TITLE: The Effects of Agro-ecological Farming Systems on Human Health

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TABLE OF CONTENTS

1. BACKGROUND AND SIGNIFICANCE

As concerns regarding the effects of agriculture on human and environmental health mount[1,2], a growing number of farmers are seeking ways to improve health from the ground up. A promising way by which a growing number farmers are seeking to improve environmental health is by using agro-ecological practices (i.e., farming more closely in harmony with natural systems), which include practices such as multi-cropping, ley rotations, and/or integrated crop-livestock systems[3]. When properly implemented, agro-ecological practices can improve soil health, nutrient cycling, and biodiversity above and below ground[3-5]. Despite potential major ecological benefits, we lack critical knowledge if consuming foods from agro-ecological systems also benefits human health. The purpose of this project is to test the hypothesis that consuming foods produced using agro-ecological practices improves consumer health compared to consuming similar foods from conventional (monoculture) farming systems.

This work will utilize a randomized cross-over design to compare an agroecological vs conventional sourced diet, and determine their effects on inflammation and cardio metabolic health signatures in middle-aged adults (35-60 y old). Diets will be isocaloric and matched for macronutrient content, and consumed for 44 days each with a 14-day washout (habitual diet) period in between. Blood, urine, and stool samples will be obtained before and after the dietary interventions, in addition to data about physical activity and questionnaires regarding quality of life.

2. STUDY OBJECTIVES

1. To determine the effects on **plasma inflammatory markers (IL-6, TNF-α, C-reactive protein, and VCAM-1)** before and after each dietary intervention.

2. To determine effects on **urinary and plasma metabolites (vitamin and mineral derivatives, polyphenols, amino acids, glucose metabolites etc.)** to provide insight into metabolic health pathways before and after each dietary intervention.

3. To determine effects on **gut microbiota communities** (alpha and beta diversity, and short-chain fatty acid producing bacteria) before and after each dietary intervention.

3. DESIGN AND PROCEDURES

3.1 Study duration

Total involvement for each participant will be 16 weeks. Each diet will be followed for 44 days (4 day rotating menus x 11 periods) with a 14-day washout period in between.

3.2 Participants

We will include a total of 40 middle aged adults (35-60 years) of all races and ethnicities in this study. Inclusion/exclusion criteria are listed below.

***Inclusion criteria*:**

- Age ≥35 and ≤60 years;
- BMI ≥25 and ≤35 kg/m^2;
- Weight stable in last 3 months (loss or gain <4%);
- Hemoglobin A1C (HbA1C ≤6.4%);
- Fasting plasma glucose concentration <126 mg/dl;
- For the safety of the participant and proper consent of the procedures, subjects must be able to speak and understand English to participate in this study;
- Stable medication/supplement use for 3 months prior to study;

***Exclusion criteria*:**

- Use of medications that are known to affect the study outcome measures (e.g., NSAIDs, corticosteroids) or increase the risk of study procedures (e.g., anticoagulants) that cannot be temporarily discontinued for this study;
- Strict dietary patterns (e.g., vegan, keto);
- Consuming >14 alcoholic drinks per week;
- Use of cigarettes (or other tobacco products) in last 3 months;
- Engaged in high level of competitive exercise (e.g., iron man, marathons, powerlifting);
- Diagnoses of active malignancy, congestive heart failure, diabetes mellitus or chronic obstructive pulmonary disease;
- Any inflammatory diseases (e.g., autoimmune diseases, coeliac disease, glomerulonephritis, hepatitis, inflammatory bowel disease, arthritis);
- Use of antibiotics in last 60 days;
- Pregnant or planning to become pregnant in the next 5 months;
- Lactating women;
- Persons who are unable or unwilling to follow the study protocol or who, for any reason, the research team considers not an appropriate candidate for this study, including non-compliance with screening appointments or study visits.

* Participants that are pregnant or planning on becoming pregnant in the next 5 months are excluded. The justification is the documented alterations in metabolism that occur during pregnancy [6], which would impact our metabolomics analysis in this study.

Exclusion criteria will primarily be identified by way of a phone/email screen and routine bloodwork (when determined eligible based on the phone-screen and/or email-screen and after being consented).

3.3 Study Schedule

ACTIVITY/VISIT	WEEK	DURATION
PHONE SCREEN A scripted phone screen will be performed to determine pre-eligibility prior to consent.	1	15 min
CONSENT A one-on-one consent visit will be conducted privately with staff either in-person or via Zoom.	1	1 h
SCREENING This visit will take place in the morning after an overnight fast and will have the following procedures: informed consent, screening blood draw (including serum pregnancy test for women of child-bearing potential), health history, height and weight, and screening questionnaires regarding food intake, health, and sleep.	1	2 h
BASELINE VISIT DIET 1 This visit will take place in the morning after an overnight fast. Participants will be asked to complete a fasted blood draw and questionnaires, and bring in urine and stool samples that were collected using at-home collection kits.	2	1 h
INTERVENTION DIET 1 Participants will be randomized to consume one of the following sourced diets: agroecological or conventional. They will follow this nutrition pattern for 44 days. During this time, participants will come to the NDFS building twice a week to pick up food.	2-8	1 h per week
POST VISIT DIET 1 On the last day of diet 1, participants will be asked to complete a fasted blood draw and questionnaires, and bring in urine and stool samples that were collected using at-home collection kits.	8	1 h
WASHOUT PERIOD Participants will consume self-selected (habitual) diets for 14 days. No involvement from research team.	9-10	none
BASELINE VISIT DIET 2 This visit will take place in the morning and will have a fasted blood draw. Participants are also asked to bring in stool and urine samples that were collected using at-home collection kits.	10	1 h
INTERVENTION DIET 2 Participants will now start their second nutritional intervention (agroecological or conventional sourced diet depending on the diet consumed during the first intervention). During this time, participants will come to the NDFS building twice a week to pick up food.	10-16	1 h per week

POST VISIT DIET 2 On the last day of diet 2, participants will be asked to complete a fasted blood draw and questionnaires, and bring in urine and stool samples that were collected using at-home collection kits.	16	1 h

3.4 Description of study visits

Participants will be asked to complete four testing visits throughout their participation in the study (pre and post visits for each of the diets) as well as complete two on-site visits per week during each of the 44-day dietary intervention periods to consume a meal and obtain a current body weight measurement. All visits will take place in clinical research space located at the Center for Human Nutrition Studies (CHNS), except for food pickup, which will take place at the NDFS building.

Phone Screen (Week 1. Duration: 15 min): Interested participants will be pre-screened by telephone, using a scripted list of questions (attached) to identify individuals that may be eligible for study entry. Suitable candidates will then be provided a REDCap survey link to complete additional screening questions that asks them further details about their health, dietary habits, and sleep habits (see inclusion/exclusion criteria). Only the minimum PHI will be collected to determine eligibility. Responses will be reviewed by a study team member (all study team members will have completed the necessary CITI training) and potentially eligible candidates will be contacted to schedule a consent/screening visit. The pre-screen is put in place to confirm that the participant meets most of the inclusion/exclusion criteria before the subject is scheduled for a consent meeting. The phone screen is thus performed to limit the amount of ineligible research participants that are consented/screened that could easily be excluded by means of a phone screening. This would also provide interested participants with the opportunity to ask additional questions about the study and to determine if they are interested in moving forward with a consent/screening visit. This will limit both subject and research staff time burden.

Consent Visit (Week 1. Duration: 1 hour): This visit can be done virtually through Zoom or occur in person in the Center for Human Nutrition Studies (CHNS) at Utah State University. Potential subjects will attend a consent session conducted by a trained member of the research staff to present the details of the study. Interested participants will complete the informed consent process privately with study staff. We will allow up to 60 minutes for the subjects to read the consent and ask questions. If a subject wants additional time to think about the study, this will be allowed. The study team will contact the subject three days after the initial consent visit ask if he or she has come to a decision. No study procedures will take place before written consent is obtained. To minimize subject burden, participants will be instructed during the phone screen that they have to opportunity to combine the consent visit with their screening visit (see below). If participants wish to combine visits, they are instructed that they will have to perform this visit in the morning after an overnight, 12-hour fast.

Screening Visit (Week 1. Duration: 2 hours): Study participants will be asked to come in during the morning after an overnight, 12-hour fast. During this visit the following will take place:

- **Blood Draw:** A trained phlebotomist (the PI) will draw a fasted blood sample for routine blood work to ensure the participant qualifies for the study. Using a small needle, 15 ml blood will be collected from a vein in the participant's forearm or hand. Blood samples will be sent to LabCorp to be analyzed for HbA1C, Glucose, Basic Metabolic Panel (BMP), and Lipid panel. The blood of women of childbearing potential will be subject to a pregnancy test (hCG) as part of this screening blood draw. This will also occur through LabCorp.

- **Body mass and height.** Height (cm) will be measured without shoes. Body mass (kg) will be measured without shoes, coats, or sweaters. BMI will be calculated as body mass (kg) / height $(m)^2$.

- **Blood pressure:** A blood pressure measurement will be taken using an automated upper arm blood pressure monitor (Omron BP5250). A total of three measurements will be taken and the average value of those will be collected for participant characterization.

- **Questionnaires:** Participants will be asked to complete the following questionnaires:

  - *7-day food log:* The seven-day food log will be filled out at home and returned during baseline visit (described below) to provide further insight into the subject's habitual diet.
  - *Food Frequency Questionnaire (FFQ):* The food frequency questionnaires will help identify any dietary restrictions and ensure that the subject's habitual diet meets the inclusion/exclusion criteria.

Baseline Visit 1 (Week 2. Duration: 1 hour): If inclusion/exclusion criteria are met, subjects will be invited for a baseline visit to complete additional measurements. We will ask participants to come in during the morning after an overnight, 12-hour fast. During this visit the following procedures will be performed:

- **Questionnaires:** Participants will be asked to complete the following questionnaires:

  - *Pittsburgh Sleep Quality Index (PSQI):* The Pittsburg Sleep Quality Index (PSQI) will be used to assess sleep quality and disturbances. This questionnaire will be repeated after each intervention period.

  - *The Perceived Stress Scale (PSS):* PSS evaluates how unpredictable, uncontrollable, and overloaded participants judge their lives. This questionnaire will be repeated after each intervention period.

- *Profile of Mood States (POMS):* The Profile of Mood States (POMS) 30-item version will determine mood changes using the following domains: Tenxion-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia and Confusion-Bewilderment. This questionnaire will be repeated after each intervention period.

- *Short Form Healthy Survey (SF-36):* The SF-36 will be used to assess quality of life using physical (functioning, role-physical, pain, general health) and mental domains (vitality, social functioning, role-emotional, and mental health). This questionnaire will be repeated after each intervention period.

- *MacArthur Socio-Economic Status Questionnaire:* This questionnaire determines participants subjective social status questions, as well as educational attainment, occupational status, income, and assets. This information is used for participant characterization and only completed at baseline.

- **Stool and Urine Collection:** Participants will be provided with a urine and stool collection kit to collect first-of-the-day stool and urine samples (see attached). Participants will receive detailed instructions on freezer storage and are provided a cooler with ice packs to bring in the frozen samples during their next visit.

- **Blood Draw:** A trained phlebotomist (the PI) will draw a fasted blood sample for metabolomics and inflammatory biomarker assays. A total of 15 mL of blood (1 TBPS) will be drawn. Our plans for measuring cytokines and metabolites are discussed in the *Analytical Procedures and Statistical Considerations subsection* (see below).

Intervention (44 days x 2 diets; 1 hour per week): After completing all baseline testing, participants will be randomized to one of the two starting diets (agroecological or conventional, for 44 days with a 14 day washout period until they start their second diet (agroecological or conventional depending on the starting diet). In accordance with established protocols, a research dietitian will provide weight-maintenance diets with a macronutrient distribution of 20% protein, 30% fat, and 50% carbohydrate based on each individual's daily energy requirement (Harris-Benedict equation). All foods that participants consume during those 4-weeks will be provided by the Metabolic Kitchen at NDFS, and dietitian-led group classes and handouts with food preparation instructions will be provided to the participants to ensure compliance. The diets will be provided as 4-day rotating menus and participants will be asked to pick-up food twice weekly at the NDFS building. This will allow the research time to interact face-to-face with the participant, address any issues, and further ensure compliance. All subjects assigned to the same diet will receive identical meals and snacks, but portion sizes will be determined based on the previously mentioned daily energy requirement. Food for the agroecological diet will be sourced predominantly from the Greenacres farm (Cincinnati, OH) and food for the conventional diet

will be sourced from local grocery stores (non-organic produce). All foods will be stored in food-grade fridges and freezers in the CHNS Metabolic Kitchen and food boxes will be prepared weekly by staff. Examples of the diets are presented in **Table 2.**

Table 2: Examples of Meals

Breakfast	**Whole milk, eggs, oatmeal, and blueberries**
Snack	**Rice cakes**
Lunch	**Ground beef, tomato salad and cherries**
Snack	**Apple with nut butter**
Dinner	**Grilled chicken with rice and steamed broccoli**

Additionally, subjects will complete the following study procedures during each intervention period:

- **Sleep, diet, and activity logs:** We will ask participants to maintain their habitual sleep levels. Participants will be asked to log their wake-up and sleep time as well as the time they spend doing physical activity in RedCap. Participants will be provided a daily link and/or reminded by phone (only if they forgot to fill these out).

- **Stool and urine samples:** Additional stool and urine samples will be collected at the end and beginning of each dietary period, using a similar approach as described in the *baseline visit 1* subsection,

- **Activity:** We will ask participants to maintain their habitual physical activity levels. A commercial Garmin activity monitor will be worn 24 hr/d to record total daily steps as a measure of physical activity. The activity monitor will connect via Bluetooth to the participant's personal smartphone or other internet-connected device loaded with the Garmin Connect app. Data will be stored in individual study accounts on the Garmin website where the study team will be able to access and review activity and monitor device function and provide feedback and encouragement to participants.

Post Diet Visits 1-2 (Weeks 8 & 16; 1 hour): On the last day of each nutrition intervention/diet phase, participants will be asked to come to the CHNS building at Utah State University to undergo a fasted blood draw (30 mL), and to complete questionnaires to determine intervention effects. The blood will be used for cytokines and metabolites and for routine blood samples analysis by LabCorp as described in the *screening visit subsection*. The questionnaires involved include the Perceived Stress Scale (PSS), Short Form Healthy Survey (SF-36), Profile of Mood States (POMS), and the Pittsburgh Sleep Quality Index (PSQI).

Baseline Visit 2 (weeks 8; 1 hour): At the beginning of the second 44-day dietary intervention period, participants will report to the lab facilities within the Center for Human Nutrition at Utah State University after an overnight fast (no eating or drinking except water for 12 h). Participants will be asked to collect fasting urine and first-of-the-day stool samples the morning before coming in, and will undergo a fasted blood draw (30 mL) while in the lab. The blood will be

used for cytokines and metabolites and for routine blood samples analysis by LabCorp as described in the *screening visit subsection*. Participants will also be given detailed instructions on food preparations and provided with their food box for the next 4 days.

4. SUBJECT RECRUITMENT AND COMPENSATION

4.1 Recruitment

Subjects will be recruited by posting flyers in designated areas around USU's campus, and through advertisement on public Facebook pages (Cache Valley Classifieds and/or Marketplace), local NextDoor pages, Craigslist, Reddit (R/Logan), Instagram, and/or Twitter. In the advertisement, potential subjects for this study will be directed to contact the study team by email or phone to obtain more information about the study, if interested. Participants who are interested will be provided with a copy of the informed consent by email. When the participant expresses interest in being in the study, the study team member will confirm, by telephone, that the participant meets most of the inclusion/exclusion criteria (ones that can be determined without the need for a blood draw) before scheduling him/her for a consent meeting. Subjects will attend a one-on-one information/consent session that will be conducted by a trained member to present the details of the study. We anticipate that we will need to telephone screen approximately 150 people and that approximately 30 will be consented. We will enroll up to 40 participants to meet our goal of having at least 18 participants complete the study.

4.2 Compensation

Participants will receive a maximum compensation of $1000. This is a fair amount, in our opinion, as meals and snacks are provided to participants. Participants will thus save on groceries by participating in this study. Participants will receive $25 for the screening visit; however, no compensation will be provided for those who only participate in the consent portion of the screening visit. Participants will receive another $975 after they complete both dietary interventions. Participants will be paid in full at the end of the study. If a participant drops out of the study before completing all two diet intervention periods they will be paid according to the degree of completion if only one diet is completed. No compensation other than the $25 for the screening is provided if participants drop out in the first week of the first diet intervention.

5. RISK/BENEFIT ASSESSMENT

This study provides no potential benefit to the participant other than the satisfaction of having contributed to the potential future benefit of others. Participants will be provided with their lab clinical results, which may be of interest to them. Society may benefit by having more information about how different farming practices impact the healthfulness of foods, which may help inform consumer food choices and food policy. Participation in this study may lead to increased risk for the following:

- **Blood draws:** Blood drawing can result in bruising, mild discomfort, and rarely, an infection where the needle enters the skin. There also is a possibility of lightheadedness and fainting. Sterile techniques and the use of trained phlebotomists will minimize these risks.

- **Questionnaires:** There is no known risks associated with various questionnaires (FFQ, PSQI, SF-36, PSS, POMS, MacArthur Socio-Economic Status Questionnaires, and Physical Activity Questionnaire) administered as part of this study; however, there is a possibility that participants may find some of the questions uncomfortable to answer.

- **Gastrointestinal Distress:** Minor gastrointestinal discomforts and/or distress may result from dietary changes. Dietary interventions that vary from habitual diets in fiber and macronutrients have the possibility of temporal discomfort to participants' gastrointestinal health and, in the event this occurs, the discomfort will be temporal and will subside.

6. COSTS TO THE SUBJECT

There will be no costs to participants for any of the treatments or testing done as part of this research study. Immediate necessary care is available if an individual is injured because of participation in a research project. However, there is no provision for free medical care or for monetary compensation for such injury. During this study, hospitalizations or additional care beyond the scope of this study will be the responsibility of patients and/or their insurance company.

7. ANALYTICAL PROCEDURES AND STATISTICAL CONSIDERATIONS

- **Inflammation:** Inflammatory biomarkers (IL-6, VCAM-1, TNF-alpha and C-reactive protein [CRP]) will be measured using ELISA kits at Dr. van Vliet's laboratory at Utah State University. Plasma samples will be stored at -80ºC until cytokine analyses are performed.

- **Dietary logs:** The Diet History Questionnaire III (NIH) and 7-day food records will be analyzed by dietetic staff within Dr. van Vliet's laboratory at Utah State University.

- **Metabolomics:** Mass spectrometry techniques will be used for untargeted and/or targeted assessment of blood and/or urinary metabolites. This analysis will give insight into the appearance of food-derived metabolites in the human body and how they may impact biomarkers of human health. This analysis will be performed using de-identified samples by Metabolon Inc (a company dedicated to metabolomics analysis) and/or the Plants for Human Health Institute. Samples will be provided to these core services as de-identified samples. No other data will be shared with these core services. Plasma and urine samples will be stored at -80ºC until metabolomics analyses are performed.

- **Gut microbiome taxonomy:** DNA will be extracted from stool samples using a QIAamp Fast DNA Stool Mini Kit following manufacturer's instructions and analyzed for taxonomic assignment using 16s rRNA sequencing at Utah State University's Genomics Core. Samples will be provided to the core service as de-identified samples. No other data will be shared with the core service.

- **Statistical analysis:** Change scores (post-dietary intervention – baseline) will be created for all inflammatory biomarkers and metabolites. Metabolic intermediate data will be reduced into Metabolic Factors using Principal Component Analysis (PCA) of the change scores. Factor scores will be computed for each individual for both diets. Change scores and factor (change) scores for both diets will be compared using paired t-tests. $P< 0.05$ will be considered statistically significant and analyses will be performed in SAS 9.4 (SAS, Cary, NC).

- **Power Calculations:** Using untargeted metabolomics for our power calculations of the number of participants we need to recruit, we assume a P-value ≤ 0.05, Q-value ≤ 0.3, and standard deviation of 0.3 based on previous work[7,8]. 600 metabolites are typically identified and a conservative estimation in longer-term dietary intervention trials is that 40-80 metabolites will be significantly different with a mean of 0.157 (18 % difference). An n=34 per group is expected to provide true discovery rates ranging from 89-93 % assuming differences in 40-80 metabolites. Assuming a drop-out of 25%, the final number of participants we anticipate to recruit is N=40.

8. DATA STORAGE AND CONFIDENTIALITY

Clinical study data (including screening data, food logs, questionnaires, satiety responses, bloodwork) will be collected and managed using REDCap (Research Electronic Data Capture). REDCap is a secure web application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. for data types and range checks), audit trails and a de-identified data export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). The system was developed by a multi-institutional consortium initiated at Vanderbilt University. REDCap servers are hosted in the College of Education & Human Services at Utah State University, where data will be stored and processed. REDCap data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team. This iterative development and testing process results in a well-planned data collection strategy for individual studies. REDCap is flexible enough to be used for a variety of types of research and provides an intuitive user interface for database design and data entry. The exception is Garmin Activity Data which will be stored on the Garmin Website and accessible to the study team by logging in to Garmin's website using a coded email in accordance with participant codes (no personal identifiers will be used). The data will be downloaded and stored (on USU's Box Drive) periodically by the research team. At the end of the study and after all data is downloaded, the account will be removed. Coded clinical data will be stored indefinitely on REDCap and/or USU's Box. The enrollment log, with participant names/addresses that can be linked back to participant codes, which is kept on the Van Vliet Lab Shared Drive on USU's box will be destroyed 3 years after completing the study. It is anticipated that all manuscripts will be published at that time and have been sent to interested study participants. Online activity always carries with them a risk of breach. Participants will be made aware of these potential threats and risks to their confidentiality.

Biospecimens will be stored in a -80ºC freezer at the Center for Human Nutrition Studies for the duration of sample collection. All samples will be stored using coded labels. This will be accomplished by removing or recoding, direct and indirect, identifiers in the bio specimen

data. Some examples of identifiers that will be removed to prevent the risk of association of a participant to their data are: names and initials, birthdate, telephone numbers, email addresses, and any other unique identifying number, code, or characteristic. Blood samples collected during screening will be sent to LabCorp for routine bloodwork. Once all biospecimens have been collected from all participants, all biospecimens will be transported to a -80ºC freezer in the Nutrition and Food Science building for long-term storage (maximum of 20 years). Any participant coded analytical data (including inflammatory biomarkers, metabolomics data, and analyzed food log data) will be kept on the Van Vliet Lab Drive at USU's Box Drive to be accessed only by Dr. Stephan van Vliet and his study staff. Study records that identify subjects will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, they will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of Utah State University. For records disclosed outside of USU, they will be assigned a de-identified code number. The key to the code will be kept on the Van Vliet Lab Drive at USU's Box Drive.

Any electronically collected data using third-party software (Garmin) will be collected using the company's platform and the data will subsequently be downloaded by the study team and stored on the Van Vliet Lab Drive at USU's Box Drive. Information collected by Garmin is subject to their terms of use and any information sent to a third-party software carries potential security risks. Some apps/third party software may be able to perform hidden functions or may have security flaws that allow unauthorized access to information. We are unable to fully assure participants that the information contained within or sent to this mobile app/third-party software are inaccessible or tamper-resistant. Nor can we guarantee that information will not be illicitly stored outside of Utah State University. Apps/third party software may include the capability of sending/receiving information with other apps/third party software, including social networking apps, or websites. If participants give permission to share their data, either inadvertently or deliberately, the terms of use for those apps/websites apply. Participants will be advised to limit personal identifiers entered into mobile applications/third party software only to those that they wish to voluntarily share with others. To protect the PHI of the participants, each participant will be assigned a unique subject ID. To collect physical activity data through Garmin, we will create a generic email account, based on the subject's ID code, which will be used to login to the activity monitor mobile app.

9. DATA SAFETY AND MONITORING

All study members will have completed CITI- and other institutional training pertinent to the study. Any unanticipated problems will be reported to the USU IRB by the PI as per institutional policy.

10. LITERATURE

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Informed Consent

The Effects of Agro-ecological Farming Systems on Human Health

Introduction

You are invited to participate in a research study conducted by Dr. Stephan van Vliet, a researcher in the Nutrition department at Utah State University. The purpose of this research is to compare the effects of foods that are sourced from conventional farming (single crops of animals and plants) and agroecological farming (using sustainable techniques such as growing multiple fruits and vegetables integrated with animals) on markers of your health. The study is funded by the Greenacres Foundation. Your participation is entirely voluntary.

As described in more detail below, we will ask you to participate in a 16-week project, which includes a screening/consent visit and four subsequent testing visits. Someone like you might be interested in participating because of the $1000 compensation, the provided food and meal plans, and the satisfaction of having contributed to the future benefit of others. Because there are some risks with blood draws, such as bleeding, bruising, clotting, minor gastrointestinal distress, or infection, you may not wish to participate. It is important for you to know that you can stop your participation at any time. More information about all aspects of this study is provided below.

This form includes detailed information on the research to help you decide whether to participate. Please read it carefully and ask any questions you have before you agree to participate.

Procedures

The table below summarizes your participation in this study. Each visit and its procedures are described in detail on the next pages. The timeframe presented in the table is a guideline only and will vary based your availability. There will be a total of 5 study visits over 16 weeks and you will pick-up a food box, twice weekly, at the Nutrition Building (650 East 1600 North, Logan, UT 84322) at Utah State University. You will consume two weight-maintenance diets for 44 days each with a 14 day break in between where you will consume your normal diet.

ACTIVITY/VISIT	WEEK	DURATION
PHONE SCREEN A scripted phone screen will be performed to determine pre-eligibility prior to consent.	1	15 min
CONSENT A one-on-one consent visit will be conducted privately with staff either in-person or via Zoom.	1	1 h
SCREENING This visit will take place in the morning after an overnight fast and will have the following procedures: informed consent, screening blood draw (including serum pregnancy test for women of child-bearing potential), health history, height and weight, and screening questionnaires regarding food intake, health, and sleep.	1	2 h
BASELINE VISIT DIET 1 This visit will take place in the morning after an overnight fast. You will be asked to complete a fasted blood draw and questionnaires, and bring in urine and stool samples that were collected using at-home collection kits.	2	1 h
INTERVENTION DIET 1 Participants will be randomized to consume one of the following sourced diets: agroecological or conventional. You will follow this nutrition pattern for 44 days. During this time, you will be asked to come to the NDFS building twice a week to pick up food.	2-8	1 h per week
POST VISIT DIET 1	8	1 h

On the last day of diet 1, you will be asked to complete a fasted blood draw and questionnaires, and bring in urine and stool samples that were collected using at-home collection kits.		
WASHOUT PERIOD Participants will consume self-selected (habitual) diets for 14 days. No involvement from research team.	9-10	none
BASELINE VISIT DIET 2 This visit will take place in the morning and will have a fasted blood draw. You will be asked to bring in stool and urine samples that were collected using at-home collection kits.	10	1 h
INTERVENTION DIET 2 You will now start your second nutritional intervention (agroecological or conventional sourced diet depending on the diet consumed during the first intervention). During this time, you will come to the NDFS building twice a week to pick up food.	10-16	1 h per week
POST VISIT DIET 2 On the last day of diet 2, you will will be asked to complete a fasted blood draw and questionnaires, and bring in urine and stool samples that were collected using at-home collection kits.	16	1 h

Consent (Week 1. Duration: 1 hour): This visit will take place at the Center for Human Nutrition Studies or will happen virtually (via a tele-call platform such as Zoom). This visit will include the following:

- Informed Consent: You will review the consent document with a member of the study team; once all your questions have been satisfactorily answered, you will be asked to sign and date the consent form if you wish to participate in the study before any of the research activities described below take place. You will be asked to sign the consent form electronically through a unique link that is provided to you.

During the phone screening, you will be offered to combine the consent visit with the screening visit described below. If you wish to do so, you will be asked to come to the Center for Human Nutrition Studies in the morning after an overnight fast, which requires you not to eat anything or chew gum (nothing but water) for 12 hours prior to your arrival time. This information will not apply if we do a virtual consent visit first. If you agree to participate you will subsequently come in for an in-person screening visit.

Screening visit (Week 1. Duration: 1 hour): Assuming you pre-qualify for the study and you are interested in participating, you will undergo an in-person screening visit. This visit will take place at the Center for Human Nutrition Studies. This visit will be performed in the morning, will last approximately 1 hour, and requires you not to eat anything or chew gum (nothing but water) for 12 hours prior to your arrival time. This visit will include the following:

- **Body mass and height**: We will measure your height and body weight to determine your body mass index.
- **Blood draw:** You will have blood drawn from a forearm or hand vein for routine lab analyses. The amount drawn will be 12 mL (1 tablespoon) total. If you are a woman of childbearing potential, a pregnancy test will be performed as part of this screening blood draw. It must be negative in order to participate in this study.
- **Questionnaires**: If you agree to participate, you will be emailed a link with questionnaires to fill out. These questionnaires will ask detailed questions about your diet (7-day food log), sleep, and physical activity patterns, which are part of the screening and/or study process.
- **Blood pressure**: A blood pressure measurement will be taken using an automated upper arm blood pressure monitor. A total of three measurements will be taken.

Baseline visits (Weeks 2 and 8. Duration: 1 hour each): These visits will be performed prior to each diet and will take place at the Center for Human Nutrition studies. They will be performed in the morning, will last approximately 1 hour, and requires you not to eat anything or chew gum (nothing but water) for 12 hours prior to your arrival time. This visit will include the following:

- **Questionnaires:** You will be provided with another set of questionnaires. These questionnaires will ask you detailed questions about your wellbeing, quality of life, mood and socioeconomic status.
- **Blood Draw:** You will have blood drawn from a forearm or hand vein for outcome measurements. The amount drawn will be 30 mL (2 tablespoons) total.
- **Stool and Urine Collection Kit:** You will be provided with a stool and urine collection kit which you will take home. You will be given instructions on how to collect your urine and stool samples. You will be asked to bring your collected stool and urine samples on your next visit, which will be the start of the intervention.
- **Physical Activity Monitor and Body Weight Scale**: You will be given a Physical Activity Monitor (Garmin), which you will wear for the remainder of the study. The study team will help you download the commercially-available Garmin Connect mobile app to your personal smartphone (you will be asked to agree to the terms and conditions listed in the Garmin Connect user agreement). You will thus need a smart phone and must be willing to download app to be able to participate in this study. You will receive an instruction sheet on how to use the Garmin and contact information in case you have any technical difficulties or questions during the study. You will also receive a scale from the research team to weigh yourself daily upon waking.

Intervention (14 weeks total: duration: 1 hour per week): After completing the screening and baseline visit, you will start the dietary intervention phase. You will be randomized (by chance, like the flip of a coin) to consume either foods sourced from conventional or agroecological farms as your first diet. You will be consuming fruits, vegetables, meat, dairy, eggs, nuts and other foods provided by the research team. Our study team will provide all food as 4-day rotating menus and you will consume each diet for 44 days with a 14 day break in between (where you consume your own preferred diet). The foods provided to you on each includes three main meals and snacks. For the purpose of this research, it is important to only consume the foods provided by the study team. If you think you will be not be able to only eat the foods that will be provided you and you will consume other foods instead, you cannot participate in this study. You are allowed to drink coffee and tea, if you desire, and mild drinking is also allowed (one-two drinks; 3 nights per week). You will be asked to come to the Nutrition Science Building on campus, twice per week during those 44 days where you will receive a box of food for the next 4 days. A dietitian will provide you with handouts that include recipe ideas and food preparation instructions. You will thus prepare these foods at home yourself, not unlike popular meal services that provide you with the ingredients and preparation instructions.

Examples of Meals

Breakfast	**Whole milk, eggs, oatmeal, fresh berries**
Snack	**Rice cakes**
Lunch	**Ground beef taco salad with fresh cherries**
Snack	**Apple with nut butter**
Dinner	**Grilled chicken with wild rice and steamed broccoli**

You are asked to maintain your usual physical activity and sleep schedule during the intervention periods. You will be asked to fill out daily sleep, activity, and food questionnaires, which will be sent to you by email every day. These questionnaires will ask you some general information about what time you went to sleep and woke up, how much physical activity you performed that day, and what your bodyweight was that morning (you will be provided with a scale). You will also wear the provided activity monitor continuously during the intervention period and are asked to sync your activity monitor with your smartphone daily. You can keep the activity monitor after the intervention.

Post Visits (Weeks 8 and 16. Duration: 1 hour): After the 44-day nutrition phase, you will be asked to complete an endpoint visit. These visits will be performed after each dietary intervention and will take place at the Center for Human Nutrition studies. These visits will be performed in the morning, will last approximately 1 hour, and requires you not to eat anything or chew gum (nothing but water) for 12 hours prior to your arrival time. This visit will include the following:

- **Blood Draw:** You will have blood drawn from a forearm or hand vein for outcome measurements. The amount drawn will be 30 mL (2 tablespoons) total.
- **Stool and Urine Collection Kit:** You will have been provided with a stool and urine collection kit which you will take home. You will be given instructions on how to collect your urine and stool samples. You will be asked to bring your collected stool and urine samples during this visit.
- **Questionnaires:** You will be asked to fill out questionnaires to determine your wellbeing, quality of life, and mood at the end of each diet so that we can compare it with when you first started the diet.

14-day washout (Weeks 9-10). Duration: n/a): During this 2-week period you will consume your self-selected diet (foods you prefer) and the research team will not provide any food. We will ask you to replicate your 7-day food log that you filled out prior to your first diet as much as possible in the last 7 days before starting the new intervention. The study team will contact you during this period by email or phone to stay in touch and to remind you of upcoming study visits.

The schedule will remain the same for the rest of the research period with one more 44-day nutrition phases with a baseline and post visit before and after each nutrition intervention period. At the beginning and end of each 44-day intervention period, you will report to the lab facilities at CHNS after an overnight fast to bring in fasting urine and first-of-the-day stool samples and to have your blood drawn in a fasted state.

This study will gather biospecimens (urine, stool, and blood) from you. This research will not include whole genome sequencing.

Risks
This is a minimal risk research study. That means that the risks of participating are no more likely or serious than those you encounter in everyday activities. The foreseeable risks or discomforts include momentary discomfort, bleeding, bruising, clotting and rarely fainting, infection or inflammation of the vein may result from the blood draw. Any change in diet might result in minor gastrointestinal distress (upset stomach). There is no known risk associated with the various questionnaires administered as part of this study; however, there is a possibility that participants may find some of the questions uncomfortable to answer. Minor gastrointestinal discomforts and/or distress may result from dietary changes. Dietary interventions that vary from habitual diets in fiber and macronutrients have the possibility of temporal discomfort to participants' gastrointestinal health and, in the event this occurs, the discomfort will be temporary and will subside. This research may involve risks that are not yet known. If you have a bad research-related experience, please contact Stephan van Vliet or his research assistants. If you are injured, medical treatment is not available from the study team. Please contact Stephan van Vliet immediately if you are injured so that further information can be provided. If physical injury or mental health risks are present, the participants will not receive treatment from the research team's resources.

Benefits
We cannot guarantee that you will directly benefit from this study but it has been designed to learn more about consumer health and the relationship with diverse methods of producing food.

COVID-19 Disclosures
Risks associated with contracting COVID-19 cannot be eliminated. Please carefully consider whether you are comfortable participating in person, particularly if you or someone in your home is at higher risk of serious illness

(https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html] from COVID-19.

COVID-19 vaccination is strongly encouraged, but not required, for Utah State University employees and students. Masking or using other face coverings is strongly encouraged, but not required, for Utah State University employees and students. This means that we cannot guarantee that the people you interact with in this research project will wear a face covering or be vaccinated. Researchers and fellow participants are not required to share vaccination information with you or to wear a facial covering. **Research participation is always completely voluntary, and you can decline or stop participating at any time.** Below, you will be permitted to *request* certain safety accommodations from the research team, but please know that they are not required to comply.

The researchers in this project are taking the following steps to ensure your safety and comfort during the in-person portions of this research project:

- Vaccinated and, in some cases, boosted with the COVID-19 vaccine
- Compliance with CDC COVID-19 safety and preventative recommendations
- Voluntarily agree to wear facial coverings, sanitizing practices, air filtration measures, social distancing, etc.

Confidentiality

Information collected about you for this research study will be kept in a research study record using de-identified participant codes. Any significant findings developed during the course of this research, which may bear upon your condition or willingness to continue participation in the study, will be provided to you. The study results will be retained in protected de-identified research records indefinitely after the study is completed. The researchers will make every effort to ensure that the information you provide as part of this study remains confidential. Your identity will not be revealed in any publications, presentations, or reports resulting from this research study.

Some of the information you will be asked to share includes health history screening questionnaires, diet logs, and food frequency questionnaires. Online activities always carry a risk of a data breach, but we will use systems and processes that minimize breach opportunities. Study data will be collected and managed using REDCap (Research Electronic Data Capture). REDCap is a secure web application designed to support data capture for research studies. The system was developed by a multi-institutional consortium initiated at Vanderbilt University. REDCap servers are hosted in the College of Education & Human Services at Utah State University. All study data will be securely stored in REDcap and/or a password-protected/restricted-access folder on Box.com which follows USU's recommendation for all digital content, or in a locked drawer in a restricted-access office which follows USU's recommendation for all physical content.

It is unlikely, but possible, that others (Utah State University, Green Acres, or state or federal officials) may require us to share the information you give us from the study to ensure that the research was conducted safely and appropriately. We will only share your information if law or policy requires us to do so.

Voluntary Participation & Withdrawal

Your participation in this research is completely voluntary. If you agree to participate now and change your mind later, you may withdraw at any time by writing to Dr. Stephan van Vliet to let him know of your decision to withdraw. His email address and contact information is provided below. If you choose to withdraw from the study, no new data about you will be collected for study purposes other than data needed to keep track of your withdrawal. If you choose to withdraw after we have already collected information about you, we ask that you indicate in writing if you want the unused identifiable blood and urine samples destroyed or if your samples could be used for another research. You may do so within three years of completion date. After that date, information samples will be de-identified and it will not be possible to know whose is whose.

The researchers may choose to terminate your participation in this research study if you experience a bad side effect or if they determine that it is no longer in your best interest to continue. USU or other regulatory agencies may stop this study at any time without your consent. You may be removed from the study if:

- You experience any bad side effects
- You are unable to keep your study visits
- You are unable to comply with the study requirements

Compensation and Costs
For your full participation in this research study, you will receive $1000. There will be no costs to participants for any of the treatments or testing done as part of this research study. If you are not able to complete all study procedures, you will receive partial payment for the portions of the study you have completed. The screening visit and first diet intervention is valued at $500; and the second diet intervention period is valued at $500. If you drop out of the study within the first two weeks of the first diet intervention you will be provided $25 for the screening. Because this study pays $1000 for participation within one calendar year, the researchers will be required to collect a W-9 for Internal Revenue Service reporting purposes. A W-9 requires that you provide your name, social security number, and address.

Immediate necessary care will be arranged if an individual is injured because of participation in a research project. However, there is no provision for free medical care or for monetary compensation from the research team for such injury.

Findings
If the researchers learn anything new during the course of this research study that might affect your willingness to continue participation, you will be contacted about those findings. This might include changes in procedures, changes in the risks or benefits of participation, or any new alternatives to participation that the researchers learn about.

Identifiers may be removed from your biospecimens. These de-identified data and biospecimens may be used or distributed for future research without additional consent from you. If you do not wish for us to use your information or biospecimens in this way, please state so below.

Once the research study is complete, a description of this clinical trial will be available on https://clinicaltrials.gov/. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this website at any time. We will also return a summary of the study to you directly.

The researchers would like to keep your contact information in order to invite you to participate in future research studies. If you would like them to keep your contact information, please initial here: _____. This information will be entered into a restricted-access file on a cloud-based service like Box that is completely separated from anything to do with this research study and maintained for up to three years. You can contact the Principal Investigator at any time to be removed from this list.

IRB Review
The Institutional Review Board (IRB) for the protection of human research participants at Utah State University has reviewed and approved this study. If you have questions about the research study itself, please contact the Principal Investigator at (435) 797-5369 or Stephan.vanvliet@usu.edu. If you have questions about your rights or would simply like to speak with someone *other* than the research team about questions or concerns, please contact the Human Research Protections Director at (435) 797-0567 or irb@usu.edu.

Dr. Stephan van Vliet

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Jennifer Cloward

Jennifer Cloward, RDN
Student Investigator
(801) 680-4678; Jennifer.cloward@usu.edu

Informed Consent
By signing below, you agree to participate in this study. You indicate that you understand the risks and benefits of participation, and that you know what you will be asked to do. You also agree that you have asked any questions you might have, and are clear on how to stop your participation in the study if you choose to do so. Please be sure to retain a copy of this form for your records.

_____	_____	_____
Participant's Signature	Participant's Name, Printed	Date
_____	_____	_____
Signature of Person Obtaining Consent	Consenter's Name, Printed	Date

☐ I do agree to allow my de-identified information/biospecimens to be used or shared for future research.

☐ I do **not** agree to allow my de-identified information/biospecimens to be used or shared for future research.

COVID-19 Safety Requests
Please note that the research team is not required to comply with these requests, but many researchers are happy to oblige where possible. **The research team will inform you** if they are unable to commit to any of your selections. You may decline to participate or withdraw your participation at any time.

☐ I would like the researchers I interact with to be fully vaccinated (two weeks after their last dose of the vaccine)

☐ I would like the researchers I interact with to use a facial covering

☐ I would like the researchers I interact with to use a facial covering only if they are not fully vaccinated

☐ I would like the researchers I interact with to take additional safety measures related to COVID-19:

_____
_____
_____