Study Title: SWAP-MEAT: Study with Appetizing Plant Food - Meat Eating Alternatives Trial

NCT #: 03718988

Document date: 12/23/19

Statistical Analysis Plan for SWAP-MEAT: Study with Appetizing Plant Food – Meat Eating Alternative Trial

Background:

Many national and global health organizations recommend limiting or reducing intake of red meat (e.g., American Heart Association, Dietary Guidelines for Americans, American Cancer Society, World Health Organization). As is always the case, reducing intake of any habitually consumed food item involves additional concomitant changes. If red meat is reduced without replacement, daily calorie intake decreases. If red meat is reduced and replaced with other food items, the health implications involve not only the components of red meat that decrease, but also the components of the replacement food item(s) that increase.

Red meat is a source of saturated fat and protein in the diet, as well as several micronutrients (e.g., choline, iron and vitamins B6 and B12); there is no fiber in meats. A replacement for red meat that has recently become more broadly available and increasingly acceptable from both a palatability and a public interest perspective is alternative meat (Alt-Meat). One of those Alt-Meats is made by the Beyond Meat company. The Beyond Meat analogues are made from pea protein and other ingredients, including coconut fat and sodium.

In comparison to red meat, plausible relative health ***benefits*** could include:

Lower tri-methyl amine oxide (TMAO) due to lower amounts of choline

Lower insulin-like growth factor (IGF-1) due to lower amounts of animal foods

Lower LDL-cholesterol levels due to modestly lower amounts of saturated fat and higher amounts of fiber.

In comparison to red meat, plausible relative health ***risks*** could include:

Higher blood pressure due to higher amounts of sodium

Higher intake of calories (and potential subsequent weight and waist circumference) due to the ultraprocessed composition of the Beyond Meat products

Another possible benefit of Alt-Meat products involves environmental issues such as greenhouse gas emissions (GHG) or water usage. These will not be addressed in the current study.

The objective of the current study is to contrast the potential beneficial and adverse health consequences of Alt-Meat vs. Red Meat products after consuming at least 2 servings/day (approximately 25% of kcal) for 8 weeks, in a crossover design, among generally healthy adults.

Study Design:

This cross-over study aims to investigate the impact of replacing meat consumption with plant-based meat alternative consumption on cardiovascular health, the gut microbiome, and metabolic status. Participants are recruited using media advertisements and email lists from previous recruitment for nutrition studies conducted by our laboratory group. Thirty-eight participants are planned to be randomized between the two arms: (1) start with 8 week Red Meat product and switch to 8 week Alt-Meat product; (2) start with 8 week Alt-Meat product and switch to 8 week Red Meat product. Clinical and metabolic measurements are taken at baseline, and weeks 2, 4, 8 (Phase I, weeks 1-8), 10, 12, and 16 (Phase II, weeks 9-16).

Primary Hypothesis: TMAO values will be lower for Alt-Meat vs. Red Meat

Our primary hypothesis is based on the fact that there is higher choline and carnitine in red meat, which are precursors to TMAO. We acknowledge that the sample size used was not based on a power calculation beforehand, but rather on available resources. We have performed an ad-hoc power analysis for a paired t-test, which assumes no effect of our covariates for the primary analysis described below. With 38 patients, the trial has 80% power to detect a -2.5 mean difference in TMAO values for Alt-Meat compared with Red Meat, assuming a standard deviation of 5.4 [1] at a 5% significance level (i.e., modest effect size of ~0.5).

Primary Outcome:
Difference in TMAO values for Alt-Meat after 8 weeks versus Red Meat after 8 weeks

Secondary Outcomes:
Difference in IGF1 values for Alt-Meat after 8 weeks versus Red Meat after 8 weeks
Hypothesis 2a: IGF1 values will be lower for Alt-Meat vs. Red Meat (rationale – IGF1 reported to be lower in vegetarians than non-vegetarians)

Difference in LDL cholesterol values for Alt-Meat after 8 weeks versus Red Meat after 8 weeks
Hypothesis 2b: LDL-C values will be lower for Alt-Meat vs. Red Meat (rationale, Alt-meat has slightly less saturated fat, and more fiber, relative to Red Meat)

Difference in blood pressure (SBP and DBP) values for Alt-Meat after 8 weeks versus Red Meat after 8 weeks
Hypothesis 2c: BP values will be higher for Alt-Meat vs. Red Meat (rationale - Alt-Meat has moderately higher sodium content than Red Meat)

Difference in fasting insulin, fasting glucose, fasting non-LDL lipids (HDL-C, triglycerides) values for Alt-Meat after 8 weeks versus Red Meat after 8 weeks
Note: No specific hypotheses for these; including as part of a set of standard clinical measures.

Exploratory Outcome:
We will compare outcomes after 8 weeks on Alt-Meat and after 8-weeks on Red Meat:

No difference expected: satisfaction consuming products (Likert scale), amount of products consumed (ave servings/day), GI symptoms (Survey), Perceived stress (PSS-10), Perceived cognitive function, fatigue and overall health (PROMIS), and perceived well-being (WHO well-being index) [2 – 5]

Expected to improve with Alt-Meat: Microbiota composition (16S)

Expected to worsen with Alt-Meat: body weight, waist circumference (rationale – due to ultraprocessed composition of the Beyond Meat products)

Primary analysis:

Patient demographics and baseline clinical characteristics will be summarized and compared between the arms with appropriate statistical methods. We will also compare rate of adherence for Alt-Meat after 8 weeks versus Red Meat after 8 weeks using a Fisher's Exact test with weekly NDSR (and Cronometer) data. A participant is determined to be adherent if the participant consumed an average of at least 2 servings per day of each product over the 8 week diet.

Characteristics found to be statistically different will be adjusted for in the sensitivity analysis described below. For our primary outcome, we will use linear regression to evaluate TMAO values for Alt-Meat versus Red Meat ('meat type'), adjusting for baseline values and the order of diet (e.g. study arm). The primary analysis will be a complete case analysis and use patients' last available lab values in each phase; patients who did not complete both phases (i.e. crossover) will be excluded from the primary analysis and will be accounted for in the exploratory analysis. To investigate no difference in TMAO values between diet types we will use a two-sided Wald test. We set a significance level of 0.05 for all analyses.

Similarly for our secondary outcomes, we will use separate linear regression models to evaluate IGF1, LDL cholesterol, systolic blood pressure, diastolic blood pressure, fasting insulin, fasting glucose, and fasting non-LDL lipids (HDL-C, triglycerides) for Alt-Meat versus Red Meat, adjusting for baseline values and the order of diet. A two-sided Wald test will be used to assess no difference between diet types.

For our exploratory outcomes, we will use separate linear regression models to evaluate microbiota composition, weight change, change in waist circumference, satisfaction consuming products, amount of products consumed, GI symptoms, perceived stress, perceived cognitive perceived function, perceived fatigue, perceived overall health, and perceived well-being for Alt-Meat versus Red Meat, adjusting for baseline values and the order of diet. Our primary microbiota composition metric will be the proportion of observed sequence variants (or species) as measured by standard 16S rRNA amplicon sequencing.

In an exploratory analysis, we will fit mixed effect regression models with TMAO values as the outcome, using all longitudinal data by incorporating an additional 'time' variable and a random effect for the correlated observations from a single participant. We will also investigate the regression models described above with additional covariates to account for differential intake of foods *other than the meat products*. In this analysis, we will adjust for the average number of calories, grams of fiber, grams of added sugars and grams of saturated fat, over each 8 week diet.

Sensitivity Analysis:

Given the limited sample size we have elected to limit the number of variable adjustments in our primary analysis. In a sensitivity analysis, we will assess the impact of including additional potentially important covariates in our models. Using the previously described models, we will assess the sensitivity of our results for each individual addition of the following covariates: gender, age, baseline weight, weight change, household (couple versus single), type of meat-product (red meat versus white meat), and dose. For dose, we will measure the proportion of calorie intake that can be attributed to the assigned meat type.

REFERENCES:

[1] Wang, Zeneng, Nathalie Bergeron, Bruce S. Levison, Xinmin S. Li, Sally Chiu, Xun Jia, Robert A. Koeth et al. "Impact of chronic dietary red meat, white meat, or non-meat protein on trimethylamine N-oxide metabolism and renal excretion in healthy men and women." *European heart journal* 40, no. 7 (2018): 583-594.

[2] Revicki, Dennis A., Martha Wood, Ingela Wiklund, and Joseph Crawley. "Reliability and validity of the Gastrointestinal Symptom Rating Scale in patients with gastroesophageal reflux disease." *Quality of life research* 7, no. 1 (1997): 75-83.

[3] Cohen, Sheldon, Tom Kamarck, and Robin Mermelstein. "A global measure of perceived stress." *Journal of health and social behavior* (1983): 385-396.

[4] Cella, David, William Riley, Arthur Stone, Nan Rothrock, Bryce Reeve, Susan Yount, Dagmar Amtmann et al. "The Patient-Reported Outcomes Measurement Information System (PROMIS) developed and tested its first wave of adult self-reported health outcome item banks: 2005–2008." *Journal of clinical epidemiology* 63, no. 11 (2010): 1179-1194.

[5] Topp, Christian Winther, Søren Dinesen Østergaard, Susan Søndergaard, and Per Bech. "The WHO-5 Well-Being Index: a systematic review of the literature." *Psychotherapy and psychosomatics* 84, no. 3 (2015): 167-176.

Database Management Plan: SWAP MEAT Study

Stanford Quantitative Sciences Unit (QSU)

Automated data checks to be done at data entry (to be verified by Stanford QSU) prior to unblinding:

- All date values restricted to certain ranges, and all dates entered that are outside of the designated range will be flagged
- Restrictions put in place for anthropomorphic values, such as height and weight, and clinical values, such as blood pressure and insulin, in order to ensure that they are valid
- Calculations directly programmed so that fields such as BMI are automatically computed to prevent errors
- Whenever possible, dropdown menus and radio buttons are provided
- Prompts to require important fields be filled (for example, to determine inclusion/exclusion criteria) or alerts to verify that a missing field is intentionally not completed
- Branching logic properly set up to conditionally display a field based on the selection of a previous response
- Each subject can be linked across data sources (Redcap, NDSR, etc.)

Quality checks implemented after data is collected (to be performed by Stanford QSU) prior to unblinding:

- Multiple data sources will be merged into Redcap for a central database
- After a sample is entered, completeness of fields will be checked
    - % of fields completed
    - relevant fields completed
- Check for common inconsistencies in data including missing values, missing expected visits, values that are out of range, hidden fields that contain values, and incorrect data type.
- For numerical data, data will be reviewed for possible outliers
    - Visual plots (scatterplots, boxplots) will be generated
    - Cross-sectional outliers
        - outliers > +/- 1.5 IQR beyond q1/q3
        - extreme outliers +/- 3 IQR beyond q1/q3
    - Longitudinal outliers (change scores between each pair of time points)
        - outliers > +/- 1.5 IQR beyond q1/q3
        - extreme outliers +/- 3 IQR beyond q1/q3
    - All outliers will be flagged and reviewed
- Check for concordance between measurements within a particular subject for consistency (e.g. consecutive waist measurements with weight change)

Possible methods for troubleshooting:

- For data collected longitudinally, are patterns similar across time points?
- Possibility that data could have been entered incorrectly (pounds instead of kg, feet instead of inches)

We will maintain a record of all data quality checks in a study repository including dates of when QC was performed and completed.