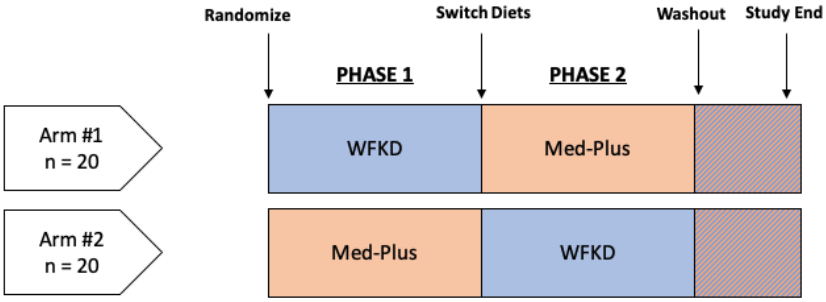
Contrasting Ketogenic and Mediterranean Diets in Individuals with Type 2 Diabetes and Prediabetes: The Keto-Med Trial

NCT #: 03810378

Date: 12/18/20

Statistical Analysis Plan for The Keto-Med Trial

Contrasting Ketogenic and Mediterranean Diets in Individuals With Type 2 Diabetes and Prediabetes



	Weeks																
	-4	-2	0	2	4	6	8	10	12	14	16	18	20	22	24	34	36	48
Blood Draw	x		x			x			x			x			x		x	
Health Education Classes			x		x	x		x	x		x	x		x				
Stool Collection	x		x			x			x			x			x		x	
Continuous Glucose Monitoring*		x	x		x	x		x	x		x	x		x	x	x	x	
Diet Assessment			x			x			x			x			x		x	x
Study Questionnaires			x			x			x			x			x		x	x

** (CGM) - to be worn for a two-week interval at each time as indicated by the thick border*

Background

The proposed randomized clinical trial will investigate differential population-specific effects of two low-carbohydrate (low-carb) diet patterns, addressing a gap in the evidence base in this area that will lead to 1) improved treatment strategies for common adverse clinical conditions, 2) improved health for these individuals, and 3) long-term decreases in health care costs. This impactful research will advance the field of personalized and precision medicine.

A Mediterranean Diet (Med-Plus), as defined in this study, maximizes the intake of vegetables, legumes, fruits and nuts, whole intact grains/cereals and fish; and minimizes the intake of meat, poultry, and dairy. This diet should exclude added sugars and refined grains.

A Well-Formulated Ketogenic Diet (WFKD) diet, as defined in this study, maximizes the intake of non-processed beef, pork, and poultry (preferably organic/grass-fed), fish, heavy cream, low-lactose, high-fat cheeses, animal fats, oils (avocado, coconut, or other nut oils), non-starchy (above ground) vegetables and limited amounts of some fruits (berries). This diet should exclude legumes, grains, sugars, starchy (below ground) vegetables, most fruits, and polyunsaturated oils (soy, sunflower, peanut, cottonseed, canola, etc.). Furthermore, this diet will aim for an intake of 20 g of carbohydrates/day at start, with the goal to have no more than 50 grams/day to maintain ketosis.

The objective of the current study is to contrast the potential beneficial and adverse health consequences of two metabolically distinct diets: Well-Formulated Ketogenic Diet (WFKD) vs Mediterranean Diet (Med-Plus), in a crossover design among generally healthy adults.

Study Design:

This cross-over study aims to investigate the impact of two metabolically distinct diets on Glycated hemoglobin, cardiovascular health, the gut microbiome, and metabolic status. Participants are recruited using media advertisements, email lists, and clinician referral (Dr. Kim) from previous recruitment for nutrition studies conducted by our laboratory group. Forty participants are planned to be randomized between the two arms: (1) start with 12 week WFKD and switch to 12 week Med-Plus; (2) start with 12 week Med-Plus and switch to 12 week WFKD. Randomization will be stratified by diabetes vs pre-diabetes status. As displayed in figure above, clinical and metabolic measurements are taken at baseline, and weeks 0, 6, 12, (Phase I, weeks 1-12), 18, 24 (Phase II, weeks 13-24) and 36 (Maintenance Phase). Continuous glucose monitoring will be collected five times during the trial, each time data will be collected every hour for ten days.

Primary Outcome:

- Difference in percent difference from baseline after 12 weeks of each phase

Secondary Outcomes:

- Difference in percent difference from baseline in LDL cholesterol after 12 weeks of each phase
- Difference in percent difference from baseline in HDL cholesterol after 12 weeks of each phase
- Difference in percent difference from baseline in triglycerides after 12 weeks of each phase
- Difference in percent difference from baseline in fasting insulin after 12 weeks of each phase
- Difference in percent difference from baseline in blood pressure after 12 weeks of each phase
- Difference in percent difference from baseline in weight after 12 weeks of each phase
- Difference in percent difference from baseline in alpha diversity after 12 weeks of each phase. Primary metric of alpha diversity will be using number of observed sequence variants ("species") determined by standard 16S rRNA amplicon sequencing (V3-V5 region followed by DADA2 to define error-corrected sequence variants). *Note: Higher alpha diversity is better. The units are the # of sequence variants*
- Difference in percent difference from baseline in composite of short-chain fatty acids (SCFA) concentration (ug/g stool: acetate + propionate + butyrate) after 12 weeks of each phase

Exploratory outcomes:

- Difference in satisfaction with WFKD and Med-Plus diets, using a 5-point Likert scale (1=not at all satisfied; 2=slightly satisfied; 3=moderately satisfied; 4=very satisfied; 5=extremely satisfied).
- Difference in adherence to diet protocols by diabetes vs. pre-diabetes status, according to 3-day food records.
- Tabulate COVID-19 Survey answers

Primary Analysis

Patient demographics and baseline clinical characteristics will be summarized by arm, as n (%) and median (interquartile range) for categorical and continuous variables, respectively. To assess any differences in each variable between the two arms, the absolute standardized differences are presented, where a value of 0.2 or less, 0.5, and 0.8 or more correspond to small, medium, and large differences, respectively. Characteristics found to display large differences will be adjusted for in a sensitivity analysis. We will also compare rate of adherence for WFKD after 12 weeks and Med-Plus diets after 12 weeks using a Fisher's Exact test with weekly NDSR (and Cronometer) data. We will develop a composite score to measure a participant's adherence to each diet.

We will use a mixed-effects linear model (1) with HbA1c percent difference from baseline for diet, *j*, as the outcome. We want to assess for a difference in diet (WFKD vs Med-Plus), while adjusting for fixed effects: order (e.g. study arm), pre-diabetes vs. diabetes status, and a random effect for each participant, *i*.

$$\left(\frac{HbA1c_{ij} - HbA1c_{i0}}{HbA1c_{i0}}\right) = \beta_o + \beta_1 Order_i + \beta_2 Diabetes_i + \beta_3 Diet_{ij} + \gamma_i \qquad (1)$$

For the primary outcome, we will use a two-sided likelihood ratio test to test for no difference between diet types. The primary analysis will include participants with baseline blood draws, and at least one blood draw in each phase (including crossing over). The primary analysis will be a complete case analysis and use participants' last available lab values in each phase or week 12, if available; patients who did not complete both phases (i.e. crossover) will be excluded. A significance level of 0.05 is set for all analyses. In a sensitivity analysis, we will adjust for baseline weight and percent weight change if weight change from baseline is found to be significant, since this is known to impact HbA1c values; and in a separate analysis, we will adjust for adherence to each diet.

Similarly, for our secondary outcomes, we will use separate mixed-effects models to evaluate fasting lipids, insulin, glucose, blood pressure and short-chain fatty acids for WFKD vs Med-Plus, adjusting for order, phase, pre-diabetes vs. diabetes status, and repeated measures. A two-sided likelihood ratio test will be used to assess no difference between diet types. For our exploratory outcome comparing diet satisfaction, we will use a Wilcoxon signed-rank test to evaluate the null hypothesis that the difference between pairs follows a symmetric distribution around zero, which indicates similar satisfaction with both diets. We will use a Fisher's Exact test to evaluate any difference in the rate of adherence due to diabetes vs pre-diabetes status, within each diet.

Sensitivity Analysis

Due to COVID-19, visits to collect blood draws were delayed for some participants. These participants report they maintained the phase's diet until the lab measurements could be collected. We will check adherence to diet based on NDSR at Week 12 and Week 12 extra visit (taken when allowed back into clinic), and also check for a change in dietary and exercise habits

during shelter-in-place. Potential impact of delays are unclear; subsequently, we will perform a number of sensitivity analyses:

Sensitivity Analysis 1 (SA1): For a simple truncated analysis, we will consider only the first phase (12 weeks) of each group and compare differences (two-sample)

Sensitivity Analysis 2 (SA2): For a simple estimate of Week 12 analysis, we will consider the average of the last lab measurement of each 12 week phase and the extra data point at Week 12 extra visit.

Sensitivity Analysis 3 (SA3): For an extended-phase analysis, we will consider using the last lab measurement from each phase, i.e. the "extra" data timepoints after week 12 in study for some participants.

Sensitivity Analysis 4 (SA4): For a pre-COVID-19 truncated analysis, we will consider the subset of data prior to the official Shelter-in-Place date for the Bay Area, March 16, 2020.

Sensitivity Analysis 5 (SA5): To assess any effect on outcomes due to COVID-19 impact on logistics, we will consider the subset of data when participants received groceries for each diet. We will also evaluate if adherence was better for the first four weeks when groceries were delivered.

Secondary Analysis

For our secondary analysis, we want to investigate: (a) a potential difference in the primary outcome by diabetes status; (b) the different trajectories of each diet; and (c) different metrics using continuous glucose monitoring data and how that compares with the gold standard HbA1c data. In a low-power analysis for (a), we will use model (1) and include an interaction term for diet and diabetes status. For (b), we will use all time points in a model similar to (1) but with a time variable. Lastly for our analysis in (c), we will consider different metrics used in the literature for continuous glucose monitoring and compare trajectories of each metric with that of the gold standard using HbA1c data.

Exploratory Analysis

For our exploratory analysis, we want to investigate long term maintenance. Upon completing both diet phases, for three months participants were instructed to eat the diet of their choosing. This analysis will be largely descriptive on what participants were consuming and their change in lab measurements.