NCT05724906

Red and Processed Meat Effects on the Metabolome and Microbiome

(Participant facing study title: EAT-WELL: Effects on metabolism of Altering Protein Sources in WELL balanced meals)

Protocol Document. Version 3, dated 1.28.22 (final)

Clinical Research Protocol

EAT-WELL: Effects on metabolism of AlTering protein sources in WELL balanced meals

Protocol Number:	RG1121543
Version Number:	3
Version Date:	January 28, 2022
Development Phase:	NA
Indication	Healthy Eating Index Diet (2015) ± red and processed meats, healthy adult volunteers
Study sites	Fred Hutchinson Cancer Research Center
Funding Organization:	National Institute of Diabetes, Digestive, and Kidney Diseases, 1 R21 DK128754-01
Principal Investigator:	Marian L. Neuhouser, PhD

FHCRC IRB Approval
02/17/2022
Document Released Date

TABLE OF CONTENTS

TITLE	EAT-WELL: Effects on metabolism of AlTering protein sources in WELL balanced meals
SPONSOR	1 R21 DK128754-01
FUNDING ORGANIZATION	National Institute of Diabetes, Digestive, and Kidney Diseases
NUMBER OF SITES	1: Fred Hutchinson Cancer Research Center
RATIONALE	Observational cohorts have found that those who self-report frequent red and processed meat intake have higher risk of several diet-related chronic diseases such as cardiovascular disease, type II diabetes and cancer compared to those with low or no intake of these foods. These findings are valuable, but self-reported diet without biological markers does not advance science towards understanding biological mechanisms. Some cohort studies have reported links between red and processed meat consumption and health related biomarkers such as lipids or food preparation-generated biomarkers such as polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HAs). However, many of these studies examined the associations of red and processed meat with blood or urine biomarkers in cross-sectional analyses, leaving the possibility for residual confounding and an inability to draw causal inference. Further, often only one pathway or one compound was measured in these studies, limiting insight into multiple pathways affected by red and processed meat consumption. And no studies have tested red and processed meat on systems-wide biomarkers using the metabolome and microbiome.
STUDY DESIGN	This controlled feeding trial will enroll 20 heathy adult females and males. Participants will consume two diets, in random order: 1) Diet A is based on the Healthy Eating Index-2015 (HEI-2015) and includes no red or processed meat; 2) Diet B is based on the Healthy Eating Index-2015 and includes red and processed meat (HEI-2015-M) as some of the protein sources. Both diet periods last 21 days and an approximately 21-day washout period occurs between diets.
PRIMARY OBJECTIVE	The study has the following primary **specific aim**: 1. Identify the extent and nature of shifts in the metabolome when consuming a standard HEI-2015 diet compared to a HEI-2015-M diet.
SECONDARY OBJECTIVES	The study has the following **secondary aim:** 1. Identify and characterize modulation of the gut microbial community when consuming a standard HEI-2015 diet compared to a HEI-2015-M diet.
NUMBER OF SUBJECTS	20
SUBJECT SELECTION CRITERIA: Inclusion Criteria	Inclusion Criteria: 1. Healthy adults 2. Age 18-50 years 3. Able to read, speak and understand English 4. Willing to come to the Fred Hutch campus twice weekly during the study
SUBJECT SELECTION CRITERIA: Exclusion Criteria	Exclusion Criteria: 1. Known allergy to red or processed meat 2. Vegetarian or vegan 3. Religious or personal reason(s) to avoid red or processed meat 4. Pregnant and/or exclusively breastfeeding 5. Alcohol or recreational drug abuse

DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will participate in the study for 11 weeks. The total duration of the study is expected to be 1.5 years
PRIMARY ENPOINTS	**Primary Aim 1:** Compare intervention effect of each feeding period on shifts in the metabolome as detected in blood and urine samples relative to baseline for each participant.
SECONDARY ENDPOINTS	**Secondary Aim:** Compare intervention effect of each feeding period on shifts in the gut microbial community as detected by 16S rRNA gene sequencing and digital droplet PCR relative to baseline for each participant.
STATISTICS **Primary Analysis Plan**	**Statistical analysis for primary Aim 1 (metabolome).** Aim 1 identifies metabolomic markers that differ between HEI-2015 and HEI-2015-M. Metabolites from NMR and LC/MS-MS platforms will be combined to allow greater coverage of the metabolome. Metabolite levels are normalized by dividing by the batch's median value followed by removing metabolites with >25% CVs in the embedded blinded duplicates as well as those with >20% missing values (expected at 5-10% of total). Next, we use unsupervised principal components analysis (PCA) to visually examine the data patterns and variability. We then apply supervised orthogonal partial least squares discriminant analysis (OPLS-DA) to identify candidate biomarkers that may differ by diet arm. VIP (variable importance in projection) scores >2 will be retained. For each identified metabolite, separate linear mixed models are fit to examine fold-changes in end of diet period metabolite levels by HEI-2015 and HEI-2015-M, adjusting for baseline. In the cross-over design each participant is their own control, minimizing noise in intra-individual variation in endogenous metabolites. The cross-over design also minimizes confounding by age, BMI, and diet order, but models are run with and without these adjustments. The Benjamini-Hochberg approach controls for false discovery rate (FDR) <0.10.
Rationale for Number of Subjects	We have set a sample size of 20 and each participant will complete both arms for this pilot study, which will provide preliminary data to support an R01. The sample size is based on the number of participants needed to observe an intervention effect while staying within the fixed budget of an R21.

1. BACKGROUND

Scientific premise and importance of the problem. The significance of this application is that mechanistic evidence by which red and processed meat affects overall systemic human metabolism is lacking. In 2019, a series of systematic reviews and meta analyses published by an *ad hoc* group[21] concluded that, contrary to US[2] and global[10, 11] dietary guidance, red and processed meat could be consumed with no harm to human health.[16-19, 21] In contrast, evidence from well-conducted observational cohorts finds that red and processed meat consumption is associated with higher risk of cardiovascular disease, type 2 diabetes, certain cancers and total mortality.[7, 26-30] The authors of the 2019 reports based some of their conclusions on the fact that US dietary guidelines rely primarily on observational cohort data instead of randomized controlled trials, a study design they viewed as 'weak'.[21] Further, the authors cited a lack of robust data connecting red and processed meat consumption to biological mechanisms linked to health outcomes. The observational data juxtaposed with the *ad hoc* group's recommendations that red and processed meat may be consumed with no ill effects, indicate that knowledge and evidence gaps remain.[31] These gaps need to be filled to inform diet-related health promotion and disease prevention recommendations. This application does not diminish observational findings of red and processed meat and disease outcomes; rather the intent is to add to the body of evidence as the totality of evidence is needed to generate robust population dietary guidance.

The problem of understanding the biological mechanisms by which red and processed meat affect human health will be explored in this project in a randomized cross-over feeding trial testing a Healthy Eating Index-2015 (HEI-2015) diet with no red and processed meat compared to a HEI-2015 diet with red and processed meat (HEI-2015-M). A controlled feeding trial is a study design optimally suited for investigating mechanisms linking food intake with metabolic response due to the ability to carefully control food intake.[32, 33] Two types of mechanistic biomarkers are outcomes for this study, both reflecting systems-wide readouts: (1) metabolomics; and (2) gut microbiota. The 2020-2030 NIH Strategic Plan for Nutrition Research[34] calls for research into food effects on both the metabolome and the microbiome, making this project a timely fit.[34, 35]

Biological effects of red and processed meat intake. Red meat is mammalian, "red pigmented flesh or organ meats including beef, pork, venison and game meats".[36] Processed meats are "any meats that have undergone salting, curing, fermentation and other methods of preservation and may include red meat, poultry and fish".[36] Red and processed meat are often grouped together as a single exposure in many investigations[29, 37, 38] and thus will be examined together in this study. A key question is: do red and processed meat generate a different metabolic and gut microbial community response than fish, poultry or plant-based proteins (e.g., nuts, beans)? Red meat has important nutrients including essential amino acids, iron, zinc and vitamin B_{12}.[39] Both red and processed meat are also high in nutrients that should be consumed in moderation such as saturated fat and sodium.[1, 38] Other red and processed meat-derived non-nutrient compounds linked to health risks include advanced glycation-end products,[40, 41] nitrosamines[42-44] and potential endocrine disruptors derived from livestock feed or from that used in processed meat preservation and packaging.[45, 46] Red meat cooking methods may also induce the formation of compounds linked to poor health including heterocyclic amines (HAs)[47, 48] such as amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhiP) [24, 49] and polycyclic aromatic hydrocarbons (PAHs).[3, 48] The evidence for HAs and PAHs supported the International Agency for Research on Cancer (IARC) declarations that red and processed meats are Group 2A and Group 1 carcinogens, respectively.[15] The presence of these cooking-derived compounds are not observed with fresh poultry and fish, but are seen in all types of processed meat, including that derived from poultry and fish.[15]

Prior interventions and controlled feeding studies testing red and processed meat. Recent interventions, including controlled feeding studies, demonstrated that red meat can be a component of a healthy diet with neutral effects or slight improvements in blood lipids, glucose tolerance and cardiometabolic risk factors.[50-52] These studies were rigorous and well-conducted, but were focused on specific health-related biomarkers, not whole-body mechanisms, leaving a gap in identifying systems-wide mechanisms. Other feeding studies identified blood and urine-based metabolomics and microbiome features that differed between lean seafood and non-seafood diets, but did not specifically test red and processed meat.[53, 54] In still another, there was a rapid microbiome change in response to 5-day controlled diets where both a plant-based diet and an animal foods-based diet were administered.[55] The plant-based diet induced bacterial diversity for key genera overall[56, 57] as well as increases in bacterial genes involved in plant polysaccharide decomposition, such as butyrate kinase (*buk*). In the animal foods-based diet bacteria involved in proteolysis and nitrogen metabolism increased, and overall bacterial diversity decreased.[55] These results support the premise that diet change and specific food components induce rapid shifts in gut microbiota.[55] However, still lacking are controlled trials explicitly testing red and processed meat while all other aspects of diet are held constant.

Rationale for metabolome and microbiome as endpoints. The 2020-2030 NIH Strategic Plan for Nutrition Research[34] calls for mechanistic studies on the relationship between foods and both the microbiome and the metabolome to allow a systems approach to nutrition research, a strategy recognized as valuable by the NIH and nutrition research community.[32, 33] Both the metabolome and microbiome are sensitive to short-term changes in diet, as shown by us and others.[53-55, 58-62] Further, both outcomes are needed for a full complement of systems-wide nutritional effects. Evidence suggests that dietary patterns,[63-68] individual foods, nutrients or food groups[62, 69-76] may display a unique metabolome or microbiome signature, including for meat and fish consumption.[75] However, many of these analyses were cross-sectional, associating baseline diet with baseline biospecimens, leaving the potential for confounding and the inability to draw causal inference. Further rationale for the microbiome endpoint is evidence that meat may expand certain bacterial groups in the colon and evidence that specific microbial metabolites are generated from the hydrolysis of meat-derived aromatic and sulfur containing amino acids.[55, 77] Moreover, red meat derived heme induces genotoxic microbial H_2S and nitrite production using *narG*, the first step in genotoxic reactions leading to DNA adducts.[77] Finally, poor diet quality (lower HEI scores) in an observational study was linked to enrichment of two microbial species, *Enterobacter* and *Escherichia-Shigella*, that reduce nitrate to nitrite using nitrate reductase (*narG*),[78, 79] findings relevant for Aim 2 as we will do 16S rRNA sequencing and identify microbial functional genes, including *narG*.

This study has the following primary **specific aim:**

1. Identify the extent and nature of shifts in the metabolome when consuming a standard HEI-2015 diet compared to a HEI-2015-M diet.

Hypothesis 1: Daily intake of red and processed meat will induce shifts in the metabolome detected in blood and urine samples using various metabolomics platforms.

This study has the following **secondary aim**

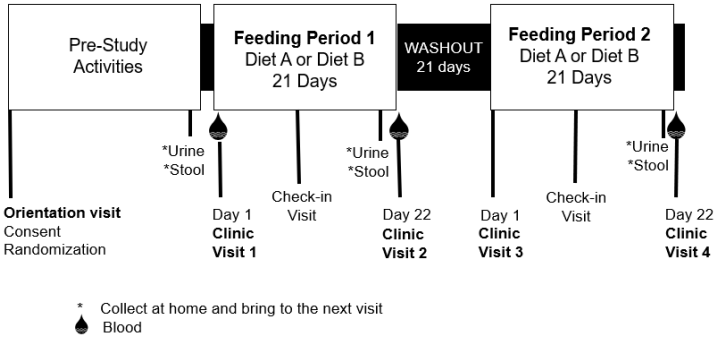
2. Identify and characterize modulation of the gut microbial community when consuming a standard HEI-2015 diet compared to a HEI-2015-M diet.

Hypothesis 2: Daily intake of red and processed meat will shift the gut microbiome such that the two diet arms will show differences in the gut microbial community structure using16S rRNA gene sequencing and digital droplet PCR for assessing microbial functional genes.

2. STUDY DESIGN

Overview. This is a randomized cross-over feeding trial to test whether red and processed meat consumption, in the context of a controlled diet based on HEI-2015, will cause shifts in the metabolome and the microbiome compared to a controlled HEI-2015 diet with no red or processed meat. Twenty healthy adults will complete two feeding periods, in random order of 21 days each with an approximately 21-day washout between feeding periods (**Figure 1**). Study meals are prepared at the Fred

Figure 1. Study design



Hutch Human Nutrition Laboratory (HNL). Participants attend seven clinic visits at the Fred Hutch Prevention Center Clinic: an orientation visit at which consent and randomization will occur; three visits to complete fasting blood draws and to deliver 24-hour urine and stool specimens collected at home; one visit to initiate feeding period 2 and two mid-point visits to check for weight stability. The collected biospecimens are used for study outcomes.

Healthy volunteers will be recruited for this study. Healthy volunteers are well-suited for this study so results will be generalizable.

The study is supported by NIH's R21 mechanism, which is designed to collect preliminary data to support larger trials.

3. ELIGIBILITY CRITERIA

Healthy adult volunteers who meet all of the inclusion and none of the exclusion criteria will be eligible for participation in this study. All races/ethnicities are eligible to participate.

3.1 Inclusion Criteria

1. Healthy adults
2. Age 18-50 years
3. Able to read, speak and understand English
4. Willing to come to the Fred Hutch campus twice weekly during the study

3.2 Exclusion Criteria

1. Known allergy to red or processed meat
2. Vegetarian or vegan
3. Religious or personal reason(s) to avoid red or processed meat
4. Pregnant and/or exclusively breastfeeding
5. Alcohol or recreational drug abuse

Participants who routinely use dietary supplements may continue to do so while on study, but they will be asked not to start new supplements. Participants may consume alcohol but will need to provide their own and report consumption. Participants will be asked to maintain their usual level of physical activity but not start new exercise routines while on study. Any over the counter medications used while on study will be recorded.

4. STUDY PROCEDURES

4.1 Recruitment

Study staff will recruit participants by traditional media (print,), social media, community organizations and local colleges/universities. Additionally, the Fred Hutch Recruitment and Retention Core may assist with recruitment of minority participants. We aim to have minority enrollment that reflects the Seattle population (2019 Seattle census estimates are 35.5% minority). . Initial recruitment and eligibility activities take place via an online screening questionnaire in response to media and flyer postings. If an individual is determined to be eligible or possibly eligible via the online screener, staff will confirm interest and eligibility by phone or email, schedule the orientation visit and provide directions to the Fred Hutch Prevention Center Clinic.

4.2 Orientation/Consent Visit

Study staff review the "study at a glance" document, the study consent and provide individuals an opportunity ask questions. Participants will be shown the study menus and asked whether they are willing and able to consume the majority of study foods. If needed, some substitutions may be possible (for example, substitute an orange for grapefruit); however, some substitutions may not be possible. Individuals who are unwilling or unable to eat the majority of study foods will be asked to not enroll in the study. Individuals will be asked to provide written informed consent for study activities. After participants sign the consent, study staff will measure height and weight and participants will complete the "Lifestyle Questionnaire" on demographic characteristics, usual dietary supplement use, medical history and usual physical activity. Information provided in this questionnaire will be used to estimate energy requirements for study diet planning. Participants will be given a four-day food record and instructions and asked to complete at home in preparation for Clinic Visit 1. Participants will be given a stool collection and a 24-hour urine collection kit as well as detailed instructions for collecting these specimens at home in preparation for Clinic Visit 1. Study staff will schedule Clinic Visit 1 and review instructions to prepare for Clinic Visit 1. Participants will be randomized (using a computerized program) to diet order (Diet A then Diet B or Diet B then Diet A).

4.3 Clinic Visit 1 (Feeding Period 1, Day 1)

At clinic visit 1, study staff will receive and process urine and stool specimens collected by participants at home. Staff will also receive the completed food record. Clinic staff will next measure weight and waist circumference and draw fasting blood. After the blood draw, participants will eat their first study meal of feeding period 1. Study staff will review instructions for the next three weeks (Feeding Period 1), meal pick-up/delivery, proper food storage and reheating, schedule Clinic Visits 2-4 and the check-in visits, provide a study calendar and answer any participant questions. Clinic visit 1 concludes with the participant being provided the first three days of study meals and daily meal check-off forms.

4.4 Feeding Period 1 (Feeding Period 1, Days 1 – 21)

A 3- to 4-day supply of study meals will be picked by the participant on site at Fred Hutch.. Participants will be asked to visit the clinic 2 times per week during each Feeding Period. Each study meal is accompanied by a check-off list for participants to indicate foods consumed and amount (if any) remaining. Participants are asked to consume only study provided foods but may use and report their own coffee and tea, (record type, amount;sweetener and cream/milk will be provided by the study). Halfway through feeding period 1, participants will attend the **Check-in Visit 1** when they pick up their next supply of study meals. At this visit, participants will be weighed and any new medication/supplements will be reported. Participants experiencing hunger or weight change of ≥±3% of clinic visit 1 weight will be placed on the next higher or lower kcal level as appropriate. A stool collection kit and 24-hour urine collection kit will be provided for use on the penultimate day of Feeding Period 1. Participants will complete urine and stool collections at home in preparation for Clinic Visit 2.

4.5 Clinic Visit 2 (Feeding Period 1, Day 22

Participants return to the clinic on day 22 of Feeding Period 1 for the second fasting blood draw and measure weight. Study staff also receive and process urine and stool specimens that were collected at-home on the penultimate day of Feeding Period 1. Study staff review instructions for the washout period (approximately the next three weeks and answer any participant questions. Staff will provide participants with $25.00 as compensation.

4.6 Clinic Visit 3 (Feeding Period 2, Day 1)

After concluding an approximately three-week washout period, participants return to the clinic on Day 1 of Feeding Period 2 for repeat anthropometric measurements (weight and waist circumference). To conserve resources, blood, urine and stool are not collected again at this visit; end of study diet samples are adjusted for baseline samples as we have done in other feeding studies.[59, 62] Using the study calendar, study staff review instructions for the next three weeks (Feeding Period 2), meal pick-up/delivery, proper food storage and reheating and answer any participant questions. Clinic visit 3 concludes with participants being provided the first three days of Feeding Period 2 meals and corresponding daily meal check-off forms.

4.7 Feeding Period 2 (Feeding Period 2, Days 1 – 221)

Similar to Feeding Period 1, a 3- to 4-day supply of study meals and daily meal check-off lists will be picked up.). Halfway through Feeding Period 2, participants will attend **check-in Visit 2** when they pick up their next supply of study meals. At this visit, participants will be weighed and any new medications/supplements will be reported. Participants experiencing hunger or weight changes of ≥±3% from Clinic Visit 3 are placed on the next higher or lower kcal level as appropriate. A stool collection kit and 24-hour urine collection kit will be provided for use on the penultimate day of Feeding Period 2. Participants will complete urine and stool collections at home in preparation for Clinic Visit 4.

4.8 Clinic Visit 4 and End of Study Visit (Feeding Period 2, Day 22)

Participants return for a final time to the clinic on Day 22 of Feeding Period 2 for the end-of-study fasting blood draw and repeat anthropometric measurements (weight and waist circumference). Study staff will receive and process the urine and stool specimens, which the participants completed at-home in preparation for Clinic Visit 4. Staff answers all participant questions and provides the End-of-Study Thank-you Letter and $75.00 as compensation.

4.9 Ongoing Study Procedures

Blood, urine and stool samples are processed at clinic visits using standard procedures and are stored at -80°C until analysis. Throughout the study, participants maintain usual physical activity, but are asked to refrain from starting new activities that may alter total energy expenditure and energy intake needs. Participants are advised to continue to take prescription medications (if needed) but should check with study staff if new prescription or over-the-counter medications are used as this can affect the metabolome. Similarly, participants may continue usual dietary supplements (if any) but should not start new supplements. Participants may consume alcohol but must provide their own alcohol and will report type and amounts. Study staff will be available by phone, or email throughout the study should questions arise and to assist with retention.

4.10 Consents

All informed consent procedures and documentation is done in compliance with Fred Hutchinson Institutional Review Office Policy.

Consent for screening. Individuals who visit the Online screening questionnaire will be explicitly asked to provide consent (via checkbox) to complete the screening questionnaire.

Study Informed Consent. At the Orientation visit, the informed consent discussion will occur and participants will be asked to sign the Fred Hutch Study Informed Consent and each participant will be provided a copy of the consent.

4.11 Design of Feeding Study Meals

Feeding study menus are developed by the Fred Hutch Human Nutrition Lab (HNL) staff using Pronutra® (Viocare Technologies, Inc., Princeton, NJ), which is a metabolic diet management system specifically designed for conducting feeding studies. Two seven-day, eucaloric repeating menu cycles are created: (1) HEI-2015 with no red and processed meat and (2) HEI-2015-M with red and processed meat as some of the protein sources. The USDA defines red meat as “any red pigmented meat and includes beef, lamb, pork, veal and game meats”.[36] This study will primarily utilize beef and pork due to budgetary and logistic restraints to obtain veal and game meats. A variety of types and cuts of red meat will be used (e.g., round, chuck, top sirloin) and cooking methods will include broiling, braising and roasting. No meats will be cooked by open flame or undergo charring. The USDA defines processed meat as “any meat that has undergone transformation through salting, curing, fermentation, or other processes to enhance flavor or improve preservation”.[36] A variety of processed meat will be used for HEI-2015-M. The HEI-2015-M menus will include at least one daily serving of red or processed meat; other protein sources (eggs, poultry, fish, legumes,) may be used if needed to meet the daily protein goals. The HEI-2015 menus will include no red or processed meat and protein sources will include eggs, poultry, fish and beans/legumes. The seven-day cycle menu will repeat three times over each 21-day feeding period. Diets will be created at 7 different kcal levels, 1800 kcal to 3000 kcal per day, in 200 kcal increments. Each day is matched within diet group for energy and for the cup and ounce equivalents for each food group, and food groups in each meal are identical across arms excluding the protein foods. Individual energy requirements for participants are estimated using the Mifflin equation.[92] Participants reporting hunger or weight changes are placed on the next higher or lower kcal level as appropriate. Weight should remain stable (± 3% of clinic visit 1 and clinic visit 3 weights) throughout the study.

All foods consumed by participants throughout the study will be prepared by HNL staff who have extensive experience with metabolic studies. All food items are individually weighed or portioned out for each participant and clearly labeled by meal. Study foods are precooked and frozen and are picked up by participants on site at Fred Hutch. Safe food handling, storage and reheating instructions are included.

4.12 Compliance.

Compliance to the intervention will be evaluated by daily meal check-off forms, the mid-feeding period check-in with the study dietitian.

5. DATA COLLECTION

This study involves a total of seven participant visits. Visits will occur as follows: 1) Orientation Visit (pre-study). 2) Clinic Visit 1 (Baseline; Day 1 of Feeding Period 1), 3) Midpoint Visit 1 (~Day 10 of Feeding Period

1), 4) Clinic Visit 2 (Day 22, the day after the end of Feeding Period 1), 5) Clinic Visit 3 (Day 1 of Feeding Period 2), 6) Midpoint Visit 2 (~Day 10 of Feeding Period 2), and 7) Clinic Visit 4 (end of study; Day 22 the day after the end of Feeding Period 2). At the Orientation Visit, the study Lifestyle Questionnaire will be completed. At all clinic visits, participants will undergo assessments of anthropometrics, and on clinic visits 1, 2 and 4 they will bring stool and urine samples collected at home and will provide fasting blood.

Twelve-hour fasting blood. Fasting blood samples will be collected on Day 1 of the first Feeding period, and on Day 22 of the first feeding period and Day 22 of the second feeding period. Vacutainers will be labeled with participants' study ID and date, processed per standard protocol and stored at -80ºC.

Stool sample: Stool samples will be collected by participants at home prior to Clinic Visits the beginning (Clinic Visit 1) and end of Feeding Period 1 (Clinic Visit 2) and the end of Feeding Period 2 (end of study, Clinic Visit 4).

Urine sample: 24-hour urine samples will be collected by participants at home prior to Clinic Visits at the beginning (Clinic Visit 1) and end of Feeding Period 1 (Clinic Visit 2) and the end of Feeding Period 2 (end of study, Clinic Visit 4).

Questionnaires:

Lifestyle Questionnaire. Date of birth, self-identified race/ethnicity, gender identity, education, smoking, alcohol use, recreational drug use, over-the-counter and prescription drug use, dietary supplement use, physical activity levels and general health history will be collected at the Visit 1 (self-administered questionnaire).

Daily meal check-off forms. All participants will complete study provided meal check-off forms to record and quantify daily meals consumed each day during both feeding periods.

5.1 Outcomes

5.1.1 Metabolome

Fasting blood, 24-hour urine, and fecal samples collected at baseline and after each diet period for each participant. Shifts in the metabolome will be characterized by conducting metabolomics assays on the blood urine, and fecal samples and comparing the readouts between the HEI-2015 and HEI-2015-M diets.

5.1.2 Microbiome

Stool DNA will be extracted from the fecal samples collected at baseline and after each diet period for each participant. Modulation of the gut microbial community will be characterized by 16S rRNA gene sequencing of the extracted genomic DNA and comparing the readouts between the HEI-2015 and HEI-2015-M diets.

In addition, digital droplet PCR assays will be conducted to measure changes in the genes for *butyrate kinase (buk)* and *nitrate reductase (narG),* and archival samples will be collected to conduct microbial growth experiments.

5.1.3 Anthropometrics

Height and waist circumference will be measured at the orientation visit only, and weight will be measured at the beginning, midpoint and end of each feeding period for each participant.

6. PARTICIPANT RISKS AND BENEFITS

6.1 Potential Risks

1. Participants may experience a little discomfort or have a temporary bruise from having blood drawn. Occasionally a participant may feel lightheaded or feel faint when having blood drawn since blood is drawn after a 12 hour fast. The amount of blood drawn for this study does not exceed 50 ml total over the 11 week study and does not occur more than twice weekly.

2. Some participants may feel that coming to study visits and consuming only study-provided foods for two 3-week periods is burdensome.
3. Some participants may find it inconvenient or uncomfortable to collect their urine and stool.

6.2 Protection Against Risks

In the event that this research activity results in an injury, medical treatment will be available, including first aid, emergency treatment and follow up care, as needed. All procedures involving blood collection and anthropometry will be done at the Fred Hutch Prevention Center Clinic. Participants will collect urine and stool samples at home. Food is carefully and safely prepared under highly controlled conditions. Trained staff will be available to assist should a medical emergency occur. An MD licensed in Washington State is the Prevention Center Director. Participants will be informed in the written consent form that payment for any such treatment must be provided by the individual and/or the individual's insurance company.

1. Participants may experience a little discomfort, bruising, fainting or phlebitis from having blood drawn. We will minimize these risks by making sure that all blood draws are conducted by well-trained nurses, medical assistants, or phlebotomists at the Fred Hutch Prevention Center. Participants will be asked before the blood draw if they have experienced problems with blood draws in the past, and they will be offered an opportunity to lie down during the procedure if they wish. After the blood draw is complete, participants will remain seated, applying pressure to the site of venipuncture. If a participant feels faint, they will be instructed to lie down until the feeling goes away. We provide a small snack and fluids after the blood draw. In our experience, blood draws performed in this manner are well tolerated, and any side effects are minimal.
2. We will make every effort to schedule participants to come to the Fred Hutch at times that are convenient for them; most participants find that morning appointments work well for the fasting blood draws. During the approximately 3-week "washout" period, participants will be allowed to eat whatever foods they like.
3. Participants will be provided all necessary supplies as well as instructions to help in the collection of stool and urine specimens. In addition, they will be provided with opaque bags to discretely carry specimens to their study visit. Study staff will be available by telephone should the participant have any questions or complications when collection these specimens.

6.3 Benefits

The data generated from this proposed clinical trial will provide essential information for participants regarding the effects of red and processed meat in the context of an overall healthy dietary pattern on the metabolome and microbiome of healthy adults.

The research we propose here is important because if a specific nutritional intervention can be shown to improve measures of the metabolome and the microbiome, then it will provide compelling preliminary data to then launch a larger clinical trial which could, potentially, have far reaching implications for thousands healthy adult members of the general population. Thus, with minimal risk to study participants, we will generate data that will be beneficial to general dietary guidance related to health promotion and disease prevention – including cancer prevention.

Overall, using this rigorous study design (randomized cross-over feeding trial), we have attempted to minimize any potential risks to study participants, whilst maximizing the amount of important scientific data that can be generated and ultimately shared with the scientific community and the general public.

6.4 Compensation

Participants will be paid $100 total, $25 at the end of the first feeding period and $75 at the end of the second feeding period to help compensate for time and travel. All food will be provided for the two feeding periods.

6.5 Alternatives

Alternatives to participating in this research study include not participating.

7. DATA ANALYSIS PLAN

This trial identifies (1) the extent and nature of metabolomic shifts and (2) as a secondary aim, modulation of the gut microbiome when consuming a standard HEI-2015 diet compared to a HEI-2015-M diet. We expect that daily intake of red and processed meat will induce shifts in the metabolome detected in blood and urine samples, and secondarily in the gut microbiome detected in extracted fecal DNA. Under the NIH R21 mechanism, it is considered a preliminary trial.

Statistical Analysis for the Primary Aim. Aim 1 identifies metabolomic markers that differ between HEI-2015 and HEI-2015-M. Metabolites from NMR and LC/MS-MS platforms will be used to allow greater coverage of the metabolome and as shown feasible by Dr. Raftery[97] and others.[98] Metabolite levels are normalized by dividing by the batch's median value followed by removing metabolites with >25% CVs in the embedded blinded duplicates as well as those with >20% missing values (expected at 5-10% of total). Next, we will use unsupervised principal components analysis (PCA) to visually examine the data patterns and variability. We then apply supervised orthogonal partial least squares discriminant analysis (OPLS-DA) to identify candidate biomarkers that may differ by diet arm. VIP (variable importance in projection) scores >2 will be retained. For each identified metabolite, separate linear mixed models will be fit to examine fold-changes in end of diet period metabolite levels by HEI-2015 and HEI-2015-M, adjusting for baseline. In the cross-over design each participant is their own control, minimizing noise in intra-individual variation in endogenous metabolites. The cross-over design also minimizes confounding by age, BMI, and diet order, but models are run with and without these adjustments. The Benjamini-Hochberg approach controls for false discovery rate (FDR) <0.10.[99]

Statistical Analysis for the Secondary Aim. To account for different sequencing depths, we will initially apply the centered-log ratio (CLR) transformation to each sample after replacing any zero counts by 1 or a small imputed value.[104] We will first gauge the overall strength of microbial signal by applying a microbiome-wide kernel-association test fusing a GLMM-MiRKAT[105] with respect to the weighted UniFrac distance.[106, 107] Analyses will account for the crossover study design using linear mixed models with outcome of either genera, alpha diversity, or genes for butyrate kinase (b*uk*) or nitrate reductase (*narG*), adjusting for baseline. As with Aim 1, we will conduct analyses with and without adjustment for age, BMI, diet order and other covariates. We will use Benjamini-Hochberg approach to account for FDR <0.10.[99] Based on our studies[62] and that of others[58] we expect to have 80% power to detect β coefficients in the linear mixed models in the range of 0.3- 04 for differences between diets at $p<0.05$. Primary analysis focuses on three genera associated with butyrate production and three genera associated with nitrate reduction. All genera will be examined in exploratory analysis.[108]

7.1 Sample Size for the Primary Aim

The sample size is based on the primary aim. For this pilot trial with a fixed sample size of 20 participants, we estimate that we expect to have 80% power to detect fold changes between diets of $\pm$ 0.20 for blood and urine metabolites. Power calculations used MetSizeR.[110] Tests are 2-sided α=5%.

8. DATA SAFETY MONITORING PLAN

Oversight for this study will be provided by the Principal Investigator, Dr. Marian Neuhouser with delegation of responsibilities to designated Co-Investigators and study personnel. Dr. Neuhouser will ensue all entry criteria are met prior to the initiation of the protocol, and all study procedures and reporting of adverse events are performed according to the IRB-approved protocol. Study participants are healthy adult volunteers, in whom the study intervention is non-therapeutic.

8.1 Adverse Event Reporting

All adverse events related to the study procedures will be fully documented on the appropriate case report form(s) and entered in a study database. For each adverse event, the investigator will provide the onset, duration, intensity, treatment required, and outcome, including documentation of need for premature termination of any study procedures. Anticipated adverse events for this study are expected to be very minimal since the diet activities have been safely used in many previous intervention studies without incident. Despite the low risk for adverse events, all study staff and investigators will carefully monitor and document any adverse events, which could include the following: bruising or fainting during the blood draws, and possible risk

of food borne illness if the participant does not follow the instructions provided for safe food handling, storage, and re-heating of the study foods

Serious Adverse Events will be reported within to the Principal Investigator within 24 hours. Adverse event reporting to the IRO will occur within the required period of time depending on the nature and severity of the event.

8.2 Plan for safety review

The PIs will perform a cumulative review of all adverse events and premature terminations review every 6 months after study initiation.

8.3 Plan for annual reporting

A summary of the investigation including all adverse events and how they were handled, enrollment, drop-outs and reason for discontinuation and any protocol modifications will be provided to the IRB on an annual basis. Annual Reports will contain:
a. The number of adverse events and an explanation of how each event was handled
b. The number of complaints and how each complaint was handled
c. The number of subject withdrawals and an explanation of why the subject withdrew or was withdrawn
d. The number of protocol deviations and how each was handled

8.4 Monitoring for data integrity and safety

Monitoring for data integrity and safety will be the responsibility of the investigators. Investigators will include the following in routine monitoring review: A) Validity and integrity of data: Data are checked for missing, unusual, or impossible values when they are entered into the study's computer database. B) Enrollment rate relative to expectation: Early lags in recruitment will be rectified with increased recruitment efforts so that recruitment will be completed on time. The investigators will monitor this closely to ensure full enrollment of appropriate participants. C) Retention of participants and adherence to protocol: The investigators will monitor retention and adherence to study protocol. Adherence to the intervention will be monitored via daily meal check-off forms and by monitoring amounts of study foods consumed/not consumed.

9. REFERENCES

1. Millen BE, Abrams S, Adams-Campbell L, Anderson CA, Brenna JT, Campbell WW, Clinton S, Hu F, Nelson M, Neuhouser ML, Perez-Escamilla R, Siega-Riz AM, Story M, Lichtenstein AH. The 2015 Dietary Guidelines Advisory Committee Scientific Report: Development and major conclusions. Adv Nutr. 2016;7(3):438-44. doi: 10.3945/an.116.012120. PubMed PMID: 27184271; PMCID: PMC4863277.

2. United States Department of Agriculture (USDA) US Department of Health and Human Services (DHHS). 2015-2020 Dietary Guidelines for Americans, 8th Edition. United States Department of Agriculture, United States Department of Health and Human Services, 2015.

3. Kruger C, Zhou Y. Red meat and colon cancer: A review of mechanistic evidence for heme in the context of risk assessment methodology. Food Chem Toxicol. 2018;118:131-53. Epub 2018/04/25. doi: 10.1016/j.fct.2018.04.048. PubMed PMID: 29689357.

4. Martin OCB, Naud N, Tache S, Debrauwer L, Chevolleau S, Dupuy J, Chantelauze C, Durand D, Pujos-Guillot E, Blas YEF, Urbano C, Kuhnle GGC, Sante-Lhoutellier V, Sayd T, Viala D, Blot A, Meunier N, Schlich P, Attaix D, Gueraud F, Scislowski V, Corpet DE, Pierre FHF. Targeting Colon Luminal Lipid Peroxidation Limits Colon Carcinogenesis Associated with Red Meat Consumption. Cancer Prev Res (Phila). 2018;11(9):569-80. Epub 2018/06/30. doi: 10.1158/1940-6207.capr-17-0361. PubMed PMID: 29954759.

5. Micha R, Shulkin ML, Penalvo JL, Khatibzadeh S, Singh GM, Rao M, Fahimi S, Powles J, Mozaffarian D. Etiologic effects and optimal intakes of foods and nutrients for risk of cardiovascular diseases and diabetes: Systematic reviews and meta-analyses from the Nutrition and Chronic Diseases Expert Group (NutriCoDE). PLoS One. 2017;12(4):e0175149. Epub 2017/04/28. doi: 10.1371/journal.pone.0175149. PubMed PMID: 28448503; PMCID: PMC5407851.

6. Ibsen DB, Warberg CK, Würtz AML, Overvad K, Dahm CC. Substitution of red meat with poultry or fish and risk of type 2 diabetes: a Danish cohort study. Eur J Nutr. 2019;58(7):2705-12. Epub 2018/09/19. doi: 10.1007/s00394-018-1820-0. PubMed PMID: 30225630.

7. Pan A, Sun Q, Bernstein AM, Manson JE, Willett WC, Hu FB. Changes in red meat consumption and subsequent risk of type 2 diabetes mellitus: three cohorts of US men and women. JAMA Intern Med. 2013;173(14):1328-35. Epub 2013/06/20. doi: 10.1001/jamainternmed.2013.6633. PubMed PMID: 23779232; PMCID: PMC3847817.

8. Guenther PM, Reedy J, Krebs-Smith SM. Development of the Healthy Eating Index-2005. J Am Diet Assoc. 2008;108(11):1896-901. Epub 2008/10/29. doi: 10.1016/j.jada.2008.08.016. PubMed PMID: 18954580.

9. Reedy J, Lerman JL, Krebs-Smith SM, Kirkpatrick SI, Pannucci TE, Wilson MM, Subar AF, Kahle LL, Tooze JA. Evaluation of the Healthy Eating Index-2015. J Acad Nutr Diet. 2018;118(9):1622-33. Epub 2018/08/28. doi: 10.1016/j.jand.2018.05.019. PubMed PMID: 30146073.

10. Pérez-Escamilla R. The Mexican Dietary and Physical Activity Guidelines: Moving Public Nutrition Forward in a Globalized World. J Nutr. 2016;146(9):1924s-7s. Epub 2016/08/12. doi: 10.3945/jn.115.218784. PubMed PMID: 27511930.

11. Monteiro CA, Cannon G, Moubarac JC, Martins AP, Martins CA, Garzillo J, Canella DS, Baraldi LG, Barciotte M, Louzada ML, Levy RB, Claro RM, Jaime PC. Dietary guidelines to nourish humanity and the planet in the twenty-first century. A blueprint from Brazil. Public Health Nutr. 2015;18(13):2311-22. Epub 2015/07/25. doi: 10.1017/s1368980015002165. PubMed PMID: 26205679.

12. Cocking C, Walton J, Kehoe L, Cashman KD, Flynn A. The role of meat in the European diet: current state of knowledge on dietary recommendations, intakes and contribution to energy and nutrient intakes and status. Nutr Res Rev. 2020:1-9. Epub 2020/01/11. doi: 10.1017/s0954422419000295. PubMed PMID: 31918784.

13. Van Horn L, Carson JA, Appel LJ, Burke LE, Economos C, Karmally W, Lancaster K, Lichtenstein AH, Johnson RK, Thomas RJ, Vos M, Wylie-Rosett J, Kris-Etherton P. Recommended dietary pattern to achieve adherence to the American Heart Association/American College of Cardiology (AHA/ACC) Guidelines: A scientific statement from the American Heart Association. Circulation. 2016;134(22):e505-e29. Epub 2016/10/30. doi: 10.1161/cir.0000000000000462. PubMed PMID: 27789558.

14. World Cancer Research Fund/American Institute of Cancer Research. Diet, Nutrition, Physical Activity and Cancer: a Global Perspective. Third Expert Report. London: World Cancer Research Fund; 2018.

15. World Health Organization, International Agency for Research on Cancer (IARC). Red and Processed Meat. IARC Monographs on the Evaluation of Carconogenic Risks to Humans: IARC; 2015.

16. Zeraatkar D, Johnston BC, Bartoszko J, Cheung K, Bala MM, Valli C, Rabassa M, Sit D, Milio K, Sadeghirad B, Agarwal A, Zea AM, Lee Y, Han MA, Vernooij RWM, Alonso-Coello P, Guyatt GH, El Dib R. Effect of Lower Versus Higher Red Meat Intake on Cardiometabolic and Cancer Outcomes: A Systematic Review of Randomized Trials. Ann Intern Med. 2019;171(10):721-31. Epub 2019/10/01. doi: 10.7326/m19-0622. PubMed PMID: 31569236.

17. Zeraatkar D, Han MA, Guyatt GH, Vernooij RWM, El Dib R, Cheung K, Milio K, Zworth M, Bartoszko JJ, Valli C, Rabassa M, Lee Y, Zajac J, Prokop-Dorner A, Lo C, Bala MM, Alonso-Coello P, Hanna SE, Johnston BC. Red and Processed Meat Consumption and Risk for All-Cause Mortality and Cardiometabolic Outcomes: A Systematic Review and Meta-analysis of Cohort Studies. Ann Intern Med. 2019;171(10):703-10. Epub 2019/10/01. doi: 10.7326/m19-0655. PubMed PMID: 31569213.

18. Vernooij RWM, Zeraatkar D, Han MA, El Dib R, Zworth M, Milio K, Sit D, Lee Y, Gomaa H, Valli C, Swierz MJ, Chang Y, Hanna SE, Brauer PM, Sievenpiper J, de Souza R, Alonso-Coello P, Bala MM, Guyatt GH, Johnston BC. Patterns of Red and Processed Meat Consumption and Risk for Cardiometabolic and Cancer Outcomes: A Systematic Review and Meta-analysis of Cohort Studies. Ann Intern Med. 2019;171(10):732-41. Epub 2019/10/01. doi: 10.7326/m19-1583. PubMed PMID: 31569217.

19. Han MA, Zeraatkar D, Guyatt GH, Vernooij RWM, El Dib R, Zhang Y, Algarni A, Leung G, Storman D, Valli C, Rabassa M, Rehman N, Parvizian MK, Zworth M, Bartoszko JJ, Lopes LC, Sit D, Bala MM, Alonso-Coello P, Johnston BC. Reduction of Red and Processed Meat Intake and Cancer Mortality and Incidence: A Systematic Review and Meta-analysis of Cohort Studies. Ann Intern Med. 2019;171(10):711-20. Epub 2019/10/01. doi: 10.7326/m19-0699. PubMed PMID: 31569214.

20. Li SA, Alexander PE, Reljic T, Cuker A, Nieuwlaat R, Wiercioch W, Guyatt G, Schünemann HJ, Djulbegovic B. Evidence to Decision framework provides a structured "roadmap" for making GRADE guidelines recommendations. J Clin Epidemiol. 2018;104:103-12. Epub 2018/09/27. doi: 10.1016/j.jclinepi.2018.09.007. PubMed PMID: 30253221.

21. Johnston BC, Zeraatkar D, Han MA, Vernooij RWM, Valli C, El Dib R, Marshall C, Stover PJ, Fairweather-Taitt S, Wójcik G, Bhatia F, de Souza R, Brotons C, Meerpohl JJ, Patel CJ, Djulbegovic B, Alonso-Coello P, Bala MM, Guyatt GH. Unprocessed Red Meat and Processed Meat Consumption: Dietary Guideline Recommendations From the Nutritional Recommendations (NutriRECS) Consortium. Ann Intern Med. 2019;171(10):756-64. Epub 2019/10/01. doi: 10.7326/m19-1621. PubMed PMID: 31569235.

22. Valli C, Rabassa M, Johnston BC, Kuijpers R, Prokop-Dorner A, Zajac J, Storman D, Storman M, Bala MM, Solà I, Zeraatkar D, Han MA, Vernooij RWM, Guyatt GH, Alonso-Coello P. Health-Related Values and Preferences Regarding Meat Consumption: A Mixed-Methods Systematic Review. Ann Intern Med. 2019;171(10):742-55. Epub 2019/10/01. doi: 10.7326/m19-1326. PubMed PMID: 31569219.

23. Guertin KA, Li XS, Graubard BI, Albanes D, Weinstein SJ, Goedert JJ, Wang Z, Hazen SL, Sinha R. Serum Trimethylamine N-oxide, Carnitine, Choline, and Betaine in Relation to Colorectal Cancer Risk in the Alpha Tocopherol, Beta Carotene Cancer Prevention Study. Cancer Epidemiol Biomarkers Prev. 2017;26(6):945-52. Epub 2017/01/13. doi: 10.1158/1055-9965.epi-16-0948. PubMed PMID: 28077427; PMCID: PMC5608021.

24. Jian SH, Yeh PJ, Wang CH, Chen HC, Chen SF. Analysis of heterocyclic amines in meat products by liquid chromatography - Tandem mass spectrometry. J Food Drug Anal. 2019;27(2):595-602. Epub 2019/04/17. doi: 10.1016/j.jfda.2018.10.002. PubMed PMID: 30987731.

25. Mosley D, Su T, Murff HJ, Smalley WE, Ness RM, Zheng W, Shrubsole MJ. Meat intake, meat cooking methods, and meat-derived mutagen exposure and risk of sessile serrated lesions. Am J Clin Nutr. 2020;111(6):1244-51. Epub 2020/02/23. doi: 10.1093/ajcn/nqaa030. PubMed PMID: 32077920; PMCID: PMC7266682.

26. Zheng Y, Li Y, Satija A, Pan A, Sotos-Prieto M, Rimm E, Willett WC, Hu FB. Association of changes in red meat consumption with total and cause specific mortality among US women and men: two prospective cohort

studies. BMJ. 2019;365:l2110. Epub 2019/06/14. doi: 10.1136/bmj.l2110. PubMed PMID: 31189526; PMCID: PMC6559336

27. Guasch-Ferré M, Satija A, Blondin SA, Janiszewski M, Emlen E, O'Connor LE, Campbell WW, Hu FB, Willett WC, Stampfer MJ. Meta-Analysis of Randomized Controlled Trials of Red Meat Consumption in Comparison With Various Comparison Diets on Cardiovascular Risk Factors. Circulation. 2019;139(15):1828-45. Epub 2019/04/09. doi: 10.1161/circulationaha.118.035225. PubMed PMID: 30958719.

28. Mattiuzzi C, Lippi G. Epidemiologic Burden of Red and Processed Meat Intake on Colorectal Cancer Mortality. Nutr Cancer. 2020:1-6. Epub 2020/05/14. doi: 10.1080/01635581.2020.1765259. PubMed PMID: 32400201.

29. Mehta SS, Arroyave WD, Lunn RM, Park YM, Boyd WA, Sandler DP. A Prospective Analysis of Red and Processed Meat Consumption and Risk of Colorectal Cancer in Women. Cancer Epidemiol Biomarkers Prev. 2020;29(1):141-50. Epub 2019/10/03. doi: 10.1158/1055-9965.epi-19-0459. PubMed PMID: 31575555; PMCID: PMC6954313.

30. Liao LM, Loftfield E, Etemadi A, Graubard BI, Sinha R. Substitution of dietary protein sources in relation to colorectal cancer risk in the NIH-AARP cohort study. Cancer Causes Control. 2019;30(10):1127-35. Epub 2019/07/22. doi: 10.1007/s10552-019-01210-1. PubMed PMID: 31327110; PMCID: PMC6736766.

31. Neuhouser ML. Red and processed meat: more with less? Am J Clin Nutr. 2020;111(2):252-5. Epub 2019/11/20. doi: 10.1093/ajcn/nqz294. PubMed PMID: 31740926.

32. Maruvada P, Lampe JW, Wishart DS, Barupal D, Chester DN, Dodd D, Djoumbou-Feunang Y, Dorrestein PC, Dragsted LO, Draper J, Duffy LC, Dwyer JT, Emenaker NJ, Fiehn O, Gerszten RE, F BH, Karp RW, Klurfeld DM, Laughlin MR, Little AR, Lynch CJ, Moore SC, Nicastro HL, O'Brien DM, Ordovas JM, Osganian SK, Playdon M, Prentice R, Raftery D, Reisdorph N, Roche HM, Ross SA, Sang S, Scalbert A, Srinivas PR, Zeisel SH. Perspective: dietary biomarkers of intake and exposure-exploration with omics approaches. Adv Nutr. 2019. Epub 2019/08/07. doi: 10.1093/advances/nmz075. PubMed PMID: 31386148.

33. Mahabir S, Willett WC, Friedenreich CM, Lai GY, Boushey CJ, Matthews CE, Sinha R, Colditz GA, Rothwell JA, Reedy J, Patel AV, Leitzmann MF, Fraser GE, Ross S, Hursting SD, Abnet CC, Kushi LH, Taylor PR, Prentice RL. Research strategies for nutritional and physical activity epidemiology and cancer prevention. Cancer Epidemiol Biomarkers Prev. 2018;27(3):233-44. Epub 2017/12/20. doi: 10.1158/1055-9965.epi-17-0509. PubMed PMID: 29254934.

34. National Institutes of Health (NIH). 2020-2030 Strategic Plan for NIH Nutrition Research. A Report of the NIH Nutrition Research Task Force2020:1-24.

35. Fleischhacker SE, Ballard RM, Starke-Reed PE, Galuska DA, Neuhouser ML. Developmental Process and Early Phases of Implementation for the US Interagency Committee on Human Nutrition Research National Nutrition Research Roadmap 2016-2021. J Nutr. 2017;147(10):1833-8. doi: 1 0.3945/jn.117.255943. PubMed PMID: 28814532; PMCID: PMC5610552.

36. https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/food-labeling/meat-and-poultry-labeling-terms/meat-and-poultry-labeling-terms. (accessed Oct 2019).

37. Wu K, Spiegelman D, Hou T, Albanes D, Allen NE, Berndt SI, van den Brandt PA, Giles GG, Giovannucci E, Goldbohm RA, Goodman GG, Goodman PJ, Hakansson N, Inoue M, Key TJ, Kolonel LN, Mannisto S, McCullough ML, Neuhouser ML, Park Y, Platz EA, Schenk JM, Sinha R, Stampfer MJ, Stevens VL, Tsugane S, Visvanathan K, Wilkens LR, Wolk A, Ziegler RG, Smith-Warner SA. Associations between unprocessed red and processed meat, poultry, seafood and egg intake and the risk of prostate cancer: A pooled analysis of 15 prospective cohort studies. Int J Cancer. 2016;138(10):2368-82. doi: 10.1002/ijc.29973. PubMed PMID: WOS:000372257300007; PMCID: PMC4837898.

38. Zeng L, Ruan M, Liu J, Wilde P, Naumova EN, Mozaffarian D, Zhang FF. Trends in Processed Meat, Unprocessed Red Meat, Poultry, and Fish Consumption in the United States, 1999-2016. J Acad Nutr Diet. 2019;119(7):1085-98.e12. Epub 2019/06/27. doi: 10.1016/j.jand.2019.04.004. PubMed PMID: 31234969; PMCID: PMC6689198.

39. Klurfeld DM. What is the role of meat in a healthy diet? Anim Front. 2018;8(3):5-10. Epub 2018/07/07. doi: 10.1093/af/vfy009. PubMed PMID: 32071794; PMCID: PMC7015455.

40. Jiao L, Stolzenberg-Solomon R, Zimmerman TP, Duan Z, Chen L, Kahle L, Risch A, Subar AF, Cross AJ, Hollenbeck A, Vlassara H, Striker G, Sinha R. Dietary consumption of advanced glycation end products and pancreatic cancer in the prospective NIH-AARP Diet and Health Study. Am J Clin Nutr. 2015;101(1):126-34. Epub 2014/12/21. doi: 10.3945/ajcn.114.098061. PubMed PMID: 25527756; PMCID: PMC4266882.

41. White DL, Collinson A. Red meat, dietary heme iron, and risk of type 2 diabetes: the involvement of advanced lipoxidation endproducts. Adv Nutr. 2013;4(4):403-11. Epub 2013/07/17. doi: 10.3945/an.113.003681. PubMed PMID: 23858089; PMCID: PMC3941820.

42. van Breda SG, Mathijs K, Sági-Kiss V, Kuhnle GG, van der Veer B, Jones RR, Sinha R, Ward MH, de Kok TM. Impact of high drinking water nitrate levels on the endogenous formation of apparent N-nitroso compounds in combination with meat intake in healthy volunteers. Environ Health. 2019;18(1):87. Epub 2019/10/19. doi: 10.1186/s12940-019-0525-z. PubMed PMID: 31623611; PMCID: PMC6796425.

43. Etemadi A, Sinha R, Ward MH, Graubard BI, Inoue-Choi M, Dawsey SM, Abnet CC. Mortality from different causes associated with meat, heme iron, nitrates, and nitrites in the NIH-AARP Diet and Health Study: population based cohort study. BMJ. 2017;357:j1957. Epub 2017/05/11. doi: 10.1136/bmj.j1957. PubMed PMID: 28487287; PMCID: PMC5423547

44. Inoue-Choi M, Sinha R, Gierach GL, Ward MH. Red and processed meat, nitrite, and heme iron intakes and postmenopausal breast cancer risk in the NIH-AARP Diet and Health Study. Int J Cancer. 2016;138(7):1609-18. Epub 2015/10/28. doi: 10.1002/ijc.29901. PubMed PMID: 26505173; PMCID: PMC4724256.

45. Pietron W, Pajurek M, Mikolajczyk S, Maszewski S, Warenik-Bany M, Piskorska-Pliszczynska J. Exposure to PBDEs associated with farm animal meat consumption. Chemosphere. 2019;224:58-64. Epub 2019/02/27. doi: 10.1016/j.chemosphere.2019.02.067. PubMed PMID: 30807914.

46. Sakhi AK, Lillegaard IT, Voorspoels S, Carlsen MH, Løken EB, Brantsæter AL, Haugen M, Meltzer HM, Thomsen C. Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults. Environ Int. 2014;73:259-69. Epub 2014/09/01. doi: 10.1016/j.envint.2014.08.005. PubMed PMID: 25173060.

47. Le NT, Michels FA, Song M, Zhang X, Bernstein AM, Giovannucci EL, Fuchs CS, Ogino S, Chan AT, Sinha R, Willett WC, Wu K. A Prospective Analysis of Meat Mutagens and Colorectal Cancer in the Nurses' Health Study and Health Professionals Follow-up Study. Environ Health Perspect. 2016;124(10):1529-36. Epub 2016/04/23. doi: 10.1289/ehp238. PubMed PMID: 27105317; PMCID: PMC5047780.

48. John EM, Stern MC, Sinha R, Koo J. Meat consumption, cooking practices, meat mutagens, and risk of prostate cancer. Nutr Cancer. 2011;63(4):525-37. Epub 2011/04/29. doi: 10.1080/01635581.2011.539311. PubMed PMID: 21526454; PMCID: PMC3516139.

49. Ren X, Wang W, Bao Y, Zhu Y, Zhang Y, Lu Y, Peng Z, Zhou G. Isorhamnetin and Hispidulin from Tamarix ramosissima Inhibit 2-Amino-1-Methyl-6-Phenylimidazo[4,5-b]Pyridine (PhIP) Formation by Trapping Phenylacetaldehyde as a Key Mechanism. Foods. 2020;9(4). Epub 2020/04/09. doi: 10.3390/foods9040420. PubMed PMID: 32260060; PMCID: PMC7230572.

50. Roussell MA, Hill AM, Gaugler TL, West SG, Heuvel JP, Alaupovic P, Gillies PJ, Kris-Etherton PM. Beef in an Optimal Lean Diet study: effects on lipids, lipoproteins, and apolipoproteins. Am J Clin Nutr. 012;95(1):9-16. Epub 2011/12/16. doi: 10.3945/ajcn.111.016261. PubMed PMID: 22170364; PMCID: PMC3238465.

51. O'Connor LE, Paddon-Jones D, Wright AJ, Campbell WW. A Mediterranean-style eating pattern with lean, unprocessed red meat has cardiometabolic benefits for adults who are overweight or obese in a randomized, crossover, controlled feeding trial. Am J Clin Nutr. 2018;108(1):33-40. Epub 2018/06/15. doi: 10.1093/ajcn/nqy075. PubMed PMID: 29901710; PMCID: PMC6600057.

52. Maki KC, Wilcox ML, Dicklin MR, Buggia M, Palacios OM, Maki CE, Kramer M. Substituting Lean Beef for Carbohydrate in a Healthy Dietary Pattern Does Not Adversely Affect the Cardiometabolic Risk Factor Profile in

Men and Women at Risk for Type 2 Diabetes. J Nutr. 2020. Epub 2020/05/03. doi: 10.1093/jn/nxaa116. PubMed PMID: 32359153.

53. Schmedes M, Aadland EK, Sundekilde UK, Jacques H, Lavigne C, Graff IE, Eng Ø, Holthe A, Mellgren G, Young JF, Bertram HC, Liaset B, Clausen MR. Lean-seafood intake decreases urinary markers of mitochondrial lipid and energy metabolism in healthy subjects: Metabolomics results from a randomized crossover intervention study. Mol Nutr Food Res. 2016;60(7):1661-72. Epub 2016/02/14. doi: 10.1002/mnfr.201500785. PubMed PMID: 26873789.

54. Schmedes M, Balderas C, Aadland EK, Jacques H, Lavigne C, Graff IE, Eng Ø, Holthe A, Mellgren G, Young JF, Sundekilde UK, Liaset B, Bertram HC. The Effect of Lean-Seafood and Non-Seafood Diets on Fasting and Postprandial Serum Metabolites and Lipid Species: Results from a Randomized Crossover Intervention Study in Healthy Adults. Nutrients. 2018;10(5). Epub 2018/05/13. doi: 10.3390/nu10050598. PubMed PMID: 29751643; PMCID: PMC5986478.

55. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505(7484):559-63. Epub 2013/12/18. doi: 10.1038/nature12820. PubMed PMID: 24336217; PMCID: PMC3957428.

56. Rios-Covian D, Gueimonde M, Duncan SH, Flint HJ, de los Reyes-Gavilan CG. Enhanced butyrate formation by cross-feeding between Faecalibacterium prausnitzii and Bifidobacterium adolescentis. FEMS Microbiol Lett. 2015;362(21). Epub 2015/10/01. doi: 10.1093/femsle/fnv176. PubMed PMID: 26420851.

57. Flint HJ, Duncan SH, Scott KP, Louis P. Links between diet, gut microbiota composition and gut metabolism. Proc Nutr Soc. 2015;74(1):13-22. Epub 2014/10/01. doi: 10.1017/s0029665114001463. PubMed PMID: 25268552.

58. Schmedes M, Brejnrod AD, Aadland EK, Kiilerich P, Kristiansen K, Jacques H, Lavigne C, Graff IE, Eng Ø, Holthe A, Mellgren G, Young JF, Sundekilde UK, Liaset B, Bertram HC. The Effect of Lean-Seafood and Non-Seafood Diets on Fecal Metabolites and Gut Microbiome: Results from a Randomized Crossover Intervention Study. Mol Nutr Food Res. 2019;63(1):e1700976. Epub 2018/03/07. doi: 10.1002/mnfr.201700976. PubMed PMID: 29509315.

59. Navarro SL, Tarkhan A, Shojaie A, Randolph TW, Gu H, Djukovic D, Osterbauer KJ, Hullar MA, Kratz M, Neuhouser ML, Lampe PD, Raftery D, Lampe JW. Plasma metabolomics profiles suggest beneficial effects of a low-glycemic load dietary pattern on inflammation and energy metabolism. Am J Clin Nutr. 2019;110(4):984-92. Epub 2019/08/23. doi: 10.1093/ajcn/nqz169. PubMed PMID: 31432072; PMCID: PMC6766441.

60. Ginos BNR, Navarro SL, Schwarz Y, Gu H, Wang D, Randolph TW, Shojaie A, Hullar MAJ, Lampe PD, Kratz M, Neuhouser ML, Raftery D, Lampe JW. Circulating bile acids in healthy adults respond differently to a dietary pattern characterized by whole grains, legumes and fruits and vegetables compared to a diet high in refined grains and added sugars: A randomized, controlled, crossover feeding study. Metabolism. 2018;83:197-204. Epub 2018/02/20. doi: 10.1016/j.metabol.2018.02.006. PubMed PMID: 29458053; PMCID: PMC5960615.

61. Miles FL, Navarro SL, Schwarz Y, Gu H, Djukovic D, Randolph TW, Shojaie A, Kratz M, Hullar MAJ, Lampe PD, Neuhouser ML, Raftery D, Lampe JW. Plasma metabolite abundances are associated with urinary enterolactone excretion in healthy participants on controlled diets. Food Funct. 2017;8(9):3209- 18. doi: 10.1039/c7fo00684e. PubMed PMID: 28808723; PMCID: PMC5607107.

62. Pan S, Hullar MAJ, Lai LA, Peng H, May DH, Noble WS, Raftery D, Navarro SL, Neuhouser ML, Lampe PD, Lampe JW, Chen R. Gut Microbial Protein Expression in Response to Dietary Patterns in a Controlled Feeding Study: A Metaproteomic Approach. Microorganisms. 2020;8(3). Epub 2020/03/12. doi: 10.3390/microorganisms8030379. PubMed PMID: 32156071; PMCID: PMC7143255.

63. Maskarinec G, Hullar MAJ, Monroe KR, Shepherd JA, Hunt J, Randolph TW, Wilkens LR, Boushey CJ, Le Marchand L, Lim U, Lampe JW. Fecal Microbial Diversity and Structure Are Associated with Diet Quality in the Multiethnic Cohort Adiposity Phenotype Study. J Nutr. 2019;149(9):1575-84. Epub 2019/06/13. doi: 10.1093/jn/nxz065. PubMed PMID: 31187868; PMCID: PMC6862930.

64. Khakimov B, Poulsen SK, Savorani F, Acar E, Gürdeniz G, Larsen TM, Astrup A, Dragsted LO, Engelsen SB. New Nordic Diet versus Average Danish Diet: A Randomized Controlled Trial Revealed Healthy Long-Term

Effects of the New Nordic Diet by GC-MS Blood Plasma Metabolomics. J Proteome Res. 2016;15(6):1939-54. Epub 2016/05/06. doi: 10.1021/acs.jproteome.6b00109. PubMed PMID: 27146725.

65. Landberg R, Hanhineva K. Biomarkers of a Healthy Nordic Diet-From Dietary Exposure Biomarkers to Microbiota Signatures in the Metabolome. Nutrients. 2019;12(1). Epub 2019/12/28. doi: 10.3390/nu12010027. PubMed PMID: 31877633; PMCID: PMC7019922.

66. Martínez-González M, Ruiz-Canela M, Hruby A, Liang L, Trichopoulou A, Hu FB. Intervention Trials with the Mediterranean Diet in Cardiovascular Prevention: Understanding Potential Mechanisms through Metabolomic Profiling. J Nutr. 2015;146(4):913s-9s. Epub 2016/03/11. doi: 10.3945/jn.115.219147. PubMed PMID: 26962184; PMCID: PMC4807639.

67. Playdon MC, Moore SC, Derkach A, Reedy J, Subar AF, Sampson JN, Albanes D, Gu F, Kontto J, Lassale C, Liao LM, Mannisto S, Mondul AM, Weinstein SJ, Irwin ML, Mayne ST, Stolzenberg-Solomon R. Identifying biomarkers of dietary patterns by using metabolomics. Am J Clin Nutr. 2017;105(2):450- 65. Epub 2016/12/30. doi: 10.3945/ajcn.116.144501. PubMed PMID: 28031192; PMCID: PMC5267308.

68. Gibbons H, Carr E, McNulty BA, Nugent AP, Walton J, Flynn A, Gibney MJ, Brennan L. Metabolomic-based identification of clusters that reflect dietary patterns. Mol Nutr Food Res. 2017;61(10). Epub 2017/06/07. doi: 10.1002/mnfr.201601050. PubMed PMID: 28586169.

69. Bondia-Pons I, Cañellas N, Abete I, Rodríguez M, Perez-Cornago A, Navas-Carretero S, Zulet M, Correig X, Martínez JA. Nutri-metabolomics: subtle serum metabolic differences in healthy subjects by NMR-based metabolomics after a short-term nutritional intervention with two tomato sauces. OMICS. 2013;17(12):611-8. Epub 2013/10/22. doi: 10.1089/omi.2013.0027. PubMed PMID: 24138070.

70. Keski-Rahkonen P, Kolehmainen M, Lappi J, Micard V, Jokkala J, Rosa-Sibakov N, Pihlajamäki J, Kirjavainen PV, Mykkänen H, Poutanen K, Gunter MJ, Scalbert A, Hanhineva K. Decreased plasma serotonin and other metabolite changes in healthy adults after consumption of wholegrain rye: an untargeted metabolomics study. Am J Clin Nutr. 2019;109(6):1630-9. Epub 2019/05/29. doi: 10.1093/ajcn/nqy394. PubMed PMID: 31136658.

71. Hanhineva K, Lankinen MA, Pedret A, Schwab U, Kolehmainen M, Paananen J, de Mello V, Sola R, Lehtonen M, Poutanen K, Uusitupa M, Mykkänen H. Nontargeted metabolite profiling discriminates diet-specific biomarkers for consumption of whole grains, fatty fish, and bilberries in a randomized controlled trial. J Nutr. 2015;145(1):7-17. Epub 2014/12/21. doi: 10.3945/jn.114.196840. PubMed PMID: 25527657.

72. Derkach A, Sampson J, Joseph J, Playdon MC, Stolzenberg-Solomon RZ. Effects of dietary sodium on metabolites: the Dietary Approaches to Stop Hypertension (DASH)-Sodium Feeding Study. Am J Clin Nutr. 2017;106(4):1131-41. Epub 2017/09/01. doi: 10.3945/ajcn.116.150136. PubMed PMID: 28855223; PMCID: PMC5611778.

73. Acar E, Gurdeniz G, Khakimov B, Savorani F, Korndal SK, Larsen TM, Engelsen SB, Astrup A, Dragsted LO. Biomarkers of individual foods, and separation of diets using untargeted LC-MS-based plasma metabolomics in a randomized controlled trial. Mol Nutr Food Res. 2019;63(1):e1800215. Epub 2018/08/11. doi: 10.1002/mnfr.201800215. PubMed PMID: 30094970.

74. Ulven SM, Christensen JJ, Nygård O, Svardal A, Leder L, Ottestad I, Lysne V, Laupsa-Borge J, Ueland PM, Midttun Ø, Meyer K, McCann A, Andersen LF, Holven KB. Using metabolic profiling and gene expression analyses to explore molecular effects of replacing saturated fat with polyunsaturated fat-a randomized controlled dietary intervention study. Am J Clin Nutr. 2019;109(5):1239-50. Epub 2019/05/06. doi: 10.1093/ajcn/nqy356. PubMed PMID: 31051508; PMCID: PMC6499508.

75. Cheung W, Keski-Rahkonen P, Assi N, Ferrari P, Freisling H, Rinaldi S, Slimani N, Zamora-Ros R, Rundle M, Frost G, Gibbons H, Carr E, Brennan L, Cross AJ, Pala V, Panico S, Sacerdote C, Palli D, Tumino R, Kühn T, Kaaks R, Boeing H, Floegel A, Mancini F, Boutron-Ruault MC, Baglietto L, Trichopoulou A, Naska A, Orfanos P, Scalbert A. A metabolomic study of biomarkers of meat and fish intake. Am J Clin Nutr. 2017;105(3):600-8. Epub 2017/01/27. doi: 10.3945/ajcn.116.146639. PubMed PMID: 28122782.

76. Scalbert A, Brennan L, Manach C, Andres-Lacueva C, Dragsted LO, Draper J, Rappaport SM, van der Hooft JJ, Wishart DS. The food metabolome: a window over dietary exposure. Am J Clin Nutr. 2014;99(6):1286-308. Epub 2014/04/25. doi: 10.3945/ajcn.113.076133. PubMed PMID: 24760973.

77. Ijssennagger N, Belzer C, Hooiveld GJ, Dekker J, van Mil SW, Müller M, Kleerebezem M, van der Meer R. Gut microbiota facilitates dietary heme-induced epithelial hyperproliferation by opening the mucus barrier in colon. Proc Natl Acad Sci U S A. 2015;112(32):10038-43. Epub 2015/07/29. doi: 10.1073/pnas.1507645112. PubMed PMID: 26216954; PMCID: PMC4538683.

78. Gregory LG, Bond PL, Richardson DJ, Spiro S. Characterization of a nitrate-respiring bacterial community using the nitrate reductase gene (narG) as a functional marker. Microbiology. 2003;149(Pt 1):229-37. Epub 2003/02/11. doi: 10.1099/mic.0.25849-0. PubMed PMID: 12576596.

79. Zhang Y, Xu Z, Li J, Liu D, Yuan Y, Chen Z, Wang G. Cooperation between two strains of Enterobacter and Klebsiella in the simultaneous nitrogen removal and phosphate accumulation processes. Bioresour Technol. 2019;291:121854. Epub 2019/07/30. doi: 10.1016/j.biortech.2019.121854. PubMed PMID: 31357041.

80. Wedekind R, Kiss A, Keski-Rahkonen P, Viallon V, Rothwell JA, Cross AJ, Rostgaard-Hansen AL, Sandanger TM, Jakszyn P, Schmidt JA, Pala V, Vermeulen R, Schulze MB, Kühn T, Johnson T, Trichopoulou A, Peppa E, La Vechia C, Masala G, Tumino R, Sacerdote C, Wittenbecher C, de Magistris MS, Dahm CC, Severi G, Mancini FR, Weiderpass E, Gunter MJ, Huybrechts I, Scalbert A. A metabolomic study of red and processed meat intake and acylcarnitine concentrations in human urine and blood. Am J Clin Nutr. 2020. Epub 2020/06/04. doi: 10.1093/ajcn/nqaa140. PubMed PMID: 32492168.

81. Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, Bright IJ, Lucero MY, Hiddessen AL, Legler TC, Kitano TK, Hodel MR, Petersen JF, Wyatt PW, Steenblock ER, Shah PH, Bousse LJ, Troup CB, Mellen JC, Wittmann DK, Erndt NG, Cauley TH, Koehler RT, So AP, Dube S, Rose KA, Montesclaros L, Wang S, Stumbo DP, Hodges SP, Romine S, Milanovich FP, White HE, Regan JF, Karlin-Neumann GA, Hindson CM, Saxonov S, Colston BW. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal Chem. 2011;83(22):8604-10. Epub 2011/11/01. doi: 10.1021/ac202028g. PubMed PMID: 22035192; PMCID: PMC3216358.

82. Huggett JF, Foy CA, Benes V, Emslie K, Garson JA, Haynes R, Hellemans J, Kubista M, Mueller RD, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT, Bustin SA. The digital MIQE guidelines: Minimum Information for Publication of Quantitative Digital PCR Experiments. Clin Chem. 2013;59(6):892-902. Epub 2013/04/11. doi: 10.1373/clinchem.2013.206375. PubMed PMID: 23570709.

83. Cao Y, Raith MR, Griffith JF. Droplet digital PCR for simultaneous quantification of general and human-associated fecal indicators for water quality assessment. Water Res. 2015;70:337-49. Epub 2014/12/30. doi: 10.1016/j.watres.2014.12.008. PubMed PMID: 25543243.

84. Nadkarni MA, Martin FE, Jacques NA, Hunter N. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology. 2002;148(Pt 1):257-66. Epub 2002/01/10. doi: 10.1099/00221287-148-1-257. PubMed PMID: 11782518.

85. Neuhouser ML, Schwarz Y, Wang C, Breymeyer K, Coronado G, Wang CY, Noar K, Song X, Lampe JW. A low-glycemic load diet reduces serum C-reactive protein and modestly increases adiponectin in overweight and obese adults. J Nutr. 2012;142(2):369-74. Epub 2011/12/23. doi: 10.3945/jn.111.149807. PubMed PMID: 22190020; PMCID: PMC3260063.

86. Prentice RL, Mossavar-Rahmani Y, Huang Y, Van Horn L, Beresford SA, Caan B, Tinker L, Schoeller D, Bingham S, Eaton CB, Thomson C, Johnson KC, Ockene J, Sarto G, Heiss G, Neuhouser ML. Evaluation and comparison of food records, recalls, and frequencies for energy and protein assessment by using recovery biomarkers. Am J Epidemiol. 2011;174(5):591-603. Epub 2011/07/19. doi: 10.1093/aje/kwr140. PubMed PMID: 21765003; PMCID: PMC3202154.

87. Lampe JW, Huang Y, Neuhouser ML, Tinker LF, Song X, Schoeller DA, Kim S, Raftery D, Di C, Zheng C, Schwarz Y, Van Horn L, Thomson CA, Mossavar-Rahmani Y, Beresford SA, Prentice RL. Dietary biomarker evaluation in a controlled feeding study in women from the Women's Health Initiative cohort. Am J Clin Nutr. 2017;105(2):466-75. doi: 10.3945/ajcn.116.144840. PubMed PMID: 28031191; PMCID: PMC5267309.

88. Runchey SS, Valsta LM, Schwarz Y, Wang C, Song X, Lampe JW, Neuhouser ML. Effect of low- and high-glycemic load on circulating incretins in a randomized clinical trial. Metabolism. 2013;62(2):188-95. Epub 2012/09/11. doi: 10.1016/j.metabol.2012.07.006. PubMed PMID: 22959497; PMCID: PMC3519963.

89. Santiago-Torres M, Kratz M, Lampe JW, Tapsoba J de D, Breymeyer KL, Levy L, Villasenor A, Wang CY, Song X, Neuhouser ML. Metabolic responses to a traditional Mexican diet compared with a commonly consumed US diet in women of Mexican descent: a randomized crossover feeding trial. Am J Clin Nutr. 2016;103(2):366-74. doi: 10.3945/ajcn.115.119016. PubMed PMID: 26718418; PMCID: PMC4733259.

90. Neuhouser ML, Clowry C, Beatty SJ, Wang CY, Drewnowski A, Perrigue MM. Rationale and design of the frequency of eating and Satiety Hormones (FRESH) study: A randomized cross-over clinical trial. Contemp Clin Trials Commun. 2019;14:100334. Epub 2019/03/06. doi: 10.1016/j.conctc.2019.100334. PubMed PMID: 30834353; PMCID: PMC6384307.

91. Staten LK, Taren DL, Howell WH, Tobar M, Poehlman ET, Hill A, Reid PM, Ritenbaugh C. Validation of the Arizona Activity Frequency Questionnaire using doubly labeled water. Med Sci Sports Exerc. 2001;33(11):1959-67. Epub 2001/11/02. PubMed PMID: 11689750.

92. Mifflin MD, St. Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh Y. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr. 1990;51(2):241-7.

93. Nagana Gowda GA, Raftery D. Overview of NMR Spectroscopy-Based Metabolomics: Opportunities and Challenges. Methods Mol Biol. 2019;2037:3-14. Epub 2019/08/30. doi: 10.1007/978-1-4939-9690-2_1. PubMed PMID: 31463836.

94. Nagana Gowda GA, Raftery D. Analysis of Plasma, Serum, and Whole Blood Metabolites Using (1)H NMR Spectroscopy. Methods Mol Biol. 2019;2037:17-34. Epub 2019/08/30. doi: 10.1007/978-1-4939- 9690-2_2. PubMed PMID: 31463837.

95. Emwas AH, Roy R, McKay RT, Ryan D, Brennan L, Tenori L, Luchinat C, Gao X, Zeri AC, Gowda GA, Raftery D, Steinbeck C, Salek RM, Wishart DS. Recommendations and standardization of biomarker quantification using NMR-based metabolomics with particular focus on urinary analysis. J Proteome Res. 2016;15(2):360-73. Epub 2016/01/09. doi: 10.1021/acs.jproteome.5b00885. PubMed PMID: 26745651; PMCID: PMC4865177.

96. Emwas AH, Luchinat C, Turano P, Tenori L, Roy R, Salek RM, Ryan D, Merzaban JS, Kaddurah- Daouk R, Zeri AC, Nagana Gowda GA, Raftery D, Wang Y, Brennan L, Wishart DS. Standardizing the experimental conditions for using urine in NMR-based metabolomic studies with a particular focus on diagnostic studies: a review. Metabolomics. 2015;11(4):872-94. Epub 2015/06/26. doi: 10.1007/s11306-014-0746-7. PubMed PMID: 26109927; PMCID: PMC4475544.

97. Fei Q, Wang D, Jasbi P, Zhang P, Nagana Gowda GA, Raftery D, Gu H. Combining NMR and MS with Chemical Derivatization for Absolute Quantification with Reduced Matrix Effects. Anal Chem. 2019;91(6):4055-62. Epub 2019/02/26. doi: 10.1021/acs.analchem.8b05611. PubMed PMID: 30801179.

98. Marshall DD, Lei S, Worley B, Huang Y, Garcia-Garcia A, Franco R, Dodds ED, Powers R. Combining DI-ESI-MS and NMR datasets for metabolic profiling. Metabolomics. 2015;11(2):391-402. Epub 2015/03/17. doi: 10.1007/s11306-014-0704-4. PubMed PMID: 25774104; PMCID: PMC4354777.

99. Benjamini Y, Hochberg Y. Controlling the false discovery rate - a practical and powerful approach to multiple testing. J Roy Stat Soc. 1995;57(1):289-300.

100. Li F, Hullar MA, Lampe JW. Optimization of terminal restriction fragment polymorphism (TRFLP) analysis of human gut microbiota. J Microbiol Methods. 2007;68(2):303-11. Epub 2006/10/31. doi: 10.1016/j.mimet.2006.09.006. PubMed PMID: 17069911; PMCID: PMC1853299.

101. Fu BC, Randolph TW, Lim U, Monroe KR, Cheng I, Wilkens LR, Le Marchand L, Hullar MA, Lampe JW. Characterization of the gut microbiome in epidemiologic studies: the multiethnic cohort experience. Ann Epidemiol. 2016;26(5):373-9. Epub 2016/04/04. doi: 10.1016/j.annepidem.2016.02.009. PubMed PMID: 27039047; PMCID: PMC4892953.

102. Fu BC, Randolph TW, Lim U, Monroe KR, Cheng I, Wilkens LR, Le Marchand L, Lampe JW, Hullar MAJ. Temporal Variability and Stability of the Fecal Microbiome: The Multiethnic Cohort Study. Cancer Epidemiol Biomarkers Prev. 2019;28(1):154-62. Epub 2018/09/13. doi: 10.1158/1055-9965.epi-18- 0348. PubMed PMID: 30206059; PMCID: PMC6625800.

103. Vogelstein B, Kinzler KW. Digital PCR. Proc Natl Acad Sci U S A. 1999;96(16):9236-41. Epub 1999/08/04. doi: 10.1073/pnas.96.16.9236. PubMed PMID: 10430926; PMCID: PMC17763.

104. Fernandes AD, Reid JN, Macklaim JM, McMurrough TA, Edgell DR, Gloor GB. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. Microbiome. 2014;2:15. Epub 2014/06/10. doi: 10.1186/2049-2618-2-15. PubMed PMID: 24910773; PMCID: PMC4030730.

105. Koh H, Li Y, Zhan X, Chen J, Zhao N. A Distance-Based Kernel Association Test Based on the Generalized Linear Mixed Model for Correlated Microbiome Studies. Front Genet. 2019;10:458. Epub 2019/06/04. doi: 10.3389/fgene.2019.00458. PubMed PMID: 31156711; PMCID: PMC6532659.

106. McDonald D, Vázquez-Baeza Y, Koslicki D, McClelland J, Reeve N, Xu Z, Gonzalez A, Knight R. Striped UniFrac: enabling microbiome analysis at unprecedented scale. Nat Methods. 2018;15(11):847-8. Epub 2018/11/01. doi: 10.1038/s41592-018-0187-8. PubMed PMID: 30377368; PMCID: PMC7250580.

107. Lozupone CA, Knight R. The UniFrac significance test is sensitive to tree topology. BMC Bioinformatics. 2015;16(1):211. Epub 2015/07/08. doi: 10.1186/s12859-015-0640-y. PubMed PMID: 26150095; PMCID: PMC4492014.

108. Randolph TW, Zhao S, Copeland W, Hullar M, Shojaie A. KERNEL-penalized Regression for analysis of micorbiome data. Ann Appl Stat. 2018;12(1):540-66. Epub 2018/09/19. doi: 10.1214/17-aoas1102. PubMed PMID: 30224943; PMCID: PMC6138053.

109. Bae S, Ulrich CM, Neuhouser ML, Malysheva O, Bailey LB, Xiao L, Brown EC, Cushing-Haugen KL, Zheng Y, Cheng TY, Miller JW, Green R, Lane DS, Beresford SA, Caudill MA. Plasma choline metabolites and colorectal cancer risk in the Women's Health Initiative Observational Study. Cancer Res. 2014;74(24):7442-52. Epub 2014/10/23. doi: 10.1158/0008-5472.can-14-1835. PubMed PMID: 25336191; PMCID: PMC4268282.

110. Nyamundanda G, Gomley IC, Fan Y, Gallagher M, Brennan L. MetSizeR: selecting the optimal sample size for metabolomic studies using an analysis based approach. BMC Bioinformatics; 2013; 14:338.