Title: **S**ymptoms of **P**olycystic Ovarian Syndrome **A**meliorated by **R**esponses of **K**eto-Adaptation

Short title: **SPARK**

PI:

Madison Kackley, PhD, CSCS
Research Scientist, Department of Human Sciences
The Ohio State University
305 Annie & John Glenn Ave
Columbus, OH 43210
Office: 614-247-9650| kackley.19@osu.edu

Co-PI/External Collaborator:

Teryn Bedell, MS, RD, LD, Co-I
Senior Research Dietician
The Ohio State University
305 Annie & John Glenn Ave
Columbus, OH 43210
Office: 614-292-4788 | bedell.387@osu.edu

Jeff S. Volek, PhD, RD
Professor, Department of Human Sciences
The Ohio State University
305 Annie & John Glenn Ave
Columbus, OH 43210
Office: 614-688-1701 | volek.1@osu.edu

Loriana Soma, MD
Physician, Obtetrics and Gynocology
The Ohio State University Wexner Medical Center
395 W 12th Ave
Columbus, OH 43210
Office: 614-293-3069 | loriana.soma@osumc.edu

ABBREVIATIONS

BHB	Beta hydroxybutyrate
KD	Ketogenic Diet
FSH	Follicle Stimulating Hormone
LBM	Lean body mass
LH	Luteinizing hormone
PCOS	Polycystic Ovarian Syndrome
DEXA	Dual-Energy X-ray Absorptiometry
mL	milliliters
mg	Milligrams
h	Hour
ANOVA	Analysis of variance
AE	Adverse event
USG	Urine Specific Gravity
yr	Year

STUDY SYNOPSIS

Study Title	Symptoms of PCOS Ameliorated by Responses to Keto-adaptation
Short Title	SPARK
Study Design	A 12-week randomized, 2 arm intervention testing responses to ketogenic diet intervention vs mixed diet with exogenous ketone supplement in women diagnosed with PCOS.
Study Participants	• Women diagnosed with PCOS between ages 18-40
Planned Study Period	August 2024-August 2025
OUTCOMES	
Primary	Changes in anovulatory status in women with PCOS
Secondary	• Changes in reproductive hormones such as progesterone, estrogen, LH, FSH • Analysis of potential predictive metabolic baseline characteristics • Differences in blood metabolites (metabolic and lipids).
Exploratory	• Examine cardiovascular function and ovarian morphology using magnetic resonance imaging (MRI).

SUMMARY AND BACKGROUND

Proposed research aims to examine the effects of ketogenic intervention on metabolic outcomes and ovulatory regulation in women with PCOS. We expect to demonstrate the novel role of low-carbohydrate, ketogenic intervention in regulating metabolic and reproductive health in PCOS-diagnosed women. Finding a therapeutic strategy that simultaneously ameliorates multiple PCOS symptoms is crucial due to the absence of a known cure. The ketogenic diet has proven safe and effective in various endocrinopathies, with or without weight loss. This study will provide foundational recommendations for PCOS-diagnosed women, irrespective of their specific symptomologies.

Impact of a Low-Carbohydrate, Ketogenic Approach. A well-formulated ketogenic diet (ketogenic diet) is a nutrient-dense lifestyle characterized by increasing blood ketones into a safe, therapeutic range of nutritional ketosis (0.5–4.0 mM beta-hydroxybutyrate; R-βHB)5. Ketosis is rapidly inducible (~3-5 days)6 with non-starchy vegetables as primary carbohydrate source (20–50 g/day), moderate protein intake (1.2–1.6 g/kg BW/day), and sufficient lipids for energy and satiety. Sustained ketosis positively modulates blood parameters during weight-loss[6–11] and weight-maintenance [12,13.] Ketogenic diets show promise for metabolic reversal and endocrine improvement in women[14], with studies indicating enhancements in glycemic control, insulin sensitivity, cholesterol profiles, hormonal profiles, and androgens. Further research is needed to understand ketogenic diets' impact on female physiology, especially pre-menopausal outcomes. In our previous work, a eucaloric low-carbohydrate, ketogenic approach was more effective in reversing metabolic syndrome compared to moderate- and high-carbohydrate diets[13]. We discovered a robust difference in circulating lipoproteins and fatty acid composition after the low-carbohydrate diet. Circulating saturated fat in both triglycerides and phospholipids were significantly lower in the groups with lower carbohydrate intake. Decreasing carbohydrate intake led to an increase in peak LDL diameter and a decrease in the concentration of small, atherosclerotic LDL particles (**Figure 1**). The positive effects of these markers may prove efficacious in decreasing dyslipidemia, both determinant factors of hyperandrogenism condition in women diagnosed with PCOS[15].

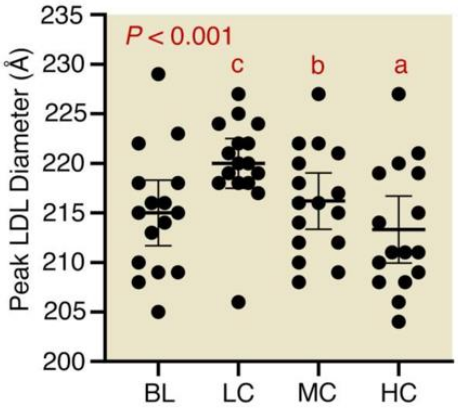


Figure 1 | Changes in Peak LDL Diameter
BL, Baseline; LC, Low carbohydrate; MC, Moderate Carbohydrate; HC, High Carbohydrate

Ketogenic Interventions in PCOS. The PCOS literature overwhelmingly supports weight-loss as an effective strategy to manage symptoms, however ketogenic diet interventions in PCOS sustained over longer durations provide even greater additional benefits. A 12-week pilot trial revealed that a ketogenic diet with no pharmaceutical assistance can regulate menses positively, and to the same extent as a mixed diet with pharmaceutically-treated PCOS, whereas the glucose control and body weight changes favored the ketogenic diet[16]. A single-arm,

12-week trial conducted in 14 women with PCOS also revealed that the ketogenic diet is a feasible, non-pharmacological intervention to improve body weight, body composition, insulin-sensitivity, cardiometabolic risk, and female-specific circulating hormones[11]. Lastly, a 24-week single-arm study examining the ketogenic diet effects on metabolic and endocrine parameters enrolled 11 women with PCOS – 5 completing all study details (~45% retention) – who demonstrated positive reductions in testosterone, luteinizing hormone: follicle stimulating hormone, and fasting insulin by the end of the diet[8]. However, the presence of menses does not confirm ovulatory function, which is a primary concern for women battling PCOS. Currently, no studies confirm the potential for reversing the anovulation that burdens women with PCOS who are desperately trying to conceive.

Women with PCOS are at an increased risk of cardiovascular complications, such as endothelial dysfunction and subclinical atherosclerosis, making precise cardiovascular measurements critical for early detection and management of potential risks (Al-Kassas et al., 2023). Similarly, MRI offers unparalleled accuracy in assessing ovarian morphology key diagnostic features of PCOS, without exposure to ionizing radiation (Wang et al., 2022). Integrating MRI for these evaluations enables a comprehensive understanding of the systemic and reproductive manifestations of PCOS, fostering improved diagnostic accuracy and the development of targeted interventions. Magnetic Resonance Imaging (MRI) provides a non-invasive and highly detailed modality to evaluate both cardiovascular function and ovarian morphology in women with PCOS. Optional baseline and post-intervention MRI measurements will be conducted to observe these trends.

As the hormone dynamics could shift when an individual is on a ketogenic intervention, testing will be moved to biweekly to capture these changes. These changes are already in effect in the SPARK001 participant in the study.

IMPACT

Our intent is to produce preliminary feasibility and efficacy data to inform the design of a future fully-powered, clinical trial that will examine whether ketogenic dietary intervention strategies are effective in ameliorating multiple adverse signs and symptoms in women with an incurable endocrinopathy. Thus, providing evidence suggesting this approach as a safe and effective treatment intervention in women diagnosed with PCOS.

OUTCOMES

Primary

Changes in anovulatory status in women with PCOS

Secondary

- Changes in reproductive hormones such as progesterone, estrogen, LH, FSH
- Analysis of potential predictive metabolic baseline characteristics
- Differences in blood metabolites (metabolic and lipids).

Exploratory

- Examine cardiovascular function using magnetic resonance imaging (MRI)
- Examine ovarian morphology through MRI.

PARTICIPANTS

This study aims to recruit 40 female participants between the ages of 18 to 40yr who have been diagnosed with PCOS. Each participant must meet all the inclusion criteria and none of the exclusion criteria at screening in order to participate.

Inclusion criteria:

- Female
- Ages 18 – 40 years
- Participant is willing and able to comply with all study procedures including the following prior to Test Day: fasting (>10 h; water only), no alcohol (>24 h), no exercise (>24 h), no acute illness and controlled feeding before the Test Day, maintain diet, exercise, medication, and supplement habits throughout the study.
- Participants with PCOS symptoms including oligomenorrhea-anovulation or spontaneous intermenstrual periods.
- BMI >18kg/m
- Participant understands the study procedures and signs forms providing informed consent to participate in the study.
- Must have access to smart phone, computer or tablet to access diet.

Exclusion criteria:

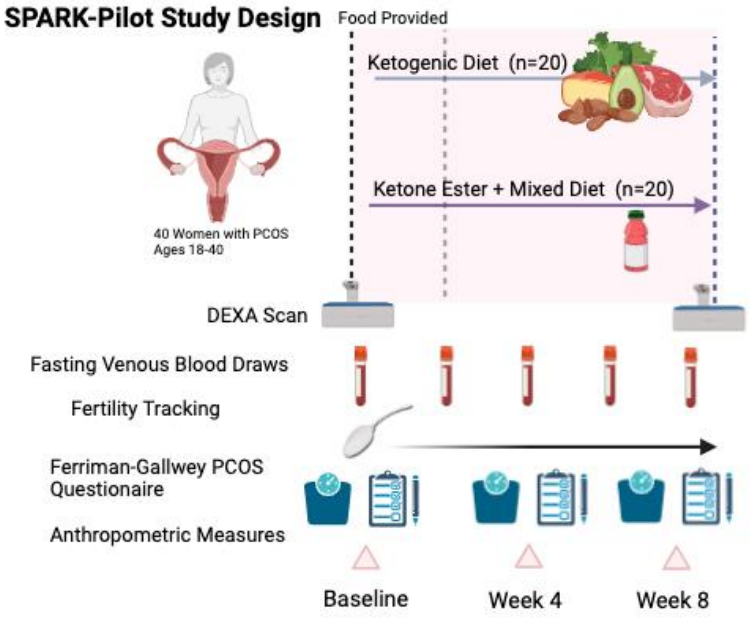
- Participant follows a low-carbohydrate diet (<30% energy from carbohydrate)
- Participant has non-PCOS etiologies of anovulation, menopause or the removal of the ovaries.
- Participant has history of type 1 diabetes
- Participant has loss of 10% bodyweight in the last 6 months
- Participant is pregnant or breast feeding
- Participant has a condition the Investigator believes would interfere with his ability to provide informed consent.

Sample Size. In our pilot trial with a sample size of 20 participants in each intervention, we acknowledge the limitations in statistical power due to the small sample. However, for this pilot trial we plan to recruit 40 participants to investigate the effects on ovulation. The results of this pilot trial will provide initial insights, but it is essential to acknowledge the limitations imposed by the small sample size.

Recruitment:

Recruitment will occur through well-established methods at OSU which include word of mouth, ResearchMatch database, social media engagement and flyers.

METHODS



Study overview:

Figure 2 | Study schematic, showing timeline for a study day.

Study Procedures

Study Day	Screening	Test Day	Daily
Visit to Study Center	X	X	
Informed consent	X		
Medical intake questionnaire, diet assessment, anthropometrics[1]	X		
Body Composition Scan[2]		X	
Basal Body Temperature [3]			X
Urine based Ovulation predication test[4]			X
Fingerstick Measurements[5]			X
Hydration Status [6]		X	
Whole blood collection[7]		X	
Surveys [8]		X	
Cardiovascular and Ovarian MRI[9]		X	

1. Medical intake questionnaire and diet assessment questionnaire are attached in **Appendix** . Anthropometric data will include: Hip and Waist circumference, BMI, height and weight.
2. Lean Body Mass and fat mass assessments will be done by DXA measurement at weeks 0 and 8.
3. Participants will also be provided with an intravaginally inserted OvuCore Sensor that is designed for measuring core body temperature which rises as progesterone is

releases. Participants will insert these monitors before sleep and wear them throughout the night
4. Participants will be provided with urine ovulation predictor kit testing strips to use during the duration of the study as a tertiary predictor of potential ovulation
5. Daily fasting glucose/ketones will be assessed using capillary finger stick lancing. A small drop of blood (i.e., the size of a grain of rice) will be obtained once daily, while fasting, using enzymatic strips fitted for a handheld analyzer (KetoMojo). All the data will be stored in the device's internal memory until retrieved by the study team during test visits.
6. Prior to blood collection, participants will be asked to completely void their bladder. And hydration status will be determined via urine specific gravity (USG) reporting <1.025. Urine will be tested for pregnancy status.
7. Blood samples will be collected according to the schedule in Figure 2.
8. Participants will complete a menstrual history survey and Ferriman-Gallwey index survery biweekly.
9. Optional assessment of cardiovascular function using MRI at weeks 0 and 12.

Screening Visit: Participants that meet the initial qualifying criteria will visit the study center for a screening meeting. The participant and a member of the research team will meet in a private office to discuss the informed consent form. The informed consent form will be provided to the participant for their review, the study will be described in full detail and any questions the interested participant has will be encouraged and responded to. If they choose to participate in the study, they will be asked to sign the consent form providing written consent. The participant will be informed that even though they signed the consent form, their participation in the study is dependent on anthropometric measures and diet and medical questionnaire answers to determine if they meet the study criteria.

If the participant provides consent, they will be provided with questionnaires including Automated Self-administered 24-hour Dietary Assessment Tool (ASA24®), and medical history. All collected samples and data will be coded to maintain participant anonymity. Height and weight will be collected. If the participant is eligible for the study and is still interested in participating then they will be scheduled to return to the study center for the testing visit.

Testing Day:
Participants will report to the study center in the morning of the Test Day. Compliance with pre-test instructions (fasted > 10h, no alcohol >24h, no exercise >24h, consumed pre-test food) will be confirmed by the Investigator. Participants will complete a baseline menstrual history and Ferriman-Gallwey index survey. Participants will be asked to completely void their bladder and a sample will be analyzed for hydration status. Participants with samples reading greater ≥ 1.025 USG will be asked to drink 160z of water and retest again in 30 minutes. Urine will be tested for pregnancy status; a positive

test would exclude participant from the study. At the same time as all whole blood samples, we will also collect capillary blood samples from a finger for real-time analysis of blood BHB and glucose concentration, using lancing device, commercially available test strips and a handheld monitor (KetoMojo, CA, USA). Body composition will be determined at each study visit by dual-energy x-ray absorptiometry (iDXA, Lunar Corporation, Madison, WI). One whole body scan will quantify lean and fat mass using encore software and visceral fat quantification will be calculated using the new CoreScan software.

Sample Processing and Analysis

Blood Processing: Whole blood collected through the IV cannula will be processed to plasma (EDTA tubes) or serum (clot activator serum collection tubes) and then snap frozen in liquid nitrogen or dry ice for storage prior to analysis (details below). We will collect capillary blood samples for real-time analysis of blood BHB and glucose concentration, using commercially available test strips and a handheld monitor (KetoMojo, CA, USA), this will also allow comparison of ketone values obtained via different methods.

Blood Sample Analytical Methods:

- *Capillary glucose and ketone concentrations* will be obtained from a finger stick using a handheld device (KetoMojo, USA).
- *Serum hormone markers* (progesterone, estradiol, FSH, LH) will be analyzed using commercially available Elecsys kits on cobas e 411(Roche, Indianapolis IN).

OvuSense Basal Body Temperature Tracking: To validate whether a ketogenic diet intervention is pivotal in reversing anovulatory status, participants enrolled will begin tracking their menstrual cycle with the OvuSense cycle tracking phone application daily. The OvuSense application is specifically targeted for participants with PCOS and is proven to be hypersensitive to the abnormal or absent ovulatory patterns of this condition. Participants will also be provided with an intravaginally inserted OvuCore Sensor that is designed for measuring core body temperature which rises as progesterone is releases. Participants will insert these monitors before sleep and wear them throughout the night. The true core temperature, which typically occurs during sleep, will automatically populate to the application and aid in predicting and confirming ovulation. This data will be shared with and downloaded by the research staff to an encrypted and secure research study laptop.

Ovulation Predictor Kit: each participant will be provided with a Per manufacturer's literature, patients were instructed to dip the wand into a sample of the first urine in the morning for 15 seconds. If two color bands are visible, and the test band is as dark or

darker than the control band, this is predictive of a positive LH surge which may predict ovulation. The participant will take a picture of the stick and upload to the OvuSense menstrual cycle tracking application.

Satiety Visual Analogue Scale: We will use a 3-item visual analogue scale, that assesses hunger, fullness and desire to eat by participant's marking on a line anchored at either end with 'not at all' and 'extremely.' Distance along the line is measured in mm.

Body Composition: Two, 7-minute whole-body scan will be conducted to assess lean-body mass and fat mass changes pre- to post-diet.

Optional Cardiovascular and Ovarian Magnetic Resonance Imaging: Magnetic resonance imaging (MRI) will be performed using a 3 Tesla system (MAGNETOM Prisma, Siemens Healthcare, Germany) to assess cardiovascular function, adipose tissue distribution, and ovarian morphology in women with PCOS. For cardiovascular and adipose measurements, chemical shift imaging will be used to generate proton density fat fraction (PDFF) maps, enabling quantification of fat content in liver, skeletal muscle, myocardium, visceral adipose tissue, subcutaneous fat, and epicardial fat. Subjects will undergo brief breath-hold scans (20–60 seconds) for liver, abdominal fat, and skeletal muscle assessments, as well as ECG-gated scans for myocardial and epicardial fat evaluation. Participants will not be required to be fasted for this procedure.

For ovarian assessment, MRI will be used to evaluate ovarian morphology, including follicle count and distribution, without ionizing radiation. All image data will be analyzed post-scan using specialized software, including in-house tools for adipose quantification and ImageJ for fat fraction and ovarian measurements. The same protocol will be repeated after the 12-week intervention period.

Diet Intervention.

Participants will be randomized into either a ketogenic diet arm or a mixed diet with ketone ester arm. During the first 2 weeks, participants will be provided food meeting either the Ketogenic diet requirements, or mixed diet (following the USDA dietary macronutrient recommendations) at a caloric level estimated at 100% of energy requirements. The participants randomized into the Mixed diet arm will also receive a ketone ester supplement. This will be provided throughout the study. Energy requirements will be determined using a combination of information obtained from testing including habitual diet assess through an NIH sponsored food frequency questionnaire (e.g., DHQ-III which will be sent to the participants email) , body mass and composition, and formulas (e.g., Harris-Benedict) that consider sex, age, height and activity patterns.

All participants will be provided all their meals and snacks selected to meet all calorie needs via individualized pre-ordered groceries and/or prepared food for the first 2 weeks of the diet. We have established the infrastructure for conducting controlled feeding studies for other funded research including development and implementation of 7-day

rotational menus, hybrid feeding approaches (providing groceries), and use of ready-made meal services (Pangea Keto, Factor, Fresh and Lean). After the initial 2 weeks, and until completion of the 8-week intervention, the participants will not be supplied with all their food but will continue to receive educational materials, recipe guides and dietitian consultation as needed. Participants will have 24-hour access to the dietetic team to answer questions. After the initial 2 weeks, participants will switch to a self-guided dietary approach. Adherence will be prospectively evaluated throughout the study period. Subjects will be asked to use the meter each morning and to submit the results to the research team through a secure KetoMojo provider/consumer sharing application. Results will be recorded by a member of the research team. Levels of ketosis and glucose will be monitored daily. Participants will be asked to maintain levels of ketones that are equal or greater than 0.5mmol/L.

Primary	Changes in anovulatory status in women with PCOS
Secondary	• Changes in reproductive hormones such as progesterone, estrogen, LH, FSH • Analysis of potential predictive metabolic baseline characteristics • Differences in blood metabolites (metabolic and lipids).
Exploratory	• Examine cardiovascular function and ovarian morphology using magnetic resonance imaging (MRI).

DATA ANALYSIS AND STATISTICAL METHODS

Statistical Plan: To evaluate the impact of the ketogenic diet intervention on menstrual cycle regularity in women with PCOS, we have the following data analysis plan:

1) Descriptive Analysis: We will provide concise summaries of menstrual cycle attributes such as cycle length, variability, and ovulation status for participants at baseline.

2) Menstrual Cycle Regularity Assessment: Survival analysis techniques, including Kaplan-Meier curves and log-rank tests, will be employed to compare the timeframe required to establish regular menstrual cycles (21-35 days range). Observations censored due to drop-outs or non-occurrences of events will be appropriately handled.

3) Ovulation Pattern Analysis: We will utilize logistic regression analysis to assess the effect of the ketogenic diet on the incidence of ovulation in PCOS women, considering each cycle as an independent statistical unit. This analysis will offer quantifiable insights into the probability of ovulation in the study population, considering potential confounders like age, BMI, and initial menstrual cycle characteristics.

4) Effect Size Estimation: Hazard ratios and their confidence intervals will be calculated to quantify the influence of the ketogenic diet on attaining regular menstrual cycles.

5) Significance Testing: The proportion of participants achieving regular menstrual cycles in each diet group will be compared using appropriate statistical tests such as the chi-square test.
Through this robust analytical strategy, we aim to unravel the influence of a ketogenic dietary intervention on menstrual cycle regularity and ovulation patterns in women with PCOS, thereby providing a compelling narrative around the potential benefits of a ketogenic diet.

STUDY MONITOTRING

Concomitant Medication/Supplements and Treatment

All concomitant medications/supplements used 1 months prior to Screening Visit and during the study will be reported to the study personnel for assessment and recorded in the participant's study documents.

Radiation

This study comprises two, whole-body DXA scans, eight-weeks apart. This radiation exposure is not necessary for medical care and is for research purposes only. The total amount of radiation is about 78 mrem and is approximately equivalent 95 days of exposure to natural background radiation. This procedure involves minimal risk and is necessary to obtain the research information about changes in lean body mass, fat mass, and bone density (i.e., 3-compartment assessment model).

Adverse Event Monitoring

An AE is defined as any untoward medical occurrence in an investigation participant following written informed consent that does not necessarily have a causal relationship with the study product. An AE can be any unfavorable or unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product. This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures (including laboratory test abnormalities).

Some side effects or GI symptoms could occur as an outcome of these dietary interventions; side effects listed in the beverage tolerability questions and reported will not be categorized as AEs but recorded as study outcomes. Side effects, outside of what is expected as a result of study product consumption, reported by participants and judged by the Investigators as medically relevant events and related to study product will be recorded as AEs.

Events should be considered AEs if they:

- Result in discontinuation from the study,
- Require treatment or any other therapeutic intervention,
- Require further diagnostic evaluation (excluding a repetition of the same procedure to confirm the abnormality),
- Are associated with clinical signs or symptoms judged by the Investigator to have a significant clinical impact.

Grading and Severity

The Investigator will evaluate all AEs with respect to their severity, and record the outcome and action taken on the AE study documents . AEs will be graded as:

Mild: Awareness of symptoms but easily tolerated
Moderate: Discomfort enough to interfere with but not prevent daily activity
Severe: Unable to perform usual activity

Relationship

The Investigator will also judge the likelihood that the AE was related to the study beverage and document this on the appropriate study documents as:

NOT RELATED	This category applies to those adverse experiences which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).
UNLIKELY	In general, this category can be considered applicable to those experiences that after careful medical consideration at the time they are evaluated, are judged to be, unlikely related to the study beverage.
POSSIBLY	This category applies to those adverse experiences for which, after careful medical consideration at the time they are evaluated, a connection with the study beverage administration appears possible but cannot be ruled out with certainty.
PROBABLY	This category applies to those adverse experiences that, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the study beverage.
DEFINITELY	This category applies to those adverse experiences which, the Investigator feels are incontrovertibly related to the study beverage.

Appropriate therapeutic action and follow-up measures will be performed by the Investigator in accordance with appropriate medical practice standard of care.

Serious Adverse Event Definition/Qualification

A SAE is defined as an AE that results in any of the following outcomes:

- Death (note that death is the outcome of a SAE and the cause of death should be listed as the AE),
- Life-threatening event,
- In-patient hospitalization or prolongation of existing hospitalization,
- A persistent or significant disability/incapacity,
- Congenital anomaly or birth defect,
- Any other important medical event that may not result in death, be life-threatening, or require hospitalization, may be considered a SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

In the event of a SAE, the participant may be dropped from the study if the Investigator deems it necessary.

Serious Adverse Event Reporting Instructions

If in the opinion of the Investigator the event meets the criteria of a SAE the following procedures will be followed:

- The Investigator will notify Institutional Review Board (IRB) of the SAE within the parameters and timeframe specified under the IRB Standard Operating Procedures (SOP). An initial report followed promptly by a complete report will be forwarded to the IRB, when applicable.
- If a participant is hospitalized or hospitalization is prolonged due to the SAE, the hospital discharge summary will be obtained if possible.
- If a death occurs and an autopsy is performed, a copy of the autopsy report will be obtained if possible.
- All efforts must be undertaken to obtain follow-up information promptly.

Recording of Adverse Events

All AEs (AE or SAE) will be recorded on the AE study documents. For participants who have an ongoing AE at their final study visit, follow-up information will be captured in the AE eCRF page which will be completed after 30 days.

Serious Adverse Event Follow-Up

For all ongoing SAEs occurring during the study, the Investigator must submit follow-up reports regarding the participant's subsequent course. All SAEs that are ongoing at the end of the study or upon discontinuation of the participant's participation must be followed until either:

- The event resolves, or
- The event/condition has stabilized (e.g., in the case of persistent impairment), or
- The event returns to baseline, if a baseline value is available, or
- The participant dies, or
- The event can be attributed to other than the study beverage, or to other than the study conduct.

CONDUCT OF THE STUDY

1. Ethics and Regulatory Considerations

This study will be conducted according to Good Clinical Practice Guidelines, the Declaration of Helsinki (2004) and United State Code of Federal Regulation Title 21. Signed written informed consent for participation in the study will be obtained from all participants before protocol-specific procedures are carried out. Participants will be informed of their right to withdraw from the study at any time. Participants will be informed that their participation in the study is completely voluntary, personal information will be both deidentified to preserve anonymity.

2. Institutional Review Board

The Investigator will ensure that an appropriately constituted IRB, in compliance with the requirements of 21 CFR 56, reviews and approves the clinical study. Before the study is started, the Investigator will forward copies of the protocol and consent form for this study to the IRB for review and approval. IRB approval must refer to the study by exact protocol title and number, identify the documents reviewed, and state the date of review. The IRB must be informed of all subsequent protocol amendments. No alterations, modifications to IRB-approved documents, including the protocol, protocol summary, consent form, recruitment materials and questionnaires will be allowed. The IRB must also be informed of all SAEs and of unexpected AEs as outlined in the IRB's SOPs or reporting guidelines.

3. Informed Consent

The study will be explained verbally as well as on the informed consent document. Each participant will be given ample opportunity to inquire about details of the study and to read and understand the consent form before signing it. It will be made clear that participants can withdraw from the study at any time.

Each participant's signed informed consent document must be kept on file by the Investigator. The participant should receive a copy of the informed consent document. A participant may not be admitted to the study unless informed consent of the participant (or his/her legally authorized representative) has been obtained.

4. **Participant Confidentiality**

The Investigator is responsible for ensuring that participants' anonymity will be maintained. For all the data collected over the course of the study for each participant (i.e., records, biological samples and questionnaires) a unique subject identifier (i.e., a code) will be assigned and used instead of the subject's name. The code for each participant which links the subject name with their identifier will only be available to research personnel. Electronic CRFs or other documents will identify participants by initials, number, or code, and not by name. The Investigator will keep a separate log showing codes, names, and addresses. Any records that contain the subject's name and identifier will either be stored in the Kinesiology file storage room in a file cabinet (locked) or protected on a computer via password protection on the individual digital file and password protection on the computer the file(s) are stored on. All other records that contain the subject identifier only will also be kept in either a file cabinet in our locked file storage room or on a password protected computer. Subject names will never be used in any presentation or publication resulting from this study. The records will be maintained until the data are published and up to a maximum of ten years after the completion of the study. All records or biological data obtained after signing of the informed consent (including the screening visit, even for subjects that are not eligible for participation in the study) are treated with the same confidentiality safety measures as those subjects who qualify. Any information obtained during the prescreening for participants that were not eligible will be deleted

5. **Withdrawal of Participants from the Study**

Participants may be removed from the study for any of the following reasons:

- A participant requests discontinuation;
- The Investigator initiates removal for medical or compliance reasons;
- Occurrence of any AE or condition that could, in the Investigator's opinion, interfere with the evaluation of the effect of the study beverage or put the participant at undue risk.

It is understood by all concerned that an excessive rate of withdrawals can render the study uninterpretable, therefore, unnecessary withdrawal of participants should be avoided. Should a participant decide to withdraw, all efforts will be made to complete and report observations as thoroughly as possible. In the event that a participant is withdrawn from the study, the reason for the withdrawal will be documented in the eCRF.

References

1. Volk, B. M. et al. Effects of Step-Wise Increases in Dietary Carbohydrate on Circulating Saturated Fatty Acids and Palmitoleic Acid in Adults with Metabolic Syndrome. PLoS One 9, e113605 (2014).
2. Hyde, P. N. et al. Dietary carbohydrate restriction improves metabolic syndrome independent of weight loss. JCI Insight 4, (2019).
3. Buga, A. et al. The Effects of a 6-Week Controlled, Hypocaloric Ketogenic Diet, With and Without Exogenous Ketone Salts, on Body Composition Responses. Front. Nutr. 8, (2021).
4. Arbour, M. W., Stec, M., Walker, K. C. & Wika, J. C. Clinical Implications for Women of a Low-Carbohydrate or Ketogenic Diet With Intermittent Fasting. Nurs. Womens. Health 25, 139–151 (2021).
5. Mavropoulos, J. C., Yancy, W. S., Hepburn, J. & Westman, E. C. The effects of a low-carbohydrate, ketogenic diet on the polycystic ovary syndrome: A pilot study. Nutr. Metab. (Lond). 2, 35 (2005).
6. Noakes, M. et al. Comparison of isocaloric very low carbohydrate/high saturated fat and high carbohydrate/low saturated fat diets on body composition and cardiovascular risk. Nutr. Metab. (Lond). 3, 7 (2006).
7. Volek, J. et al. Comparison of energy-restricted very low-carbohydrate and low-fat diets on weight loss and body composition in overweight men and women. Nutr. Metab. (Lond). 1, 13 (2004).
8. Paoli, A., Mancin, L., Giacona, M. C., Bianco, A. & Caprio, M. Effects of a ketogenic diet in overweight women with polycystic ovary syndrome. J. Transl. Med. 18, 104 (2020).
9. Gupta, L. et al. Ketogenic diet in endocrine disorders: Current perspectives. J. Postgrad. Med. 63, 242 (2017).
10. Hestiantoro, A. et al. Triglycerides, independent of Ferriman Gallwey Score, is a main determinant of free testosterone index in PCOS. F1000Research 8, 94 (2019).
11. Li, J. et al. Ketogenic diet in women with polycystic ovary syndrome and liver dysfunction who are obese: A randomized, open-label, parallel-group, controlled pilot trial. J. Obstet. Gynaecol. Res. 47, 1145–1152 (2021).