Title

Role of resistant starch on diabetes risk factors in people with prediabetes: Design, conduct, and baseline results of the STARCH trial

Authors

Marlatt, Kara L
White, Ursula A
Beyl, Robbie A
et al.

Publication Date

2018-02-01

DOI

10.1016/j.cct.2017.12.005

Peer reviewed

Contemporary Clinical Trials 65 (2018) 99–108

Contents lists available at ScienceDirect

Contemporary Clinical Trials

journal homepage: www.elsevier.com/locate/conclintrial

Role of resistant starch on diabetes risk factors in people with prediabetes: Design, conduct, and baseline results of the STARCH trial

Kara L. Marlatt[a], Ursula A. White[a], Robbie A. Beyl[a], Courtney M. Peterson[a,b], Corby K. Martin[a], Maria L. Marco[c], Michael J. Keenan[d], Roy J. Martin[a,d], Kayanush J. Aryana[d], Eric Ravussin[a,*]

[a] Pennington Biomedical Research Center, Baton Rouge, LA 70808, United States
[b] Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL 35233, United States
[c] School of Food Science and Technology, College of Agriculture and Environmental Sciences, University of California-Davis, Davis, CA 95616, United States
[d] School of Nutrition & Food Sciences, College of Agriculture, Louisiana State University, Baton Rouge, LA 70803, United States

ARTICLE INFO

Keywords:
Resistant starch
Microbiota
Prediabetes
Gut permeability
Insulin sensitivity
Energy metabolism

ABSTRACT

Dietary resistant starch (RS) might alter gastrointestinal tract function in a manner that improves human health, particularly among adults at risk for diabetes. Here, we report the design and baseline results (with emphasis on race differences) from the STARCH trial, the first comprehensive metabolic phenotyping of people with prediabetes enrolled in a randomized clinical trial testing the effect of RS on risk factors for diabetes. Overweight/obese participants (BMI ≥ 27 kg/m^2 and weight ≤ 143 kg), age 35–75 y, with confirmed prediabetes were eligible. Participants were randomized to consume 45 g/day of RS (RS = amylose) or amylopectin (Control) for 12 weeks. The study was designed to evaluate the effect of RS on insulin sensitivity and secretion, ectopic fat, and inflammatory markers. Secondary outcomes included energy expenditure, substrate oxidation, appetite, food intake, colonic microbial composition, fecal and plasma levels of short-chain fatty acids, fecal RS excretion, and gut permeability. Out of 280 individuals screened, 68 were randomized, 65 started the intervention, and 63 were analyzed at baseline (mean age 55 y, BMI 35.6 kg/m^2); 2 were excluded from baseline analyses due to abnormal insulin and diabetes. Sex and race comparisons at baseline were reported. African-Americans had higher baseline acute insulin response to glucose (AIRg measured by frequently sampled intravenous glucose tolerance test) compared to Caucasians, despite having less visceral adipose tissue mass and intrahepatic lipid; all other glycemic variables were similar between races. Sleep energy expenditure was ~90–100 kcal/day lower in African-Americans after adjusting for insulin sensitivity and secretion. This manuscript provides an overview of the strategy used to enroll people with prediabetes into the STARCH trial and describes methodologies used in the assessment of risk factors for diabetes.

Clinicaltrials.gov identifier: STARCH (NCT01708694). The present study reference can be found here: https://clinicaltrials.gov/ct2/show/NCT01708694.

Submission Category: "Study Design, Statistical Design, Study Protocols".

1. Introduction

Prediabetes is characterized by insulin resistance and impaired glucose tolerance and is a significant predictor for developing type 2 diabetes. Proposed mechanisms for the development of prediabetes include the ectopic accretion of lipid in tissues such as liver, skeletal muscle, and pancreas [1]. Lifestyle interventions to treat prediabetes and stop/delay its progression to frank diabetes are necessary to prevent a deleterious disease that is often difficult to manage once developed.

High-amylose Type 2 resistant starch (RS) is a dietary ingredient that has garnered interest for its ability to slow digestion and improve metabolic health markers in rodents and humans. In rodents, a fermentable carbohydrate such as RS has been shown to reduce abdominal

Abbreviations: AIRg, Acute insulin response to glucose; AUC, Area under the curve; BMD, Bone mineral density; BMI, Body mass index; DI, Disposition index; DXA, Dual-energy X-ray absorptiometry; EMCL, Extramyocellular lipid; FFM, Fat-free mass; FM, Fat mass; FSIGTT, Frequently sampled intravenous glucose tolerance test; HbA1c, Hemoglobin A1c; HDL, High-density lipoprotein cholesterol; IHL, Intrahepatic lipid; IMCL, Intramyocellular lipid; RFPM, Remote food photography method; RQ, Respiratory quotient; RS, Resistant starch; SI, Insulin sensitivity index; Sg, Glucose effectiveness; SleepEE, Sleep energy expenditure; TAT, Total adipose tissue; TC, Total cholesterol; VAS, Visual analog scale; VAT, Visceral adipose tissue; ^{1}H-MRS, Proton magnetic resonance spectroscopy

* Corresponding author at: Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge 70808, United States.
E-mail address: eric.ravussin@pbrc.edu (E. Ravussin).

https://doi.org/10.1016/j.cct.2017.12.005
Received 5 July 2017; Received in revised form 11 December 2017; Accepted 20 December 2017
Available online 21 December 2017
1551-7144/

fat [2–4] and cholesterol [5], as well as increase gut bacteria [2] and improve insulin sensitivity [4,6]. The mechanism for the observed reduction in abdominal fat may be due to an increase in energy expenditure and fat oxidation [7]. In humans, RS fermentation can enrich gut microbiota species such as *Bifidobacterium*, *Ruminococcus bromii*, and *Eubacterium rectale* [8]; improve cardiometabolic endpoints such as insulin sensitivity, body fat storage, and cholesterol levels [9–14]; and may suppress appetite [15–17].

To our knowledge, few studies have investigated the impact of dietary RS supplementation on metabolic risk factors in humans. Moreover, few studies have metabolically phenotyped adults with prediabetes given the challenges associated with recruiting this population [18–19]. Detailing successful recruitment strategies and retention methods in studies of individuals with prediabetes is critical to improve future recruitment and retention of similar populations. The clinical trial entitled *Role of Resistant Starch on Diabetes Risk Factors (STARCH)* was designed to examine the effects of daily RS supplementation in adults with prediabetes on metabolic outcomes—including insulin sensitivity and secretion, ectopic fat, energy expenditure and substrate oxidation, inflammation, food intake, and gut microbiota. While the effect of daily RS supplementation on metabolic health in adults with prediabetes will be assessed in future analyses, the primary goals of the present manuscript were to: 1) to describe the recruitment and screening process of adults with prediabetes for the STARCH trial; 2) to outline the study methods and procedures; and 3) to provide a comprehensive metabolic phenotype of adults with prediabetes (pre-intervention) in cross-sectional baseline analyses of sex and race.

2. Study design & methods

2.1. Study design

The STARCH trial was designed as a randomized, double-blind, placebo-controlled, parallel-arm trial conducted at Pennington Biomedical Research Center (PBRC). Participants were recruited, screened, and randomized with a 1:1 allocation to 45 g/day of resistant starch (RS = amylose) or amylopectin (Control) for 12 weeks. Both the RS and placebo were consumed in yogurt packets that were provided to the participants. Multi-stage screening (3 clinic visits) was implemented to identify eligible participants with confirmed prediabetes. Week 0 (baseline, pre-intervention) and Week 12 (end of intervention) study visits involved 2.5 days of inpatient testing conducted within a one-week period and included: (1) dual-energy X-ray absorptiometry (DXA) to measure body composition; (2) proton magnetic resonance spectroscopy (^{1}H-MRS) to measure lipid in the liver and skeletal muscle; (3) 12-h overnight respiratory chamber to assess energy metabolism including energy expenditure and macronutrient oxidation; (4) frequently sampled intravenous glucose tolerance test (FSIGTT) to assess insulin sensitivity and insulin secretion; (5) standardized meal test to assess glucose, insulin, and incretins; (6) Visual Analog Scales (VAS) to measure appetite; (7) food intake tests and remote food photography to measure food intake; (8) gut permeability testing (sugar absorption); (9) fecal collection to measure gut microbiota, short-chain fatty acids, and RS; (10) blood markers of endotoxemia, inflammation, and hormones; and (11) breath hydrogen and methane analyses to assess microbial fermentation. The study was approved by the PBRC Institutional Review Board, and participants provided written informed consent before participating. All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2. Study population and eligibility criteria

Males and females of all races and ethnicities between 35 and 75 years of age, with a body mass index (BMI) ≥ 27 kg/m^2 and weight ≤ 143 kg, were eligible to participate. Confirmed prediabetes as assessed by either impaired fasting glucose or elevated HbA1c was required. Prior to enrollment, participants underwent detailed screening assessments, completed diet and physical activity records for a 7-day period, and were encouraged to maintain the same level of exercise and body weight throughout the study. Eligibility criteria are detailed in Table 1.

Table 1
Eligibility criteria for the STARCH trial.

Inclusion criteria
Are 35–75 years of age
Have a body mass index ≥ 27 kg/m^2, and weight ≤ 143 kg
Have pre-diabetes, as confirmed by having *either*: (1) impaired fasting glucose (IFG), i.e. fasting glucose of 100–125 mg/dL, or (2) elevated HbA1c level between 5.7 and 6.4%.
Are willing to maintain the same level of exercise throughout the trial
Exclusion criteria
Medical criteria
History or clinical manifestation of a significant medical condition
Blood pressure > 150/100 mm Hg (at screening)
Have metal objects in the body (e.g., pacemaker, metal pins, bullet)
Have clinically significant GI malabsorption, chronic diarrhea, or use antibiotics within 1 month of study
Abnormal laboratory markers (e.g., elevated potassium, low hemoglobin or hematocrit)
Psychiatric and behavioral criteria
Clinical depression or other psychological conditions
Chronically consume alcohol (> 4 servings per day) or actively smoke cigarettes (> 1/4 pack per day)
Medication criteria
Chronic use of medications (e.g., diuretics, steroids, and adrenergic-stimulating agents)
Short-term (less than a month) treatment with any other medications
Use contraceptives, oral/parenteral glucocorticoids, or meds influencing glucose or insulin within 1 month of study
Use of proton pump inhibitors
Other criteria
Breastfeeding or pregnant women, or women intending to become pregnant.
Pre-menopausal women lacking a regular menstrual cycle
Are required to perform any kind of heavy physical activity

2.3. Sample size determination

The primary intent of the present analysis was to provide a comprehensive metabolic phenotype of adults with prediabetes (pre-intervention); therefore, we provide here only overall baseline descriptive characteristics of those participants who started the intervention, as well as sex and race comparisons. No power analysis was done for these baseline descriptive comparisons. In future analyses of primary outcomes from the STARCH trial, the following sample size determination criteria were applied: the primary outcome of the STARCH trial was the change in insulin sensitivity (SI). Assuming a maximum 15% loss due to attrition, a minimum of 94 participants were targeted for enrollment and randomization. Power calculations revealed that 40 completers per group provides 85% and 95% power (two-tailed, $\alpha = 0.05$) to detect 15% and 18% improvements, respectively, in insulin sensitivity relative to the control group, assuming a within-group standard deviation of 22% [20]. Unfortunately, because of slow recruitment rates, the study was prematurely ended. Specifically, 59 participants (29 RS, 30 Control) of the total 65 participants who started the intervention actually completed the trial. These numbers provided 80% statistical power to detect a 16.3% improvement in insulin sensitivity, which is equivalent to an effect size of $d = 0.74$.

2.4. Recruitment and screening strategies

Participants were recruited by the PBRC recruitment core via media advertising (e.g. radio, online, and television ads), health promotion

events, use of databases, and referral sources between November 2012 and January 2016, with the goal of achieving 80 completers (i.e., 40 per group). We aimed to recruit an ethnically diverse group based on the demographics of Baton Rouge (i.e., approximately 30% African-Americans). From past experience, we expected to find 1 potential participant with impaired fasting glucose out of every 5 individuals screened (i.e., 20%).

During initial online or telephone screening, participants' age, height, and weight (from which body mass index (BMI) is calculated) were collected, as well as basic medical information. Volunteers who were clearly ineligible were excluded. Next, a staged screening process consisting of a variety of biologic and behavioral assessments was conducted over a series of 3 clinic visits. During screening, a standardized psychological interview focusing on barriers to adherence and retention during the intervention was also administered. Lastly, each participant had the opportunity to sample the blueberry yogurt containing the starches to determine if s/he would be willing to eat the prescribed yogurt every day for 12 weeks. At the end of the screening process, a multidisciplinary team of behavioral experts, nutritionists, and other clinical staff determined if the volunteer was suitable for inclusion in the STARCH trial. The study protocol and the timing of the procedures are detailed in Fig. 1.

2.5. Study procedures

2.5.1. Anthropometric characteristics

Metabolic weight was measured in the morning after an overnight fast (Scale Tronix 5200, Welch Allyn, Inc.; Skaneateles Falls, NY) while wearing a surgical gown, which was subtracted from total weight. Height, waist and hip circumference, resting pulse, blood pressure, and body temperature were also measured. All anthropometrics were measured at screening and bi-weekly thereafter (i.e., Weeks 0, 2, 4, 6, 8, 10, and 12).

2.5.2. Behavioral counseling

In-person behavioral counseling was provided bi-weekly by a health coach to foster protocol adherence and participant retention and to promote weight stability (≤ 1.5 kg deviation from baseline weight) during the trial. Participants were weighed at all PBRC visits and instructed to maintain, increase, or decrease food intake in order to maintain their baseline body weight. To monitor adherence to yogurt consumption, participants were required to return the lids/labels of the yogurts and empty packets that they consumed to each session and were queried about their adherence.

2.5.3. Body composition

Fat mass (FM), fat-free mass (FFM), percent body fat, visceral adipose tissue (VAT), and bone mineral density (BMD) were measured using dual X-ray absorptiometry whole-body scanner (Lunar iDXA; General Electric, Milwaukee, WI, USA). All scans were analyzed with the enCORE software version 13.60.033.

2.5.4. Ectopic fat accumulation

Lipid in the liver (i.e., intrahepatic lipid (IHL)) and skeletal muscle (i.e., soleus and anterior tibialis extramyocellular lipid (EMCL) and intramyocellular lipid (IMCL)) were measured using ^{1}H magnetic resonance spectroscopy (^{1}H-MRS) on a 3.0-Tesla whole body imaging and spectroscopy system (GE Medical Systems, Milwaukee, WI) using the Point Resolved Spectroscopy (PRESS) box technique [21]. Lipid peaks were normalized to an external oil phantom of known constant concentration [22]. Oil-adjusted IHL, EMCL, and IMCL data were analyzed using the jMRUi software package.

2.5.5. 12-h overnight respiratory chamber

Participants entered the respiratory chamber at approximately 1900 h and exited at 0700 h the following morning. Upon entering, participants were immediately fed a standardized meal prepared by the metabolic kitchen that was 30% of their resting metabolic rate (RMR) [23] multiplied by an activity factor of 1.5. At 2100 h, participants were fed a snack (20% of their RMR × 1.5 at Week 0). Macronutrient contents for both the meal and snack were 15%, 35%, and 50% from protein, fat, and carbohydrate, respectively. Participants received the same meal and snack at Week 0 and Week 12. Energy expenditure and the oxidation of carbohydrate, fat, and protein was calculated as outlined in [24]. Respiratory quotient (RQ) was measured as CO_2 production divided by O_2 consumption throughout the test and used in conjunction with urinary nitrogen excretion to calculate macronutrient oxidation rates. Sleep energy expenditure (SleepEE) was assessed between 0200 and 0500 h for those minutes where activity was less than 1% as measured by radar and was extrapolated to 24 h.

2.5.6. Frequently sampled intravenous glucose tolerance test

To determine insulin sensitivity, a frequently sampled intravenous glucose tolerance test (FSIGTT) was performed [25]. Subjects were

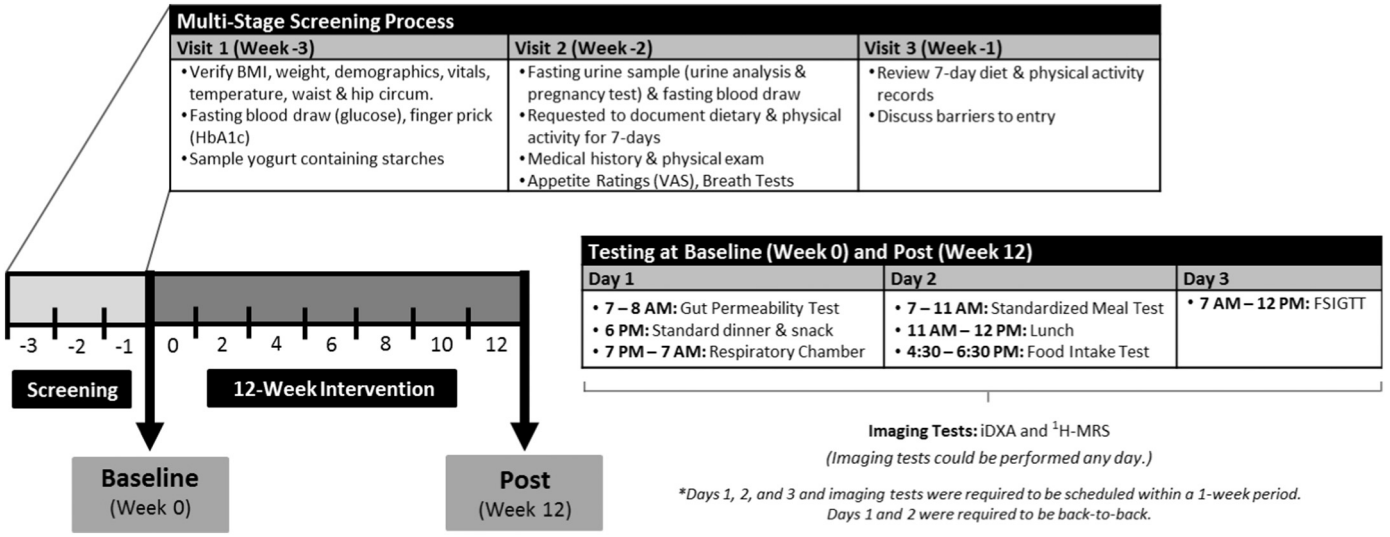

Fig. 1. Protocol and procedures.
BMI, body mass index; DXA, dual-energy X-ray absorptiometry; FSIGTT, frequently sampled intravenous glucose tolerance test; ^{1}H-MRS, proton magnetic resonance spectroscopy; HbA1c, hemoglobin A1c; VAS, visual analog scale.

studied after an overnight fast. After 30 mins of rest, two IV lines were placed. Briefly, after a baseline blood sample, glucose (300 mg/kg body weight) was injected at Time 0 (min), followed by collection of blood samples at 2, 4, 8, and 19 mins. At Time = 20 min, a bolus of insulin (0.03 U/kg body weight) was given, and frequent sampling resumed at 22, 25, 30, 40, 50, 70, 100, 120, and 180 min. Each blood sample was analyzed for glucose and insulin. The Minimal Model was used to calculate the insulin sensitivity index (SI), disposition index (DI), glucose effectiveness (Sg), and acute insulin response to glucose (AIRg) using MinMod software [26] (MINMOD-PC, ©R. Bergman).

2.5.7. Standardized meal test

Following an overnight fast, an intravenous catheter was inserted into an antecubital vein to obtain venous blood samples. During a 5-min period, subjects consumed a 400-kcal test meal (smoothie) consisting of 20% protein, 40% fat, and 40% carbohydrate under supervision. Blood was collected at −15, −5, 15, 30, 45, 60, 90, 120, 180 min to measure glucose and insulin. Additional blood was collected in tubes with protease inhibitor at −15, 30, 60, 90, 120, and 180 time points to measure Peptide YY (PYY), ghrelin (active and total), and glucagon-like peptide 1. Plasma samples were analyzed by radioimmunoassay for active and total ghrelin (Millipore, Wizard 2470 gamma counter). Glucose and insulin area under the curve (AUC), as well as the AUC ratio (insulin AUC/glucose AUC) were calculated.

2.5.8. Appetite rating and food intake testing

Self-reported appetite (hunger, fullness, satisfaction, desire to eat, and motivation to eat) were measured with Visual Analogue Scales (VAS) at screening, and bi-weekly thereafter. VAS provides reliable and valid measures of subjective states related to energy intake [27]. Participants rated their “average” appetite over the previous week, which has proven to be consistent with daily assessments of appetite but are less burdensome [28]. At Weeks 0 and 12, the Eating Inventory was used to measure dietary restraint (the intent to restrict food intake), disinhibition (the tendency to overeat), and perceived hunger [29], which are associated with eating behavior and body mass [30–31]. Higher scores indicate greater levels of the construct being measured. Participants also completed a Food Intake Test to quantify energy and macronutrient intake at Weeks 0 and 12.

2.5.9. Remote food photography

Quantification and monitoring of dietary intake using the Remote Food Photography Method© (RFPM) and SmartIntake® app was conducted at Weeks 0 and 12. The RFPM has been found to reliably and validly estimate the energy intake (kcal) of adults in free-living conditions, and the method also accurately estimates nutrient intake [32–33].

2.5.10. Gut permeability testing

Gut barrier function was assessed by a differential sugar absorption test, as described previously [34–35] but with minor modifications. A fasting urine sample was collected upon arrival. Participants then drank 30 mL of distilled water containing 5 g sucralose (Sigma-Aldrich, St Louis, MO, USA) and immediately followed by 150 mL of distilled water containing 7.5 g lactulose (Sigma-Aldrich) and 2 g mannitol (Sigma-Aldrich). Participants were allowed to drink water only for the first 5 h and were allowed to eat and drink freely thereafter. Participants were instructed to collect all urine during the next 24 h in provided containers as three separate samples (0700 to 1200 h, and 1200 to 1900 h on their own, and 1900 to 0700 h while in the respiratory chamber) and to keep them refrigerated until the samples were returned to the center. Concentrations of sucralose, lactulose and mannitol in the urine were determined using HPLC. The ratio of urinary excretion rates of sucralose/mannitol over 24 h indicates whole-gut permeability, while that of lactulose/mannitol over 0 to 5 h indicates small intestinal permeability.

2.5.11. Fecal collection & bacterial diversity

Fecal samples were collected to measure the amount and types of bacteria in the gut. If a participant did not have at least 2 bowel movements during Week 0 and 12 visits, the participant was instructed to collect his/her next stool sample at home, freeze it, and bring back to the center in an insulated container that was provided. The stools were homogenized and prepared for 16S rRNA gene amplicon sequencing as previously described that included DNA extraction from stool samples [36].

2.5.12. Other blood markers

Blood markers of endotoxemia and inflammation, as well as hunger hormones were analyzed. Specifically, plasma lipopolysaccharide (LPS), IL-6, tumor necrosis factor alpha (TNF-α), high-sensitivity CRP (hs-CRP), high molecular weight (HMW) adiponectin, and leptin were assayed. IL-6 and TNF-α were measured using immunoassay with fluorescent detection (Millipore, Luminex), while hs-CRP was measured using immunoassay with chemiluminescent detection (Siemens, Immulite 2000). HMW adiponectin and leptin were assayed using ELISA methodology (Millipore, Bio Rad Microplate reader) and radioimmunoassay (Millipore, Wizard 2470 gamma counter), respectively.

2.5.13. Breath hydrogen and methane

Bi-weekly breath tests were conducted for the purposes of assessing gut fermentation byproducts (hydrogen and methane). Participants fasted for at least 12-h prior to testing, and alveolar air samples were collected after participants exhaled through a mouthpiece connected to a dual-bag system by a three-way valve. Breath samples were analyzed for hydrogen and methane by gas chromatography (MicroLyzer Model SC, Quintron Instrument Co., Inc., Milwaukee, WI).

2.6. Statistical analysis

All analyses were performed using SAS version 9.4 software (SAS Institute, Inc.). Means with 95% confidence intervals were performed on all participants (n = 63) to test for describe differences between males and females at baseline (Tables 2 and 3). *P*-values are additionally provided as an alternative way to evaluate the analyses. Race means are expressed only for a dichotomous sample (n = 59) of African-Americans and Caucasians since only four participants identified as Asian/Asian Indian or other/bi-racial (Tables 4 and 5). Sex and race means and confidence intervals in carbohydrate, fat, and protein oxidation are based on the least-squares means of the linear model with SleepEE as a covariate to adjust for differences in metabolic body size (Tables 2 and 4). Linear models were also used to show sex means and confidence intervals in SleepEE with FFM, FM, age, and race as covariates. Additional adjustment for metabolic characteristics of FSIGTT (e.g., AIRg, DI, SI, and Sg) or standardized meal test (e.g., glucose AUC, insulin AUC, and AUC ratio) was further applied (individually, one-by-one) to separately run models. Race means and confidence intervals in SleepEE adjusting for FFM, FM, age, and sex were also assessed, using similar additional adjustments for metabolic characteristics derived from FSIGTT and standardized meal test. Sex and race means in FSIGTT and/or standardized meal test variables were also assessed following adjustment for age, race (or sex), and VAT/FM ratio. All covariates in models were chosen a prior and are the standard method for adjusting glucose and energy metabolism characteristics [37]. Accounting for VAT/FM ratio is important when examining differences between races [38].

3. Results

3.1. Screening, enrollment, and retention

A total of 2863 individuals screened online or by telephone, of which a total of 1770 individuals were contacted by our recruiting core

Table 2
Baseline characteristics of STARCH participants by sex[a].

	Everyone (N = 63)		Male (N = 21)		Female (N = 42)		*P*-value
	Mean	[95% CI]	Mean	[95% CI]	Mean	[95% CI]	
Anthropometrics							
Age (y)	55	[52, 57]	56	[51, 60]	54	[51, 57]	0.45
Race (*n*, C/AA/Other)	27/33/3		11/9/1		16/23/3		0.31
Weight (kg)	100.7	[97.2, 104.1]	104.3	[98.8, 109.8]	98.9	[94.4, 103.4]	0.15
BMI (kg/m^2)	35.6	[34.4, 36.8]	34.0	[32.6, 35.3]	36.4	[34.8, 38.0]	0.02
Waist circumference (cm)	110.7	[108.0, 113.4]	113.5	[109.1, 117.9]	109.3	[105.9, 112.8]	0.14
Cardiometabolic risk factors							
Fasting glucose (mg/dL)	106	[103, 108]	105	[100, 110]	106	[102, 109]	0.62
Fasting insulin (μU/mL)	21.7	[19.2, 24.2]	20.7	[16.9, 24.6]	22.2	[18.8, 25.5]	0.59
HOMA-IR	5.77	[5.01, 6.53]	5.45	[4.28, 6.61]	5.93	[4.92, 6.94]	0.55
HbA1c (%)	5.7	[5.6, 5.8]	5.7	[5.6, 5.8]	5.7	[5.6, 5.8]	0.75
Total cholesterol (mg/dL)	188	[179, 196]	187	[170, 203]	189	[178, 199]	0.84
HDL (mg/dL)	52	[49, 55]	45	[41, 49]	55	[52, 59]	< 0.001
LDL (mg/dL)	114	[107, 121]	114	[102, 127]	114	[105, 123]	0.95
Triglycerides (mg/dL)	109	[91, 128]	132	[85, 178]	98	[82, 114]	0.16
TC/HDL ratio	3.8	[3.5, 4.0]	4.3	[3.8, 4.8]	3.5	[3.3, 3.8]	< 0.01
Body composition							
Body fat (%)	43.6	[41.8, 45.4]	35.6	[33.1, 38.1]	47.6	[46.5, 48.7]	< 0.001
Fat mass (kg)	44.0	[41.4, 46.6]	37.4	[33.3, 41.5]	47.3	[44.5, 50.2]	< 0.001
Lean mass (kg)	53.7	[51.4, 56.0]	63.3	[60.6, 66.0]	49.0	[47.0, 50.9]	< 0.001
VAT mass (kg)	2.0	[1.8, 2.3]	2.7	[2.2, 3.3]	1.7	[1.4, 1.9]	< 0.001
VAT/TAT mass	0.05	[0.04, 0.05]	0.07	[0.06, 0.08]	0.04	[0.03, 0.04]	< 0.001
BMD (g/cm^2)	1.26	[1.23, 1.29]	1.32	[1.27, 1.37]	1.23	[1.20, 1.27]	0.01
Ectopic fat							
IHL (%)	6.8	[4.5, 9.1]	5.6	[2.6, 8.6]	7.4	[4.2, 10.5]	0.40
Soleus EMCL (%)	3.1	[2.1, 4.1]	3.4	[1.7, 5.2]	2.9	[1.7, 4.1]	0.61
Soleus IMCL (%)	1.4	[0.8, 2.0]	1.6	[1.0, 2.2]	1.3	[0.4, 2.2]	0.63
Tibialis anterior EMCL (%)	3.4	[2.6, 4.3]	2.8	[1.4, 4.2]	3.7	[2.6, 4.8]	0.34
Tibialis anterior IMCL (%)	0.6	[0.4, 0.8]	0.7	[0.3, 1.1]	0.5	[0.3, 0.7]	0.30
Breath testing[b]							
Breath hydrogen (ppm)	9.0	[6.3, 11.8]	8.4	[4.1, 12.8]	9.4	[5.8, 12.9]	0.75
Breath methane (ppm)	7.8	[3.6, 12.0]	9.0	[1.4, 16.5]	7.2	[1.9, 12.5]	0.70
12-h respiratory chamber[b]							
SleepEE (kcal/d)	1630	[1553, 1706]	1853	[1747, 1959]	1515	[1431, 1600]	< 0.001
Sleep RQ	0.83	[0.82, 0.85]	0.83	[0.81, 0.86]	0.83	[0.82, 0.85]	0.98
npRQ	0.82	[0.81, 0.84]	0.81	[0.78, 0.84]	0.83	[0.81, 0.84]	0.37
CHO oxidation (g/d)[c]	143.6	[125.6, 161.7]	133.3	[98.2, 168.3]	148.9	[125.1, 172.7]	0.49
Fat oxidation (g/d)[c]	96.6	[87.1, 106.2]	94.3	[78.0, 110.6]	97.8	[86.7, 108.9]	0.74
Protein oxidation (g/d)[c]	99.9	[90.7, 109.1]	112.3	[98.1, 126.6]	93.5	[83.9, 103.2]	0.05
FSIGTT[b]							
AIRg (mU/L/min)	732	[479, 985]	679	[458, 899]	760	[384, 1136]	0.70
DI (S_I × AIRg)	1066	[788, 1345]	987	[653, 1321]	1108	[711, 1505]	0.64
SI (mU/L/min)	1.97	[1.58, 2.36]	1.86	[1.23, 2.48]	2.03	[1.51, 2.55]	0.68
Sg (min^{-1})	0.02	[0.01, 0.02]	0.02	[0.01, 0.02]	0.02	[0.01, 0.02]	0.49
Basal glucose (mg/dL)	94	[91, 96]	95	[89, 100]	93	[90, 96]	0.65
Standard meal test[b]							
Glucose AUC (mg/dL × hr)	310.3	[299.6, 321.0]	309.6	[287.5, 331.8]	310.6	[298.4, 322.8]	0.93
Insulin AUC (μU/mL × hr)	164.5	[146.4, 182.7]	154.9	[130.5, 179.2]	169.6	[144.5, 194.7]	0.44
AUC ratio	0.53	[0.47, 0.60]	0.50	[0.42, 0.58]	0.55	[0.46, 0.64]	0.39

AA, African-American; AIRg, acute insulin response to glucose; AUC, area under the curve; BMD, bone mineral density; BMI, body mass index; BP, blood pressure; C, Caucasian; CHO, carbohydrate; DI, disposition index; EE, energy expenditure; EMCL, extramyocellular lipid; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; LDL, low-density lipoprotein cholesterol; npRQ, non-protein respiratory quotient; RQ, respiratory quotient; SI, insulin sensitivity index; Sg, glucose effectiveness; TAT, total adipose tissue; TC, total cholesterol; VAT, visceral adipose tissue.

[a] Values are raw means with 95% confidence intervals.

[b] Data from the 12-h respiratory chamber and breath testing was available for n = 62, and data from FSIGTT and Standard Meal Test data was available for n = 61.

[c] Data for male and female participants are adjusted for SleepEE and values are least-squares means with 95% confidence intervals.

for further telephone screening. Of the 1770 individuals who completed telephone screening, 280 (16%) were eligible and remained interested in participating in the trial (Fig. 2). 280 volunteers began in-clinic screening, of whom 212 (76%) were ineligible. Approximately 50% of those who were rejected did not fit the diagnostic criteria for pre-diabetes, with others excluded for taking exclusionary medications or other exclusion criteria (e.g., high blood pressure). 68 individuals were randomized, and 65 started the intervention (i.e. initiated baseline evaluation), as shown in the CONSORT diagram (Fig. 2). Of the 65 participants who started the intervention, 2 participants were removed from the baseline analyses: one participant displayed abnormally high insulin levels (perhaps indicative of a congenital condition), and another was diabetic yet was mistakenly enrolled. Baseline data (Week 0) from 63 participants (21 males, 42 females) aged 55 y [95% CI: 52, 57] and BMI 35.6 kg/m^2 [95% CI: 34.4, 36.8] were available and therefore analyzed. Of the 63 participants analyzed, there were 32 African-Americans, 27 Caucasians, 1 Asian-Asian Indian, and 3 identified as other/bi-racial. Of the 63 participants enrolled, 9 (6 males, 3 females)

Table 3
Baseline eating behavior and ratings of all STARCH participants by sex[a].

	Everyone (N = 63)		Male (N = 21)		Female (N = 42)		*P*-value
	Mean	[95% CI]	Mean	[95% CI]	Mean	[95% CI]	
Eating inventory							
Cognitive restraint	9.11	[8.00, 10.22]	7.62	[5.93, 9.31]	9.86	[8.43, 11.29]	0.06
Disinhibition	7.78	[6.79, 8.77]	6.29	[4.79, 7.78]	8.52	[7.26, 9.78]	0.03
Hunger	5.21	[4.39, 6.03]	4.76	[3.12, 6.40]	5.43	[4.47, 6.39]	0.45
VAS							
Feelings of hunger	40	[35, 45]	45	[33, 56]	38	[32, 43]	0.26
Feelings of fullness	63	[57, 68]	58	[45, 71]	65	[59, 71]	0.33
Desire to eat	58	[52, 63]	62	[52, 73]	55	[48, 62]	0.25
Perceived amount of food able to eat	63	[58, 68]	66	[58, 74]	61	[55, 68]	0.37
Feelings of satisfaction	60	[55, 65]	56	[47, 65]	62	[56, 68]	0.30
Food intake test							
Fat intake (kcal)	250	[227, 274]	313	[265, 361]	219	[197, 242]	< 0.001
Carbohydrate intake (kcal)	399	[364, 435]	483	[409, 557]	357	[323, 392]	< 0.01
Protein intake (kcal)	157	[145, 169]	191	[173, 210]	140	[128, 153]	< 0.001
Total intake (kcal)	795	[729, 862]	973	[840, 1106]	707	[645, 768]	< 0.001
Fat consumed (%)	31.0	[30.1, 32.0]	31.8	[30.4, 33.3]	30.6	[29.4, 31.9]	0.20
Carbohydrate consumed (%)	50.1	[48.8, 51.4]	49.2	[47.3, 51.1]	50.6	[48.8, 52.3]	0.28
Protein consumed (%)	20.3	[19.3, 21.3]	20.4	[18.8, 22.0]	20.2	[18.9, 21.6]	0.85

VAS, visual analog scale.
[a] Values are raw means with 95% confidence intervals.

were on stable statins or cholesterol-lowering medications.

3.2. Anthropometrics, cardiometabolic risk factors, body composition, and ectopic fat

Baseline sex differences are presented in Table 2. Enrolled females had higher BMI and high-density lipoprotein (HDL) cholesterol, and lower total cholesterol TC/HDL ratio compared to enrolled males. As expected, females had higher percent body fat, as well as lower VAT/TAT mass and BMD. No differences in fasting glucose, fasting insulin, HbA1c, ectopic fat (liver and muscle), or breath hydrogen or methane at baseline were observed between males and females.

With regards to race (n = 59 because 4 participants reported other races), Caucasians were older and had higher fasting glucose, VAT mass, VAT/TAT mass, and IHL compared to African-Americans (Table 4). Conversely, African-Americans had higher HbA1c and BMD compared to Caucasians. No differences in BMI, HOMA-IR, fasting lipids, percent body fat, intramyocellular or extramyocellular lipid, or breath hydrogen or methane were observed between the races.

3.3. FSIGTT and standardized meal test

Data from two (2) participants were deemed unusable at Week 0 due to poor model fit as a result of missing data or were non-physiologic; therefore, only FSIGTT and standardized meal test data on 61 subjects were analyzed. No differences in mean insulin sensitivity or insulin secretion at baseline were observed between males and females (Table 2). Specifically, no differences in AIRg, DI, SI, Sg, or basal glucose via FSIGTT, or glucose AUC, insulin AUC, or AUC ratio via the standard meal test, were observed between males and females. However, African-Americans had higher AIRg than Caucasians (Table 4).

Because of the known impact of age and body shape on carbohydrate metabolism, we confirmed the absence of differences between males and females for SI (p = 0.29) or AIRg (p = 0.74) after adjusting for age, race, and VAT/FM; however, females trended towards having a lower DI compared to males (799 vs. 1720, [−1907.7, 65.9]; p = 0.07). Additionally, an interaction effect between race and sex was observed for insulin AUC via the standardized meal test. Specifically, African-American females had significantly higher insulin AUC compared to African-American males (208 vs. 136 μU/mL × hr, [4.8, 149.7]; p = 0.04), as well as trend towards higher insulin AUC compared to Caucasian females (208 vs. 162 μU/mL × hr, [−4.0, 98.3]; p = 0.07). In addition, Caucasian females tended to have significantly higher insulin AUC compared to Caucasian males (162 vs. 91 μU/mL × hr, [−10.7, 160.9]; p = 0.09).

Similarly, African-American females also trended towards having higher AUC ratios, indicative of increased insulin resistance, compared to African-American males (0.66 vs. 0.43, [0.0, 0.5]; p = 0.06), as well as compared to Caucasian females (0.66 vs. 0.50, [0.0, 0.3]; p = 0.07).

3.4. Respiratory chamber

Data from one participant was deemed unusable due to environmental concerns (e.g., humidity, temperature) at Week 0 that resulted in a non-physiological (low) RQ value; therefore, only respiratory chamber data on 62 subjects were analyzed. SleepEE was significantly lower in females at baseline compared to males. Protein oxidation, adjusted for SleepEE, was also significantly lower in females at baseline compared to males (Table 2). No race differences in respiratory chamber characteristics, including carbohydrate, fat, and protein oxidation adjusted for SleepEE, were observed (Table 4). In further analyses, SleepEE was significantly lower in females (~270 kcal/day) compared to males after adjusting for FFM, FM, age, and race (1533 vs. 1804 kcal/day, [−451.1, −90.4]; p = 0.004). Similarly, after additional adjustments for carbohydrate metabolism characteristics determined by FSIGTT (i.e., AIRg, DI, SI, or Sg) or by the standardized meal (i.e. glucose AUC, insulin AUC, or AUC ratio), SleepEE was still significantly lower in females (~260 kcal/day) compared to males (all models: p < 0.05). Furthermore, African-Americans had significantly lower SleepEE (~100 kcal/day) compared to Caucasians after adjusting for FFM, FM, age, and sex (1619 vs. 1718 kcal/day, [−185.1, −13.1]; p = 0.02) and even after further adjustments for FSIGTT outcomes (i.e., AIRg, DI, SI, or Sg; ~90 kcal/day; p < 0.05) or mixed meal outcomes (glucose AUC, insulin AUC, and AUC ratio; ~100 kcal/day; p < 0.05).

3.5. Eating behavior & ratings

Baseline appetite and food intake measurements are displayed in Table 3 and Table 5. Females reported significantly higher levels of disinhibition (i.e., reported a greater tendency to overeat) compared to males and trended towards having higher dietary restraint (i.e., reported intent to restrict food intake) compared to males. No differences

Table 4
Baseline characteristics of STARCH participants by race[a].

	Everyone (N = 59)		Caucasian (N = 27)		African-American (N = 32)		*P*-value
	Mean	[95% CI]	Mean	[95% CI]	Mean	[95% CI]	
Anthropometrics							
Age (y)	55	[52, 57]	57	[53, 61]	52	[49, 55]	0.048
Gender (*n*, male/female)	20/39		11/16		9/23		0.31
Weight (kg)	100.1	[96.5, 103.6]	98.7	[93.5, 104.0]	101.2	[96.2, 106.2]	0.49
BMI (kg/m^2)	35.4	[34.2, 36.6]	34.7	[33.0, 36.4]	36.0	[34.3, 37.7]	0.28
Waist circumference (cm)	110.4	[107.7, 113.1]	111.0	[107.0, 115.1]	109.8	[106.0, 113.6]	0.66
Cardiometabolic risk factors							
Fasting glucose (mg/dL)	104	[102, 107]	107	[103, 111]	102	[99, 105]	0.048
Fasting insulin (μU/mL)	20.9	[18.4, 23.4]	22.1	[18.3, 25.9]	19.9	[16.5, 23.3]	0.38
HOMA-IR	5.49	[4.78, 6.20]	5.94	[4.85, 7.02]	5.11	[4.14, 6.08]	0.25
HbA1c (%)	5.7	[5.6, 5.7]	5.6	[5.5, 5.7]	5.7	[5.7, 5.8]	0.01
Total cholesterol (mg/dL)	189	[180, 198]	188	[175, 201]	189	[176, 202]	0.86
HDL (mg/dL)	52	[50, 55]	51	[47, 55]	54	[50, 58]	0.32
LDL (mg/dL)	114	[106, 122]	111	[100, 123]	116	[105, 127]	0.57
Triglycerides (mg/dL)	109	[90, 128]	126	[98, 154]	95	[67, 122]	0.10
TC/HDL ratio	3.7	[3.5, 4.0]	3.8	[3.5, 4.2]	3.7	[3.3, 4.1]	0.56
Body composition							
Body fat (%)	43.4	[41.6, 45.3]	42.5	[39.5, 45.5]	44.2	[41.7, 46.7]	0.37
Fat mass (kg)	43.6	[41, 46.2]	42	[38, 46]	45	[41, 49]	0.26
Lean mass (kg)	53.6	[51.2, 56.0]	54.0	[49.9, 58.0]	53.3	[50.1, 56.4]	0.78
VAT mass (kg)	2.0	[1.7, 2.3]	2.4	[2.0, 2.8]	1.6	[1.3, 1.9]	0.002
VAT/TAT mass	0.05	[0.04, 0.05]	0.06	[0.05, 0.07]	0.04	[0.03, 0.04]	< 0.001
BMD (g/cm^2)	1.26	[1.23, 1.30]	1.22	[1.17, 1.27]	1.30	[1.26, 1.34]	0.02
Ectopic fat							
IHL (%)	6.6	[4.3, 8.9]	11.7	[7.4, 16.0]	2.25	[1.5, 3.1]	< 0.001
Soleus EMCL (%)	3.1	[2.1, 4.2]	3.3	[1.3, 5.4]	3.0	[2.0, 4.0]	0.73
Soleus IMCL (%)	1.4	[0.8, 2.1]	1.7	[0.3, 3.1]	1.2	[0.8, 1.6]	0.52
Tibialis anterior EMCL (%)	3.5	[2.6, 4.5]	3.6	[2.1, 5.1]	3.5	[2.3, 4.7]	0.93
Tibialis anterior IMCL (%)	0.6	[0.4, 0.8]	0.7	[0.4, 1.0]	0.5	[0.2, 0.8]	0.38
Breath testing[b]							
Breath hydrogen (ppm)	8.7	[5.9, 11.4]	6.7	[3.9, 9.5]	10.4	[5.7, 15.0]	0.17
Breath methane (ppm)	8.0	[3.5, 12.4]	6.5	[0.3, 12.6]	9.3	[2.5, 16.0]	0.54
12-h respiratory chamber[b]							
SleepEE (kcal/d)	1623	[1543, 1703]	1659	[1530, 1788]	1592	[1487, 1696]	0.41
Sleep RQ	0.83	[0.82, 0.85]	0.84	[0.82, 0.86]	0.83	[0.81, 0.85]	0.28
npRQ	0.82	[0.81, 0.84]	0.83	[0.81, 0.85]	0.82	[0.79, 0.84]	0.45
CHO oxidation (g/d)[c]	143.0	[123.8, 162.3]	153.2	[124.9, 181.5]	134.2	[107.8, 160.6]	0.33
Fat oxidation (g/d)[c]	96.2	[86.2, 106.2]	92.6	[79.6, 105.6]	99.3	[87.2, 111.4]	0.46
Protein oxidation (g/d)[c]	98.6	[89.1, 108.0]	96.9	[85.2, 108.6]	100.0	[89.1, 111]	0.70
FSIGTT[b]							
AIRg (mU/L/min)	762	[492, 1031]	506	[358, 653]	992	[498, 1485]	0.06
DI (S_I × AIRg)	1122	[830, 1415]	975	[567, 1383]	1255	[821, 1689]	0.34
SI (mU/L/min)	2.04	[1.63, 2.45]	2.22	[1.51, 2.93]	1.87	[1.38, 2.36]	0.40
Sg (min^{-1})	0.02	[0.01, 0.02]	0.02	[0.01, 0.02]	0.02	[0.01, 0.02]	0.98
Basal glucose (mg/dL)	93	[90, 95]	94	[90, 98]	91	[88, 94]	0.21
Standard meal test[b]							
Glucose AUC (mg/dL × hr)	307.4	[296.7, 318.1]	315.9	[298.9, 332.9]	300.4	[286.4, 314.3]	0.15
Insulin AUC (μU/mL × hr)	161.8	[142.9, 180.8]	151.1	[135.2, 167.0]	170.9	[138.0, 203.7]	0.27
AUC ratio	0.53	[0.46, 0.60]	0.48	[0.43, 0.53]	0.57	[0.46, 0.68]	0.16

AIRg, acute insulin response to glucose; AUC, area under the curve; BMD, bone mineral density; BMI, body mass index; BP, blood pressure; CHO, carbohydrate; DI, disposition index; EE, energy expenditure; EMCL, extramyocellular lipid; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; LDL, low-density lipoprotein cholesterol; npRQ, non-protein respiratory quotient; RQ, respiratory quotient; SI, insulin sensitivity index; Sg, glucose effectiveness; TAT, total adipose tissue; TC, total cholesterol; VAT, visceral adipose tissue.

[a] Values are raw means with 95% confidence intervals.

[b] Data from the 12-h respiratory chamber and breath testing was available for n = 58, and data from FSIGTT and Standard Meal Test data was available for n = 57.

[c] Data for African-American and Caucasian participants are adjusted for SleepEE and values are least-squares means with 95% confidence intervals.

in perceived hunger between females and males were present. Over the previous week, ratings of hunger, fullness, desire to eat, prospective food consumption, and satisfaction were also not different between females and males. Additionally, Caucasian females and males consumed similar percentages of fat, carbohydrate, and protein during the Food Intake Test, although males consumed significantly more calories for fat, carbohydrate, protein, and the total amount eaten compared to females (Table 3).

There were no differences between African-Americans and Caucasians for any of the eating behavior and appetite ratings, as well as food intake measures. Specifically, no differences in Eating Inventory behaviors (dietary restraint, disinhibition, or perceived hunger) or ratings of hunger, fullness, desire to eat, prospective food consumption, or satisfaction via VAS over the previous week were observed between African-Americans and Caucasians. Similarly, no differences between food intake measures were observed (Table 5).

Table 5
Baseline eating behavior and ratings of all STARCH participants by race[a].

	Everyone (N = 59)		Caucasian (N = 27)		African-American (N = 32)		*P*-value
	Mean	[95% CI]	Mean	[95% CI]	Mean	[95% CI]	
Eating inventory							
Cognitive restraint	9.19	[8.01, 10.37]	9.89	[8.47, 11.31]	8.59	[6.73, 10.45]	0.28
Disinhibition	7.88	[6.86, 8.90]	8.30	[6.50, 10.10]	7.53	[6.34, 8.72]	0.46
Hunger	5.29	[4.44, 6.14]	5.00	[3.57, 6.43]	5.53	[4.46, 6.60]	0.54
VAS							
Feelings of hunger	39	[34, 45]	41	[33, 49]	38	[30, 45]	0.59
Feelings of fullness	62	[56, 68]	64	[58, 71]	60	[50, 70]	0.43
Desire to eat	57	[51, 63]	53	[44, 63]	61	[53, 68]	0.22
Perceived amount of food able to eat	63	[58, 68]	60	[51, 68]	66	[59, 73]	0.24
Feelings of satisfaction	59	[54, 65]	58	[51, 64]	61	[53, 69]	0.52
Food intake test							
Fat intake (kcal)	252	[227, 278]	244	[197, 290]	260	[231, 288]	0.55
Carbohydrate intake (kcal)	401	[362, 439]	385	[318, 451]	414	[368, 460]	0.45
Protein intake (kcal)	157	[144, 169]	152	[131, 173]	161	[145, 177]	0.40
Total intake (kcal)	798	[727, 869]	769	[643, 896]	822	[741, 903]	0.46
Fat consumed (%)	31.1	[30.1, 32.2]	30.8	[29.0, 32.6]	31.5	[30.3, 32.6]	0.50
Carbohydrate consumed (%)	50.1	[48.7, 51.5]	50.1	[48.1, 52.1]	50.1	[48.0, 52.1]	0.97
Protein consumed (%)	20.2	[19.1, 21.3]	20.5	[18.8, 22.1]	20.0	[18.5, 21.4]	0.66

VAS, visual analog scale.
[a] Values are raw means with 95% confidence intervals.

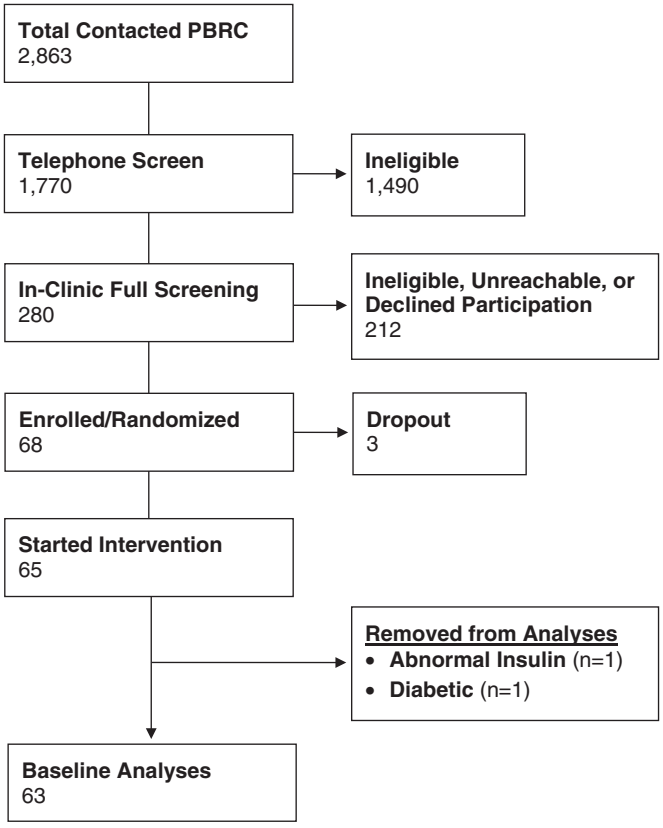

Fig. 2. CONSORT diagram.

4. Discussion

To our knowledge, this is the first trial to conduct comprehensive metabolic phenotyping of adults with prediabetes, as well as to identify potential sex and racial differences in metabolism in those at-risk for diabetes. As expected, females had a higher mean BMI, percent body fat, fat mass, HDL cholesterol, yet had lower TC/HDL ratio, lean mass, visceral adiposity, BMD, sleep energy expenditure, and protein oxidation compared to males with prediabetes. Similar to previously published literature [38–40], African-Americans had higher acute insulin response to glucose compared to Caucasians, despite having less visceral adiposity and intrahepatic lipid. Indeed, visceral adiposity is an important determinant of insulin sensitivity particularly when examining racial differences [38]. Even after adjusting for visceral adiposity, however, females (notably African-American females) tended to have higher insulin AUC and AUC ratio compared to other groups. An extensive meta-analysis by Kodama et al. [39] revealed that individuals of African descent often have higher acute insulin response yet lower levels of insulin sensitivity compared to Caucasians who have lower acute insulin response for variable degrees of insulin sensitivity. In contrast to this observation, African-American and Caucasian participants in our trial did not exhibit differences in their relationship between acute insulin response and insulin sensitivity by FSIGTT. Interestingly, we did observe differences in sleep energy expenditure. Sleep energy expenditure was ~90–100 kcal/day greater in Caucasians compared to African-Americans, after accounting for differences in age, sex, fat mass, lean mass and respective degree of insulin sensitivity via FSIGTT and mixed meal test results.

An important goal of the present manuscript is also to detail our extensive recruitment and screening strategies used to successfully enroll individuals with prediabetes. Recruiting adults with prediabetes is challenging. For this reason, reporting our extensive recruitment and enrollment strategies, as well as our methods for obtaining excellent retention following study initiation will prove valuable to others planning to design and implement trials in populations with prediabetes. Despite successful recruitment and retention, several challenges arose throughout the STARCH trial. First, the majority (~50%) of individuals who completed the required screening visits but who were rejected were ineligible because they were not diagnosed with prediabetes. Indeed, the majority of adults with prediabetes do not know they have prediabetes [18]. Our past experience with enrolling people with prediabetes in PBRC studies yielded 1 participant with prediabetes out of every 5 screened individuals; therefore, we anticipated that at least 50 (20%) of an estimated target of 250 screened subjects would be eligible. While recruiting at local health fairs or healthcare education events that offered free blood glucose screening was a useful recruitment tool to stimulate enrollment, the most successful recruitment tools were sustained media coverage, including radio, online, and television ads, as well as repeatedly launched listserv

email blasts advertising the STARCH trial. We found that newspaper advertising was not useful. Additionally, we expanded our recruitment reach by modifying the inclusion criteria for age and BMI partway through trial recruitment. Specifically, an age range of 35–65 years was expanded to include individuals up to 75 years and an initial BMI range of 30 to 44.9 kg/m^2 was later expanded to include subjects with BMI $\geq$ 27 kg/m^2. We also relaxed our required 3 "consecutive" days at baseline and Week 12 testing to be only 2 consecutive days plus an additional day to be completed within a single week time frame to accommodate working professionals. As a result of our extensive and flexible recruitment strategies, 68 subjects (24%) were eligible out of 280 subjects screened.

Trial adherence and retention were equally as important to ensure trial success. Our strategies to maximize protocol adherence and retention with bi-weekly, in-person behavioral counseling sessions and weighing to ensure weight remained steady throughout the trial were also effective. All 63 participants (100%) who completed baseline testing (Week 0) were retained throughout the entirety of the trial, quite an achievement for such a demanding protocol.

Despite the inevitable recruitment challenges that arise with enrolling people with prediabetes into such a demanding clinical trial, our comprehensive team of researchers and highly trained support staff developed recruitment, adherence and retention methods that resulted in the successful enrollment of participants with prediabetes into the present STARCH trial. The design and conduct of future clinical trials in individuals with prediabetes would benefit from our findings. Future reporting of the STARCH trial will detail the impact of our 12-week dietary intervention (resistance starch supplementation) on metabolic risk factors for the development of type 2 diabetes.

Funding

This research is funded by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (R01DK092575 to Ravussin). CMP was supported by grant KL2TR001419 from the National Center for Advancing Translational Sciences. This work is supported by the Louisiana Clinical and Translational Science Center (LA CaTS) Center Pilot Grant Project (U54 GM104940) and a NORC Center Grant (P30DK072476).

Conflict of interest

Louisiana State University and Pennington Biomedical Research Center have an interest in the intellectual property surrounding the SmartIntake app and Remote Food Photography Method and Corby Martin is an inventor of the technology. Michael Keenan has also received research funding from Ingredion Incorporated and gifts of their products for use in his future research. None of the other authors reported a conflict of interest related to the study.

Acknowledgements

KLM (interpreted the data, wrote paper); UAW (interpreted the data, wrote paper); RAB (analyzed data, performed statistical analysis); CMP (conducted research, wrote paper); CKM (designed the study, conducted research, wrote paper); MLM (designed the study, wrote paper); MJK (designed the study, wrote paper); RJM (designed the study, wrote paper); KJA (designed the study, wrote paper); ER (designed the study, conducted research, wrote paper, has primary responsibility for final content). All authors approved the final manuscript before submission.

References

[1] C. Lara-Castro, W.T. Garvey, Intracellular lipid accumulation in liver and muscle and the insulin resistance syndrome, Endocrinol. Metab. Clin. N. Am. 37 (4) (2008) 841–856.
[2] M.J. Keenan, M. Janes, J. Robert, R.J. Martin, A.M. Raggio, K.L. McCutcheon, et al., Resistant starch from high amylose maize (HAM-RS2) reduces body fat and increases gut bacteria in ovariectomized (OVX) rats, Obesity (Silver Spring) 21 (5) (2013) 981–984.
[3] L. Shen, M.J. Keenan, R.J. Martin, R.T. Tulley, A.M. Raggio, K.L. McCutcheon, et al., Dietary resistant starch increases hypothalamic POMC expression in rats, Obesity (Silver Spring) 17 (1) (2009) 40–45.
[4] P.W. So, W.S. Yu, Y.T. Kuo, C. Wasserfall, A.P. Goldstone, J.D. Bell, et al., Impact of resistant starch on body fat patterning and central appetite regulation, PLoS One 2 (12) (2007) e1309.
[5] E.A. de Deckere, W.J. Kloots, J.M. van Amelsvoort, Resistant starch decreases serum total cholesterol and triacylglycerol concentrations in rats, J. Nutr. 123 (12) (1993) 2142–2151.
[6] L. Shen, M.J. Keenan, A. Raggio, C. Williams, R.J. Martin, Dietary-resistant starch improves maternal glycemic control in Goto-Kakazaki rat, Mol. Nutr. Food Res. 55 (2011) 1499–1508.
[7] J. Zhou, R.J. Martin, R.T. Tulley, A.M. Raggio, L. Shen, E. Lissy, et al., Failure to ferment dietary resistant starch in specific mouse models of obesity results in no body fat loss, J. Agric. Food Chem. 57 (2009) 8844–8851.
[8] I. Martinez, J. Kim, P.R. Duffy, V.L. Schlegel, J. Walter, Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects, PLoS One 5 (11) (2010) e15046.
[9] K.C. Maki, C.L. Pelkman, E.T. Finocchiaro, K.M. Kelley, A.L. Lawless, A.L. Schild, et al., Resistant starch from high-amylose maize insulin sensitivity in overweight and obese men, J. Nutr. 142 (2012) 717–723.
[10] B.A. Gower, R. Bergman, D. Stefanovski, B. Darnell, F. Ovalle, G. Fisher, et al., Baseline insulin sensitivity affects response to high-amylose maize resistant starch in women: a randomized, controlled trial, Nutr. Metab. (Lond.) 13 (2) (2016) (eCollection 2016).
[11] I.L. Brown, Applications and uses of resistant starch, J. AOAC Int. 87 (3) (2004) 727–732.
[12] J.A. Higgins, Resistant starch: metabolic effects and potential health benefits, J. AOAC Int. 87 (3) (2004) 761–768.
[13] M.D. Robertson, A.S. Bickerton, A.L. Dennis, H. Vidal, K.N. Frayn, Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism, Am. J. Clin. Nutr. 82 (3) (2005) 559–567.
[14] M.D. Robertson, J.M. Currie, L.M. Morgan, D.P. Jewell, K.N. Frayn, Prior short-term consumption of resistant starch enhances postprandial insulin sensitivity in healthy subjects, Diabetologia 46 (5) (2003) 659–665.
[15] R.A. Samra, G.H. Anderson, Insoluble cereal fiber reduces appetite and short-term food intake and glycemic response to food consumed 75 min later by healthy men, Am. J. Clin. Nutr. 86 (4) (2007) 972–979.
[16] C.L. Bodinham, G.S. Frost, M.D. Robertson, Acute ingestion of resistant starch reduces food intake in healthy adults, Br. J. Nutr. 103 (6) (2010) 917–922.
[17] H.J. Willis, A.L. Eldridge, J. Beiseigel, W. Thomas, J.L. Slavin, Greater satiety response with resistant starch and corn bran in human subjects, Nutr. Res. 29 (2) (2009) 100–105.
[18] A. Menke, S. Casagrande, L. Geiss, C.C. Cowie, Prevalence of and trends in diabetes among adults in the United States, 1988–2012, JAMA 314 (10) (2015) 1021–1029.
[19] R.R. Rubin, W.Y. Fujimoto, D.G. Marrero, T. Brenneman, J.B. Charleston, S.L. Edelstein, et al., DPP Research Group, The diabetes prevention program: recruitment methods and results, Control. Clin. Trials 23 (2) (2002) 157–171.
[20] K.L. Johnston, E.L. Thomas, J.D. Bell, G.S. Frost, M.D. Robertson, Resistant starch improves insulin sensitivity in metabolic syndrome, Diabet. Med. 27 (4) (2010) 391–397.
[21] D.E. Larson-Meyer, B.R. Newcomer, G.R. Hunter, Influence of endurance running and recovery diet on intramyocellular lipid content in women: a 1H NMR study, Am. J. Physiol. Endocrinol. Metab. 282 (1) (2002) E95–E106.
[22] G. Perseghin, P. Scifo, F. De Cobelli, et al., Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents, Diabetes 48 (1999) 1600–1606.
[23] M.D. Mifflin, S.T. St Jeor, L.A. Hill, B.J. Scott, S.A. Daugherty, Y.O. Koh, A new predictive equation for resting energy expenditure in healthy individuals, Am. J. Clin. Nutr. 51 (2) (1990) 241–247.
[24] E. Jéquier, K. Acheson, Y. Schutz, Assessment of energy expenditure and fuel utilization in man, Annu. Rev. Nutr. 7 (1987) 187–208.
[25] S. Welch, S.S. Gebhart, R.N. Bergman, L.S. Phillips, Minimal model analysis of intravenous glucose tolerance test-derived insulin sensitivity in diabetic subjects, J. Clin. Endocrinol. Metab. 71 (6) (1990) 1508–1518.
[26] R.N. Bergman, Y.Z. Ider, C.R. Bowden, C. Cobelli, Quantitative estimation of insulin sensitivity, Am. J. Phys. 623 (6) (1979) E667–77.
[27] A. Flint, A. Raben, J.E. Blundell, A. Astrup, Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies, Int. J. Obes. Relat. Metab. Disord. 124 (2000) 38–48.
[28] L.G. Womble, T.A. Wadden, J.M. Chandler, A.R. Martin, Agreement between weekly vs. daily assessment of appetite, Appetite 40 (2) (2003) 131–135.
[29] A.J. Stunkard, S. Messick, Eating Inventory Manual (The Psychological Corporation), Harcourt Brace & Company, San Antonio, TX, 1988.
[30] O.J. Lawson, D.A. Williamson, C.M. Champagne, J.P. DeLany, E.R. Brooks, P.M. Howat, et al., The association of body weight, dietary intake, and energy expenditure with dietary restraint and disinhibition, Obes. Res. 3 (2) (1995) 153–161.
[31] C.F. Smith, P.J. Geiselman, D.A. Williamson, C.M. Champagne, G.A. Bray, D.H. Ryan, Association of dietary restraint and disinhibition with eating behavior, body mass, and hunger, Eat. Weight Disord. 3 (1) (1998) 7–15.

[32] C.K. Martin, H. Han, S.M. Coulon, H.R. Allen, C.M. Champagne, S.D. Anton, A novel method to remotely measure food intake of free-living individuals in real time: the remote food photography method, Br. J. Nutr. 101 (2009) 446–456.
[33] C.K. Martin, J.B. Correa, H. Han, H.R. Allen, J.C. Rood, C.M. Champagne, et al., Validity of the Remote Food Photography Method (RFPM) for estimating energy and nutrient intake in near real-time, Obesity 20 (2012) 891–899.
[34] A. Farhadi, A. Keshavarzian, E.W. Holmes, J. Fields, L. Zhang, A. Banan, Gas chromatographic method for detection of urinary sucralose: application to the assessment of intestinal permeability, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 784 (1) (2003) 145–154.
[35] A.D. Anderson, P.K. Jain, S. Fleming, P. Poon, C.J. Mitchell, J. MacFie, Evaluation of a triple sugar test of colonic permeability in humans, Acta Physiol. Scand. 182 (2) (2004) 171–177.
[36] Y.H. Hsieh, C.M. Peterson, A. Raggio, M.J. Keenan, R.J. Martin, E. Ravussin, M.L. Marco, Impact of different fecal processing methods on assessments of bacterial diversity in the human intestine, Front. Microbiol. 7 (2016) 1643 (eCollection 2016).
[37] Y.Y. Lam, L.M. Redman, S.R. Smith, G.A. Bray, F.L. Greenway, D.L. Johannsen, E. Ravussin, Determinants of sedentary 24-h energy expenditure: equations for energy prescription and adjustment in a respiratory chamber, Am. J. Clin. Nutr. 99 (4) (2014) 834–842.
[38] M.A. Banerji, R.L. Chaiken, D. Gordon, J.G. Kral, H.E. Lebovitz, Does infra-abdominal adipose tissue in black men determine whether NIDDM is insulin-resistant or insulin-sensitive? Diabetes 44 (1995) 141–146.
[39] K. Kodama, D. Tojjar, S. Yamada, K. Toda, C.J. Patel, A.J. Butte, Ethnic differences in the relationship between insulin sensitivity and insulin response: a systematic review and meta-analysis, Diabetes Care 36 (2013) 1789–1796.
[40] K.C. Chiu, N.P. Lee, P. Cohan, L.-M. Chuang, Insulin sensitivity differs among ethnic groups with a compensatory response in β-cell function, Diabetes Care 23 (2000) 1353–1358.