does not have a research-related HIPAA policy. If you will be working with a HIPAA covered entity (e.g., Poudre Valley Health System), you will need to follow their HIPAA guidelines. If your project will involve a HIPAA-regulated entity, in the Attachment section (#16) please attach that entity's required HIPAA consent and/or each waiver of authorization or alteration of authorization requested (e.g., waiver of authorization for access to medical records). Include HIPAA authorization language in the consent document(s) as appropriate (e.g., when enrolling subjects).

*Protected Health Information (PHI) is health information with one or more of the following identifiers. For more information see: http://www.hhs.gov/ocr/hipaa/

1. Names
2. Social Security numbers
3. Telephone numbers
4. All geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code, and their equivalent geocodes, except for the initial three digits of a zip code, if, according to the current publicly available data from the Bureau of the Census: (1) The geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people; and (2) The initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000
5. All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death; and all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older
6. Fax numbers
7. Electronic mail addresses
8. Medical record numbers
9. Health plan beneficiary numbers
10. Account numbers
11. Certificate/license numbers
12. Vehicle identifiers and serial numbers, including license plate numbers
13. Device identifiers and serial numbers
14. Web Universal Resource Locations (URLs)
15. Internet Protocol (IP) address numbers
16. Biometric identifiers, including finger and voice prints
17. Full face photographic images and any comparable images; and
18. Any other unique identifying number, character, or code (note this does not mean the unique code assigned by the Investigator(s) to code the research data)

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* * * Attachments * * *

16. Attachments

Attach relevant documents here. These could include: Collaborating Investigator's IRB approval and approved documents; Conflict of Interest information; Debriefing Script; Grant/Sub-contract; HIPAA Authorization Form from HIPAA-covered entity; Interview/Focus Group Questions; Investigator's Brochure; Letters of Agreement/Cooperation from organizations who will help with recruitment; Methodology section of associated Thesis or Dissertation project; Questionnaires; Radiation Control Office approval material; Recruitment Material (e.g., flyers, email text, verbal scripts); Sponsor's Protocol; Surveys; Other files associated with the protocol (you can upload most standard file formats: xls, pdf, jpg, tif, etc.) Please be sure to attach all documents associated with your protocol. Failure to attach the files associated with the

protocol may result in this protocol being returned to you for completion prior to being reviewed. Students: Be sure to attach the Methods section of your thesis or dissertation proposal. If this protocol is associated with a grant proposal, please remember to attach your grant.

To update or revise any attachments, please delete the existing attachment and upload the revised document to replace it.

Document Type	**Recruitment Material (e.g., flyers, email text, verbal scripts)**
Attachment	1 page summary for clinicians v. 14May2015
Document Name	1 page summary for clinicians v. 14May2015

Document Type	**Recruitment Material (e.g., flyers, email text, verbal scripts)**
Attachment	Verbal Recruitment 14May2015
Document Name	Verbal Recruitment 14May2015

Document Type	**Questionnaire/Survey**
Attachment	medical questionaire
Document Name	medical questionaire

Document Type	**SOP**
Attachment	B-01-R1 Biopsy SOP
Document Name	B-01-R1 Biopsy SOP

Document Type	**SOP**
Attachment	B-02-R1 Biopsy Needle Cleaning
Document Name	B-02-R1 Biopsy Needle Cleaning

Document Type	**SOP**
Attachment	HP-05-R1 Reporting AEs SOP
Document Name	HP-05-R1 Reporting AEs SOP

Document Type	**SOP**
Attachment	D2O-01-R1 Prep and Dispensing SOP
Document Name	D2O-01-R1 Prep and Dispensing SOP

Document Type	**Recruitment Material (e.g., flyers, email text, verbal scripts)**
Attachment	Dairy Metformin Ad
Document Name	Dairy Metformin Ad

Document Type	**Letters of Agreement.Cooperation**
Attachment	Physician Support Letter
Document Name	Physician Support Letter

Document Type	**Recruitment Material (e.g., flyers, email text, verbal scripts)**
Attachment	CTS_Materials_Ad Examples
Document Name	CTS_Materials_Ad Examples

Document Type	**Questionnaire/Survey**
Attachment	BARSE
Document Name	BARSE

Document Type	**Questionnaire/Survey**
Attachment	EXSE_Baseline
Document Name	EXSE_Baseline

Document Type	**Questionnaire/Survey**
Attachment	EXSE
Document Name	EXSE

Document Type	**Questionnaire/Survey**
Attachment	IPAQ_English_self-admin_short
Document Name	IPAQ_English_self-admin_short

Document Type	**Questionnaire/Survey**
Attachment	NEWS
Document Name	NEWS

Document Type	**Questionnaire/Survey**
Attachment	Socioeconomic questionnaire
Document Name	Socioeconomic questionnaire

Document Type	**Questionnaire/Survey**
Attachment	Planning, Self-Regulation and Expectancy
Document Name	Planning, Self-Regulation and Expectancy

Document Type	**Other Protocol Material**
Attachment	Report - Draft 4
Document Name	Report - Draft 4

Document Type	**Questionnaire/Survey**
Attachment	15-5837H Medical Screening form 19Feb2016
Document Name	15-5837H Medical Screening form 19Feb2016

Document Type	**Other Protocol Material**
Attachment	AE DC464 GI
Document Name	AE DC464 GI

Document Type	**Other Protocol Material**
Attachment	AE SR312 GI
Document Name	AE SR312 GI

Document Type	**Other Protocol Material**
Attachment	AE TA334 GI
Document Name	AE TA334 GI

Document Type	**Other Protocol Material**
Attachment	AE TA334 pink colored urine
Document Name	AE TA334 pink colored urine

Document Type	**IBC Approval Letter**
Attachment	IBC approval
Document Name	IBC approval

Document Type	**Other Protocol Material**
Attachment	AE AC693 GI present prior to start of intervention
Document Name	AE AC693 GI present prior to start of intervention

Document Type	**Investigator's Brochure**
Attachment	G6 Investigator Brochure
Document Name	G6 Investigator Brochure

Document Type	**Investigator's Brochure**
Attachment	CONTOUREZ-User-Guide-ENGLISH
Document Name	CONTOUREZ-User-Guide-ENGLISH

Document Type	**SOP**
Attachment	CGM SOP 9.1.16
Document Name	CGM SOP 9.1.16

Document Type	**Investigator's Brochure**
Attachment	Microlet2
Document Name	Microlet2

Document Type	**Investigator's Brochure**

Attachment	G6 Blinded US_Study Instructions_User Guide
Document Name	G6 Blinded US_Study Instructions_User Guide
Document Type	**Questionnaire/Survey**
Attachment	Participant_Pre Screening 01Sept2016 v.2
Document Name	Participant_Pre Screening 01Sept2016 v.2
Document Type	**Recruitment Material (e.g., flyers, email text, verbal scripts)**
Attachment	Dairy-Metformin Advertisement Example
Document Name	Dairy-Metformin Advertisement Example
Document Type	**Recruitment Material (e.g., flyers, email text, verbal scripts)**
Attachment	Participant Testimonial
Document Name	Participant Testimonial
Document Type	**Recruitment Material (e.g., flyers, email text, verbal scripts)**
Attachment	new recruiment flyer 1
Document Name	new recruiment flyer 1
Document Type	**Recruitment Material (e.g., flyers, email text, verbal scripts)**
Attachment	new recruiment flyer 2
Document Name	new recruiment flyer 2
Document Type	**Recruitment Material (e.g., flyers, email text, verbal scripts)**
Attachment	VIDEO PRODUCTION OUTLINE
Document Name	VIDEO PRODUCTION OUTLINE
Document Type	**Other Protocol Material**
Attachment	GCP protocol v 1.0 7 Decmber2016 v1.0
Document Name	GCP protocol v 1.0 7 Decmber2016 v1.0
Document Type	**Other Protocol Material**
Attachment	DOA CGM 03Feb2017
Document Name	DOA CGM 03Feb2017
Document Type	**Grant/Sub-Contract**
Attachment	Colorado State University - CTSA IIIS-2016-039 (11-01-2016) (003)

Document Name	Colorado State University - CTSA IIIS-2016-039 (11-01-2016) (003)
Document Type	**Grant/Sub-Contract**
Attachment	CSU Dexcom Proposal 8.4.16
Document Name	CSU Dexcom Proposal 8.4.16
Document Type	**Other Protocol Material**
Attachment	15.5837H NSR DETERMINATION 02242017
Document Name	15.5837H NSR DETERMINATION 02242017
Document Type	**Other Protocol Material**
Attachment	15.5837 SR-NSR determination
Document Name	15.5837 SR-NSR determination
Document Type	**Other Protocol Material**
Attachment	Adverse Event Log 21April2017
Document Name	Adverse Event Log 21April2017
Document Type	**Other Protocol Material**
Attachment	CGM Adverse Event Log 03Feb2017
Document Name	CGM Adverse Event Log 03Feb2017
Document Type	**Other Protocol Material**
Attachment	Protocol Deviation CGM applied twice for pre measurements 04April2017 signed
Document Name	Protocol Deviation CGM applied twice for pre measurements 04April2017 signed
Document Type	**Other Protocol Material**
Attachment	Protocol Deviation consent signed 04March2017signed
Document Name	Protocol Deviation consent signed 04March2017signed
Document Type	**Other Protocol Material**
Attachment	Adverse Event Log 01June2017
Document Name	Adverse Event Log 01June2017
Document Type	**Other Protocol Material**
Attachment	CGM Adverse Event Log 01June2017
Document Name	CGM Adverse Event Log 01June2017

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* * * Obligations * * *

Obligations (Researcher's Responsibilities)

The Principal Investigator is ultimately responsible for the conduct of the project. Obligations of the Principal Investigator are:

Conduct the research involving human subjects as presented in the protocol, including modifications, as approved by the Department and Institutional Review Board. Changes in any aspect of the study (for example project design, procedures, consent forms, advertising materials, additional key personnel or subject populations) will be submitted to the IRB for approval before instituting the changes (PI will submit the "Amendment/Revision" form);

Provide all subjects a copy of the signed consent form, if applicable. Investigators will be required to retain signed consent documents for three (3) years after close of the study;

Maintain an approved status for Human Subjects Protection training. Training must be updated every three (3) years (Contact RICRO to check your current approval/renewal dates). For more information: Human Subjects Training Completed?

Submit either the "Protocol Deviation Form" or the "Report Form" to report protocol Deviations/Violations, Unanticipated Problems (UPs) and/or Adverse Events (AEs) that occur in the course of the protocol. Any of these events must be reported to the IRB as soon as possible, but not later than five (5) working days. Note that if an event resulted in life threatening injury or death OR an event resulted in substantive harm to the safety, rights or welfare to human subjects, this must be reported to the IRB within 24 hours;

Submit the "Continuing Review" Form in order to maintain active status of the approved protocol. This form must be submitted annually at least four (4) weeks prior to expiration, five (5) weeks for protocols that require full review. If the protocol is not renewed before expiration, all activities must cease until the protocol has been re-reviewed;

Notify the IRB that the study is complete by submitting the "Final Report" form.

Dr. Karyn Hamilton

X The Principal Investigator has read and agrees to abide by the above obligations.

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* * * Event History * * *

Event History

Date	Status	View Attachments	Letters
04/29/2015	NEW FORM CREATED		
05/15/2015	NEW FORM SUBMITTED	Y	
05/15/2015	NEW FORM PANEL ASSIGNED		

05/15/2015	NEW FORM REVIEWER(S) ASSIGNED		
05/18/2015	NEW FORM REVIEWER(S) ASSIGNED		
07/01/2015	NEW FORM REVIEWER(S) ASSIGNED		
07/27/2015	NEW FORM REVIEWER(S) ASSIGNED		
08/13/2015	NEW FORM MOVED		
08/13/2015	NEW FORM APPROVED	Y	Y
08/24/2015	AMENDMENT 1 FORM CREATED		
08/24/2015	AMENDMENT 1 FORM SUBMITTED	Y	
08/24/2015	AMENDMENT 1 FORM SUBMITTED	Y	
08/25/2015	AMENDMENT 1 FORM REVIEWER(S) ASSIGNED		
10/07/2015	AMENDMENT 1 FORM MOVED		
10/07/2015	AMENDMENT 1 FORM APPROVED	Y	Y
10/27/2015	AMENDMENT 2 FORM CREATED		
10/27/2015	AMENDMENT 2 FORM SUBMITTED	Y	
11/04/2015	AMENDMENT 2 FORM REVIEWER(S) ASSIGNED		
12/03/2015	AMENDMENT 2 FORM APPROVED	Y	Y
12/03/2015	AMENDMENT 2 FORM UNDO APPROVED		
12/03/2015	AMENDMENT 2 FORM MOVED		
12/03/2015	AMENDMENT 2 FORM APPROVED	Y	Y
12/14/2015	AMENDMENT 3 FORM CREATED		
12/14/2015	AMENDMENT 3 FORM SUBMITTED	Y	
12/15/2015	AMENDMENT 3 FORM REVIEWER(S) ASSIGNED		
01/06/2016	AMENDMENT 3 FORM REVIEWER(S) ASSIGNED		
01/21/2016	AMENDMENT 3 FORM MOVED		
01/21/2016	AMENDMENT 3 FORM APPROVED	Y	Y
02/19/2016	AMENDMENT 4 FORM CREATED		
02/19/2016	AMENDMENT 4 FORM SUBMITTED	Y	
02/22/2016	AMENDMENT 4 FORM REVIEWER(S) ASSIGNED		
03/09/2016	AMENDMENT 4 FORM REVIEWER(S) ASSIGNED		

03/16/2016	DEVIATION 1 FORM CREATED			
03/16/2016	DEVIATION 1 FORM SUBMITTED	Y		
03/17/2016	DEVIATION 2 FORM CREATED			
03/17/2016	DEVIATION 2 FORM SUBMITTED	Y		
03/18/2016	DEVIATION 2 FORM REVIEWER(S) ASSIGNED			
03/18/2016	DEVIATION 2 FORM REVIEWER(S) ASSIGNED			
03/18/2016	AMENDMENT 4 FORM REVIEWER(S) ASSIGNED			
03/21/2016	AMENDMENT 4 FORM MOVED			
03/21/2016	AMENDMENT 4 FORM APPROVED	Y		Y
04/15/2016	DEVIATION 2 FORM REVIEWER(S) ASSIGNED			
04/15/2016	DEVIATION 1 FORM REVIEWER(S) ASSIGNED			
04/20/2016	CONTINUING REVIEW 1 FORM CREATED			
04/27/2016	CONTINUING REVIEW 1 FORM SUBMITTED	Y		
05/13/2016	CONTINUING REVIEW 1 FORM REVIEWER(S) ASSIGNED			
05/17/2016	DEVIATION 1 FORM APPROVED	Y		N
05/17/2016	DEVIATION 2 FORM APPROVED	Y		N
06/03/2016	CONTINUING REVIEW 1 FORM APPROVED	Y		Y
07/08/2016	REPORT 1 FORM CREATED			
07/11/2016	REPORT 1 FORM SUBMITTED	Y		
07/18/2016	REPORT 1 FORM REVIEWER(S) ASSIGNED			
07/18/2016	REPORT 1 FORM REVIEWER(S) ASSIGNED			
08/10/2016	REPORT 1 FORM APPROVED	Y		N
09/01/2016	AMENDMENT 5 FORM CREATED			
09/02/2016	AMENDMENT 5 FORM SUBMITTED	Y		
09/09/2016	AMENDMENT 5 FORM REVIEWER(S) ASSIGNED			
09/12/2016	AMENDMENT 5 FORM REVIEWER(S) ASSIGNED			
10/10/2016	AMENDMENT 5 FORM REVIEWER(S) ASSIGNED			
10/13/2016	REPORT 2 FORM CREATED			

10/14/2016	REPORT 2 FORM SUBMITTED	Y	
10/14/2016	REPORT 2 FORM REVIEWER(S) ASSIGNED		
11/02/2016	AMENDMENT 5 FORM MOVED		
11/02/2016	AMENDMENT 5 FORM APPROVED	Y	Y
11/02/2016	AMENDMENT 5 FORM UNDO APPROVED		
11/02/2016	AMENDMENT 5 FORM APPROVED	Y	Y
11/30/2016	AMENDMENT 6 FORM CREATED		
11/30/2016	AMENDMENT 6 FORM SUBMITTED	Y	
12/07/2016	AMENDMENT 6 FORM REVIEWER(S) ASSIGNED		
12/14/2016	AMENDMENT 6 FORM MOVED		
12/14/2016	AMENDMENT 6 FORM APPROVED	Y	Y
01/05/2017	DEVIATION 3 FORM CREATED		
01/05/2017	DEVIATION 3 FORM SUBMITTED	Y	
01/06/2017	DEVIATION 3 FORM REVIEWER(S) ASSIGNED		
01/07/2017	REPORT 2 FORM APPROVED	Y	N
01/11/2017	DEVIATION 3 FORM REVIEWER(S) ASSIGNED		
02/02/2017	AMENDMENT 7 FORM CREATED		
02/03/2017	AMENDMENT 7 FORM SUBMITTED	Y	
02/10/2017	AMENDMENT 7 FORM REVIEWER(S) ASSIGNED		
02/13/2017	DEVIATION 4 FORM CREATED		
02/13/2017	DEVIATION 4 FORM SUBMITTED	Y	
02/22/2017	AMENDMENT 7 FORM REVIEWER(S) ASSIGNED		
02/22/2017	AMENDMENT 7 FORM REVIEWER(S) ASSIGNED		
02/24/2017	AMENDMENT 7 FORM REVIEWER(S) ASSIGNED		
02/27/2017	AMENDMENT 7 FORM MOVED		
02/27/2017	AMENDMENT 7 FORM APPROVED	Y	Y
03/23/2017	AMENDMENT 8 FORM CREATED		
04/24/2017	AMENDMENT 8 FORM SUBMITTED	Y	

04/25/2017	AMENDMENT 8 FORM REVIEWER(S) ASSIGNED		
04/26/2017	AMENDMENT 8 FORM APPROVED	Y	Y
05/04/2017	CONTINUING REVIEW 2 FORM CREATED		
05/04/2017	CONTINUING REVIEW 2 FORM SUBMITTED	Y	
05/12/2017	CONTINUING REVIEW 2 FORM REVIEWER(S) ASSIGNED		
05/19/2017	CONTINUING REVIEW 2 FORM APPROVED	Y	Y
05/25/2017	DEVIATION 3 FORM APPROVED	Y	N
07/05/2017	AMENDMENT 9 FORM CREATED		
07/11/2017	AMENDMENT 9 FORM SUBMITTED	Y	
07/14/2017	AMENDMENT 9 FORM REVIEWER(S) ASSIGNED		
07/18/2017	DEVIATION 4 FORM MOVED		
07/18/2017	DEVIATION 4 FORM APPROVED	Y	N
07/24/2017	AMENDMENT 9 FORM REVIEWER(S) ASSIGNED		
07/27/2017	DEVIATION 5 FORM CREATED		
07/27/2017	AMENDMENT 9 FORM APPROVED	Y	Y
07/27/2017	AMENDMENT 9 FORM UNDO APPROVED		
07/27/2017	AMENDMENT 9 FORM APPROVED	Y	Y
07/27/2017	DEVIATION 5 FORM SUBMITTED	Y	
08/09/2017	DEVIATION 5 FORM REVIEWER(S) ASSIGNED		
08/28/2017	DEVIATION 5 FORM APPROVED	Y	N
04/04/2018	CONTINUING REVIEW 3 FORM CREATED		
05/09/2018	CONTINUING REVIEW 3 FORM SUBMITTED	Y	
05/15/2018	CONTINUING REVIEW 3 FORM MOVED		
05/15/2018	CONTINUING REVIEW 3 FORM APPROVED	Y	Y
10/03/2018	AMENDMENT 10 FORM CREATED		
10/04/2018	AMENDMENT 10 FORM SUBMITTED	Y	
10/04/2018	AMENDMENT 10 FORM APPROVED	Y	Y

Statistical Plan

Data are presented as mean ± standard error of the mean (SEM) and a significance was set a priori at $P<0.05$. Normality was confirmed by the D'Agostino and Pearson test. A two-way ANOVA (Treatment Group x Time) with repeated measures for time was performed. Upon a significant effect, a Holm-Sidak's post-hoc test was used for multiple comparisons. When comparing the change (Δ) from pre to post-intervention between groups, data were analyzed using an unpaired t-test. Pearson's correlation coefficient (r) was used to determine associations between dependent variables. GraphPad Prism 7.0 was used to perform statistical analysis