The Effect of SLC19A3 Inhibition on the Pharmacokinetics of Thiamine

NCT03746106

Version Date: 08/05/2020

STUDY TITLE:

The Effect of SLC19A3 Inhibition on the Pharmacokinetics of Thiamine

STUDY SPONSOR:

National Institute of Diabetes and Digestive and Kidney Diseases

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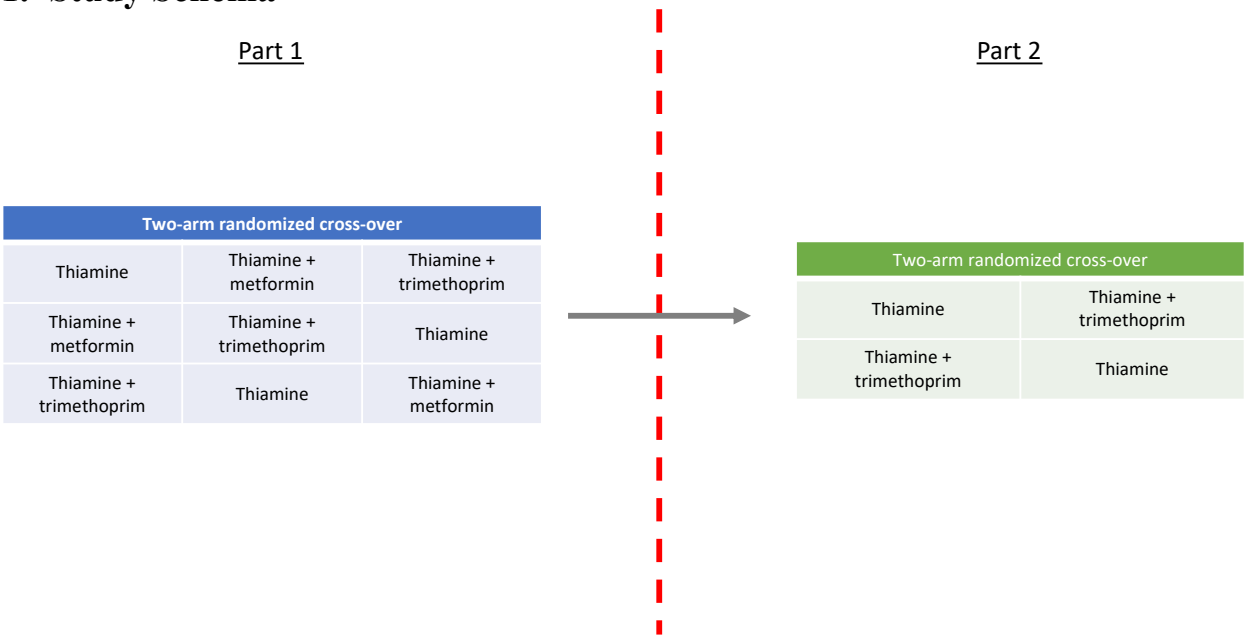

2. Introduction

2.1 Background and Rationale

Thiamine is an essential vitamin utilized as a cofactor in several enzymatic pathways. Thiamine deficiency is thought to occur primarily as a result of malnutrition and can result in severe or even fatal symptoms such as delirium and heart failure. Though linked to malnutrition, thiamine deficiency has been discovered in a variety of patient populations where malnutrition is not present including diabetes, HIV, and various cancers amongst others. Currently, the contribution of therapeutic drugs to thiamine deficiency in disease states is not understood. Trimethoprim is a synthetic antibacterial that inhibits the production of tetrahydrofolic reductase by binding to dihydrofolate reductase and is a known inhibitor of SLC19A3 the major intestinal absorptive transporter for thiamine[21]. Metformin is an orally prescribed drug that is used as a first line of therapy for Type II diabetes patients and is a known inhibitor of SLC19A3. This study aims to investigate the potential interaction between thiamine and drugs known to inhibit thiamine transporter SLC19A3 such as trimethoprim and metformin.

Wet and dry beriberi, classical forms of thiamine deficiency, and Wernicke's encephalopathy, a severe neurological syndrome associated with thiamine deficiency, have been described in various settings associated with poor nutrition: developing countries, prison populations, and post-bariatric surgery patients[8-10].

Though the prevalence of thiamine deficiency in the general U.S. population is not known, thiamine deficiency appears to occur commonly in certain patient populations including those with alcoholism, various cancers, and patients infected with HIV or in the intensive care unit as well as those with diabetes[11-14]. In fact, thiamine supplementation has been proposed as adjuvant therapy for patients with diabetes and alcoholism[15, 16]. Notably, although thiamine deficiency has been described in patients with diabetes, the contribution of metformin (first line therapy) to the thiamine deficiency is not known. Similarly, the contribution of therapeutic drugs to thiamine deficiency in other diseases is not understood. Additionally, thiamine pyrophosphate (TPP), one of the major metabolites of thiamine, is a co-factor for multiple metabolic pathways. It has been shown that TPP added to in vitro enzyme systems significantly increases the metabolic

rates of TPP-dependent enzymes (e.g., pyruvate dehydrogenase (PDH))[45, 46]. Thus, it has been speculated that thiamine deficiency could affect the concentration of key metabolites and metabolic ratios of TPP-dependent enzymes and pathways.

Thus, drugs that potently inhibit the transporter, such as fedratinib, may "phenocopy" this disorder, and less potent prescription drug inhibitors may increase susceptibility of patients to thiamine deficiency. Increased susceptibility may be particularly apparent in patients with other comorbidities such as gastric bypass surgery, alcoholism or patients with diabetes and cancer.

The recent disastrous clinical trial with fedratinib, a novel JAK2 inhibitor, highlights the potential importance of pharmaceutical agents as causative of thiamine deficiency. In the fedratinib trial, a handful of myelofibrosis patients experienced Wernicke's encephalopathy leading to termination of the trial[20]. Subsequent studies showed that fedratinib was a potent inhibitor of SLC19A3, providing a mechanism for the observed side-effect of fedratinib. Using SLC19A3 stably expressing cell lines, Zhang et al. showed that fedratinib potently inhibited thiamine uptake by SLC19A3 (IC50 = 2.1 μM) and suggested that a 4-aminopyrimidine group is a critical moiety for SLC19A3 inhibitors.

Recent data from our lab as well as the literature provide an explanation for why such profound thiamine deficiency—leading to Wernicke's encephalopathy— has not been observed previously during clinical drug trials. That is, currently marketed prescription drugs may not be as potent as fedratinib in inhibiting SLC19A3. As part of our preliminary studies, we created a stably transfected HEK293 cell line expressing SLC19A3 and identified prescription drugs as inhibitors of SLC19A3-mediated thiamine uptake. Inhibition studies suggested that both metformin and pyrimethamine could inhibit SLC19A3 after oral doses, though neither compound was as potent as fedratinib (IC50 = 492 μM (metformin), 100 μM (pyrimethamine) and 2.1 μM (fedratinib)). Similarly, other studies in collaboration with our lab have shown coinciding results, with recent inhibition studies suggesting that trimethoprim could inhibit SLC19A3 after oral doses as well (IC50 = 5.56 μM).

There have been no clinical studies performed assessing the effect of SLC19A3 inhibition on thiamine absorption and distribution to date.

Given the potency experiments, metformin and trimethoprim are estimated to reach concentrations in the intestinal fluid that would exceed (>10-fold) the in vitro IC50 values for SLC19A3, and therefore may cause drug-vitamin interactions[22, 23]. Thus, it is important to evaluate the hypothesis of whether co-administration of SLC19A3 inhibitors, such as metformin and trimethoprim, and thiamine reduces thiamine absorption and distribution in a healthy population to inform dosing of concomitant medications, and adverse events, in at-risk populations.

- Is there an active control group?

  ☐ **Yes** ☒ **No**

2.2 Risks to Subjects

Risk to subjects should be minimal. Potential discomforts include mild side effects related to administered drugs and/or vitamins including but not limited to abdominal or stomach discomfort, decreased appetite, nausea, and diarrhea. Everyone taking part in this study will be watched carefully for any side effects that may happen. Most, if not all, side effects should dissipate shortly after the drug administration is ceased. All drugs and vitamins used in this trial

have been administered to healthy volunteers at the same, if not higher, doses with mild to no side effects.

Any other inconveniences may include traveling to the research site for study visits, eating a strict controlled diet, and/or blood draws. If any of these affect the subject's psychological, social, or physical well-being, the subject may ask to withdraw their consent from the study.

All subjects will provide at most 157.5mL of blood per cycle. Since the common standard for collection is approximately 1 pint (i.e. 473mL) at blood drives and/or donation centers, we feel that we will not putting the patient in danger. The total blood volume provided for screening and all three cycles will be approximately 484mL for females and 480.5mL for males for Part 1. For Part 2 all subjects will provide at most 147.5mL of blood per cycle. The total blood volume provided for screening and all 2 cycles will be approximately 315mL. Loss of 550mL is not harmful and is typically replenished by the body within two to three weeks.

To protect patients from loss of privacy and confidentiality, only the research staff will have access to the samples and records obtained. Information from this study used for scientific publication will not contain any identifying information.

2.3 Potential Benefits to Subjects

The benefit of participating in this study is that subjects will be able to add to our knowledge about how SLC19A3 inhibitors such as metformin and trimethoprim affect the absorption and disposition of thiamine with relatively little inconvenience to them. While these types of studies are unlikely to offer direct benefit to participants at the time of their involvement, the information gained may be used to develop new therapies and/or revise dosing guidelines to improve survival or quality of life for them or other patients like them in the future.

2.4 Alternatives

The only alternative is to not participate in the study. Subjects can receive the research drugs and supplement used in this study over-the-counter (thiamine) or with a prescription (metformin and trimethoprim) at their local pharmacy.

3 Objectives

Part 1:

- Primary Objective

  The primary objective of Part 1 is to assess the effects of SLC19A3 inhibitors, metformin and trimethoprim, on the absorption and distribution of thiamine as measured by the change in AUC, and other pharmacokinetic parameters, between the thiamine and inhibitor (combination) arms and thiamine (single agent) arm.

- Secondary Objective

  The secondary objective of Part 1 is to investigate metabolic signatures reflecting the activity of TPP-dependent enzymes by comparing levels of individual metabolites and metabolic ratios after the administration of thiamine or a combination of the thiamine and SLC19A3 inhibitor which may lead to the discovery of biomarkers that can be used as signatures of reduced thiamine absorption.

- Exploratory Objective

  An exploratory objective for Part 1is to determine the effect of genetic variants of thiamine transporters including, but not limited to, organic cation transporter 1 (OCT1) and solute carrier family 19 member 3 (SLC19A3), on thiamine disposition and absorption.

Part 2:

- Primary Objective

  The primary objective for Part 2 is to further asses the effects of the SLC19A3 inhibitor, trimethoprim, on the absorption and distribution of thiamine as measured by the change in AUC, and other pharmacokinetic parameters, between the thiamine and trimethoprim (combination) arm and thiamine (single agent) arm.

- Secondary Objective

  The secondary objective of Part 2 is to further investigate metabolic signatures reflecting the activity of TPP-dependent enzymes by comparing levels of individual metabolites and metabolic ratios after the administration of thiamine or a combination of thiamine and trimethoprim which may lead to the discovery of biomarkers that can be used as signatures of reduced thiamine absorption.

- Exploratory Objective

  An exploratory objective for Part 2 is to determine the effect of genetic variants of thiamine transporters including, but not limited to, organic cation transporter 1 (OCT1) and solute carrier family 19 member 3 (SLC19A3), on thiamine disposition and absorption.

4 Enrollment and Withdrawal

4.1 Inclusion Criteria

1. Male or female between the ages of 18-65 years old.

2. Eats a wide variety of food and willing to consume study diet (i.e. not on a specific diet such as Atkins, Fodmap, etc.).

3. Written informed consent obtained from the subject and ability for subject to comply with the requirements of the study.

4.2 Exclusion Criteria

1. Subjects who are pregnant, breastfeeding, or unwilling to practice birth control during participation in the study.

2. Self-reported severe food allergies or diet restrictions (vegans, vegetarians, Atkins, Fodmap, etc.) that would prevent consumption of study diets.

3. Subjects with extreme obesity (BMI > 35).

4. Subjects who are smokers or have smoked in the past year and/or have smoked or ingested THC/marijuana in the past week, or who are unwilling to comply with a 1-week washout.

5. Subjects with any disease affecting or impairing the function of the liver, kidney or heart.

6. Subjects with moderate to severe hypertension.

7. Subjects with diabetes mellitus, hyperthyroidism, hypothyroidism, cardiovascular disease, glaucoma.

8. Subjects with gastrointestinal disease, gastrointestinal disorder, or gastrointestinal surgery.

9. Subjects with known infection with HIV, Hepatitis B (HBsAg) or Hepatitis C (no laboratory diagnostics concerning these diseases will be performed within the present study. Volunteers who are cured of past HepC infection are eligible to participate with doctor’s approval letter).

10. Alcohol use on average > 2 servings/day or > 14 servings/wk (Serving size: 12oz beer/4oz wine/2oz hard liquor) or self-reported binge drinking.

11. Subjects that are on vitamin B supplements or multi-vitamins or who have taken vitamin B supplements or multi-vitamins in the past 30 days, or are not willing to comply with a 30-day washout of vitamin B supplements.

12. Subjects with possible folate deficiency.

13. Subjects taking any other clinically significant drugs as judged by the investigator.

14. Subjects with a condition, disease, or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.

15. Female subjects undergoing treatment for infertility or hormone replacement therapy (Volunteers using hormonal birth control will not be excluded).

16. Subjects who have taken antimalarials in the past 60 days.

17. Participating in another research study while participating in this research study.

18. Non-English speaking

19. Subjects with abnormal laboratory results at screening as judged by the investigator or study physician.

4.3 Withdrawal of Subjects

A subject may be discontinued from either part of the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent
- Subject is not compliant with study procedures or center regulations
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment
- Sponsor request for early termination of the study
- Positive pregnancy test (females)

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. However, if a subject is withdrawn or does withdraw from the study, the data collected until that point will still be used in future analyses.

If a subject is withdrawn from treatment due to an adverse event, the subject will be told to follow up with their doctor, contacted to gather information on symptom outcome, and the AE will be reported according to protocol.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents.

4.4 Recruitment and Retention

4.4.1 Local Recruitment Methods

Participants will be recruited from the general public using several recruitment strategies including, but are not limited to: local print and electronic media (such as advertisements in newspapers, craigslist, local newsletters, bulletin boards and different websites, [i.e., university jobsites at Tufts University, Boston University]), social media (i.e. Facebook, Twitter, LinkedIn), general mailings, as well as posting flyers in public places such as local YMCAs, supermarkets, libraries, laundromats, local community organizations and health centers. See attachment for recruitment flier (**Recruitment**

Flier) and listing for HNRCA and Tufts Medical Center Clinical Trials websites. Recruitment, screening, and enrollment procedures as well as documents related to MRU standard policies and procedures, approved under IRB #6701, will be used for this protocol.

Additionally, the Metabolic Research Unit (MRU) resources include a roster of >20,000 names of subjects from which potentially qualifying women and men within the specified age and BMI can be identified and contacted through the use of direct mailings. Only subjects in the MRU database who have agreed to be contacted for future studies will be contacted for this study (see attachment – **HNRCA Recruitment Letter**). Subjects who do not wish to be contacted are flagged in this database (Protocol Manager) with "DO NOT CONTACT." Previous subjects with this flag in their record will not receive any unsolicited recruitment materials, i.e., the letter to previous volunteers. All advertisements, fliers, and letters will be pre-approved by the IRB.

Although employees will not be targeted for recruitment and enrollment, employees of Tufts University and/or the HNRCA (employee-subjects) who voluntarily want to participate in the study will be eligible for screening and enrollment. In order to qualify for the study, employee-subjects must respond to IRB-approval advertisement of their own accord and will not be directly approached by any person seeking to recruit them for participation in the study. Members of the research team, as direct-report subordinates of the PI and anyone who is direct-report subordinate to any of the research team members in any other capacity will not be eligible to participate in the study. If employee-subjects qualify to participate in the study, they cannot participate as volunteers during hours in which they are being compensated by Tufts University for their regular work. This includes use of vacation, personal days and sick time.

Individuals who express interest in the study will be called by the team to describe the purpose, design, blinding, obligations and study stipend payments for participating in the study. Prospective volunteers will provide oral consent form prior to completing a screening questionnaire, and if they appear to be qualified, they will be invited to the HNRCA for a full screening (see attachment – **Telephone Prescreening Questionnaire**). This screening telephone interview will ensure that the volunteers are fully aware of all study activities and the full participant study burden. This will allow for the recruitment of only those who are confident they are able to complete all study activities. The goal is to minimize drop outs as well as to minimize the number of potential participants scheduled for screening. If they are eligible based on the Telephone Prescreening Questionnaire, subjects will be mailed a health questionnaire, to bring this completed questionnaire to the screening visit (see IRB submission #6701 – **Screening Health Questionnaire**).

The full screening informed consent process will be conducted in a quiet location by a research nurse or coordinator at the Metabolic Research Unit (MRU) at the HNRCA, and those interested will be asked to sign the screening consent form prior to the start of all screening procedures. Nurses will be immediately available to answer any medical questions that the research coordinator cannot address, and the PI will be available via email or phone should more clarification be necessary. Study candidates will be given ample time to thoroughly read study consents, ask questions or get further clarifications on any study protocol. They will be provided with answers to their satisfaction and understanding. Potential participants will be asked to restate in their own words, their understanding of information to confirm comprehension. The screening process will likely take 3 hours to complete. Once all of the participant's questions and concerns have been addressed, the designated staff will obtain informed consent. Subjects will then undergo blood tests and a review of their medical history and general health, use of medications and nutritional supplements. Once these tests have been completed, subjects will be provided with a standard continental breakfast (muffin or bagel, fruit, coffee) at the HNRCA.

Study candidates that need time to think over their participation or consult with family members will be given all the study consent forms (screening consent, study instructions and study FAQ form to take home with for further consideration and possible consultation with family members. They will be asked to contact a study representative if they have any further questions at any time following that, or if they decide to proceed with participation, another screening appointment will be scheduled. If they decide to proceed with the screening procedures during their screening visit, these will take place following signing of the screening consent form (see attachment – **Screening Consent Form**). The informed consent procedure will detail out what their involvement would be. They will be provided with a copy of all the study consent forms to take home with them for their review and consideration; again, they are asked to feel free to contact a study representative with any further questions.

Results of blood testing will be available at least 1-2 weeks after screening. Once an individual has gone through the screening process and is deemed eligible to participate, he/she will be informed by telephone by a staff at the MRU that they are eligible. If they still agree to participate, they will be scheduled to come to the HNRCA for the baseline study visit. During this phone call, eligible subjects will be reminded that they are to fast for 12 hours prior to their screening visit. They will be sent a letter in the mail reminding them of their participation in the study and their baseline visit and of the requirement to fast 12 hours before (see IRB submission #6701 – **HNRCA Study Admission Letter**). During this visit they will be asked if they still have any questions concerning their participation. All questions will be responded to by an experienced MRU registered nurse to the participant's satisfaction. Candidates will be asked to restate in their own words, their understanding of information to confirm comprehension. Once the participant is ready to proceed, he/she will be asked to sign the appropriate consent forms (main informed consent form including optional tissue banking) before participation begins. All subjects are informed that they are never bound to participate in the study and are free to withdraw their consent at any time for any reason. Blood test results will be mailed to each individual after they are available, at least 1-2 weeks after screening. A separate letter will be mailed to individuals deemed ineligible during screening, assessed by the study physician or nurse, and will be informed that they do meet eligibility requirements, will be disqualified and may be provided with a recommendation to see their physician (see IRB submission #6701 – **Letter for Excluded Subjects, Letter for Lab Results, Letter for Abnormal Lab Results**).

4.4.2 Study-Wide Recruitment Methods

Materials that will be used to recruit subjects are being submitted as part of the IRB package. When subjects respond to recruitment material, they will have a phone prescreen and the information requested from them is described in the prescreen form.

Is this a multicenter study where subjects will be recruited by methods not under the control of the local Tufts site (e.g., call centers, national advertisements)?

☐ **Yes** ☒ **No**

4.4.3 Payment

Will subjects receive money, gifts, or any other incentive for participating in this study?
This does not include reimbursement for expenses, which is considered in the next section.

☒ **Yes** ☐ **No**

Part 1
Participants will receive $25 upon completion of the screening process. Participants will receive a total compensation of $1000 upon completion of the full study. If a participant does not complete the entire study, payment will be prorated accordingly according to the chart below.

Subjects will receive $250 for completing first group (Days 0, 1, 2). Subjects will receive an additional $350 for completing the second group (Days 0, 1, 2) and will receive $400 for completing Group 3 (Day 0, 1, 2). The stipend payments will be mailed to the participant as a check, depending on their duration of participation in the study. The participant should expect to receive the check within 2-3 weeks after mailing.

Cycle	Day 0	Day 1	Day 2	Total	Running Total
1	$50	$50	$150	$250	$250
2	$70	$70	$210	$350	$600
3	$80	$80	$240	$400	$1000

Part 2

Participants will receive $25 upon completion of the screening process. Participants will receive a total compensation of $715 upon completion of the full study. If a participant does not complete the entire study, payment will be prorated accordingly, depending on where the participant is in the study prior to withdrawal. Subjects will receive $300 for completing first group (Days 0, 1, 2). Subjects will receive an additional $415 for completing the second group (Days 0, 1, 2). The stipend payments will be mailed to the participant as a check, depending on their duration of participation in the study. The participant should expect to receive the check within 2-3 weeks after mailing.

Cycle	Day 0	Day 1	Day 2	Total	Running Total
1	$50	$50	$200	$300	$300
2	$80	$80	$255	$415	$715

4.4.4 Reimbursement

Will subjects be reimbursed for their expenses, such as travel, parking, meals, or any other study related costs?

☐ **Yes** ✖ **No**

5 Study Design

5.1 Study Timelines

Part 1

As many as eighty healthy volunteers will be screened, with the goal of enrolling seven volunteers into Part 1 of the study, and six finishing the study. Each subject will be administered the same three treatments (thiamine only, thiamine and metformin combination, and thiamine and trimethoprim combination), simply in a different order, depending on his/her group assignment. There will be three cycles in this study, each separated by at least 5 days but no more than two weeks, unless previously approved by principal investigator. Subjects will be assigned to the treatments in a random order. Subjects will come three times during each cycle in which they will receive drug, vitamin, or a combination of the two and will be evaluated through multiple blood samples and urine collection.

Screening data, including the MRU Nursing Health History Questionnaire (see IRB submission #6701), urine sample, and a blood sample, will be reviewed to determine

subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

Subjects will be randomized into one of three groups and will be administered a different sequence of drugs dependent on their group assignment:

Group 1: 5mg thiamine only (Cycle 1), 5mg thiamine and 1000mg metformin (Cycle 2), 5mg thiamine and 300mg trimethoprim (Cycle 3)

Group 2: 5mg thiamine and 1000mg metformin (Cycle 1), 5mg thiamine and 300mg trimethoprim (Cycle 2), 5mg thiamine only (Cycle 3)

Group 3: 5mg thiamine and 300mg trimethoprim (Cycle 1), 5mg thiamine only (Cycle 2), 5mg thiamine and 1000mg metformin (Cycle 3)

Despite the different sequence order, administration and sample collection for both drugs will remain the same for each group. Subjects will arrive on Day 0 of each cycle to pick-up three thiamine deficient meals (prepared by the site) that they will be asked to consume throughout the day without any other food intake. Female subjects will be asked to provide a urine sample to ensure non-pregnant status. On Day 1, subjects will arrive following an overnight fast and will be asked to complete a fasting weight, vitals, and a RN-administered health history questionnaire to review any change in medications or health status. Subjects will then be asked to empty their bladders and provide a baseline blood sample before being administered any drug(s). Subjects will receive vitamin or a combination of vitamin and drug. Subjects will be provided limited water, less than 500mL, following the dose and will be given no food or additional water for 4 hours post-dose to avoid excessive dilution of the dose or a food-drug interaction. After the 4-hour period, subjects will be provided their first meal and water ad libitum. Blood and urine collection will proceed on Day 1 as outlined below in Schedule of Study Outcomes. Subjects will be provided three thiamine deficient meals during their visit on Day 1. Subjects will be permitted to leave after the 12-hour post-dose sample collection and will be asked to fast overnight. On Day 2 of each cycle, subjects will arrive at the site, submit their urine collection kit, and have one more blood collection (24-hour post-dose). They will then be served a light standard breakfast. See Schedule of Study Outcomes for more details.

Total duration of the study including time to achieve complete enrollment and complete 9 study visits will be approximately 19-37 days depending on when the subject schedules their next cycle visit.

All subjects will provide at most 157.5mL of blood per cycle. Since the common standard for collection is approximately 1 pint (i.e. 473mL) at blood drives and/or donation centers, we feel that we will not putting the subject in danger. Loss of 550mL is not harmful and is typically replenished by the body within two to three weeks.

Cycle Schedule (Repeat for a total of three cycles – 9 total visits)

Visit	Activities	Expected Duration
Day -1 to 0 (Day -1 to 0)	-Consent (cycle 1 only) -Randomization -Pregnancy test for females -Weight, vitals (cycle 1 only) -Health/med/AE review -Meet with RD -Breakfast on site (optional)	1-2 hrs

	-Pick-up study meals to be consumed the rest of the day (or following day) -Fast overnight for 8 hours	
Day 1	-Vitals measured -Health/med/AE review/ -Baseline urine sample -Drug/vitamin administration -Blood collections, drawn from IV (0h, 0.25h, 0.5h, 1h, 1.5h, 2h, 2.5h, 3h, 3.5h, 4h, 6h, 8h, 10h, 12h) -Urine collections (0-4 h, 4-8 h, 8-12 h) -Consume study diets -Instructions for urine and fasting overnight	14 hrs
Day 1 Overnight	-Urine Collection at Home (12-24 h)	
Day 2, *24-hours post-dose*	-Arrive fasting -Submit urine kit -Blood collection (24-hr post-dose) -Urine collection (if necessary) -Eat a standard breakfast	1 hr
Washout (5-14 Days) between Cycle 1 and 2 and between Cycle 2 and 3	-No visit required -Resume usual diet -Complete two food diaries	20-30 minutes

Part 2

Based on the results from Part 1, trimethoprim will be taken forward in a two-arm, randomized crossover design in Part 2 as follows:

After screening 60 volunteers, 14 subjects are expected to be enrolled at the Tufts site with the goal of having 12 healthy volunteers complete the study. Each subject will be administered the same two treatments (thiamine only and a combination of thiamine + trimethoprim), simply in a different order depending on arm assignment. There will be two cycles in this study, each separated by at least 5 days. Subjects will come in three times within a 72-hour period during each cycle.

Screening data, including a background questionnaire, urine sample, and a blood sample, will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

Subjects will be randomized into one of two groups and will be administered a different sequence of treatments dependent on their group assignment:

Group 1: thiamine only (Cycle 1), thiamine and trimethoprim (Cycle 2)

Group 2: thiamine and trimethoprim (Cycle 1), thiamine only (Cycle 2)

Subjects will arrive on Day 0 of each cycle to pick-up three thiamine deficient meals (prepared by the site) that they will be asked to consume throughout the day without any other food intake. Female subjects will be asked to provide a urine sample to ensure there is no change in pregnancy status. On Day 1, subjects will arrive following an overnight fast and will be asked to complete a fasting weight, vitals, and a RN-administered health history questionnaire to review any changes in medications or health status. Subjects will

then be asked to empty their bladders and provide a baseline blood sample before being administered any drug(s). Subjects will receive vitamin or a combination of the drug + vitamin. Subjects will be provided limited water, less than 500mL, following the dose and will be given no food or additional water for 4 hours post-dose to avoid excessive dilution of the dose or a food drug interaction. After the 4-hour period, subjects will be provided a naturally thiamine deficient snack and water ad libitum. Blood and urine collection will proceed on Day 1 as outlined below in Schedule of Study Outcomes. Subjects will be provided thiamine deficient snacks and one thiamine deficient meal during their visit on Day 1. Subjects will be permitted to leave after the 10-hour post-dose sample collection and will be asked to fast overnight. On Day 2 of each cycle, subjects will arrive at the site, submit their urine collection kit, and have one more blood collection (24-hour post-dose). See Schedule of Study Outcomes for more details.

Total duration of subject participation will be approximately 6 days. Total duration of the study will include time to achieve complete enrollment plus 11-20 days of the study depending on when the subject schedules their next cycle visit.

Cycle Schedule (Repeat for a total of two cycles – 6 total visits)

Visit	Activities	Expected Duration
Day -1 to 0	-Consent (cycle 1 only) -Randomization -Pregnancy test for females -Weight, vitals (cycle 1 only) -Health/med/AE review -Meet with RD -Breakfast on site (optional) -Pick-up study meals to be consumed the rest of the day (or following day) -Fast overnight for 8 hours	1-2 hrs
Day 1	-Vitals measured -Health/med/AE review -Baseline urine sample -Drug/vitamin administration -Blood collections, drawn from IV (0h, 0.25h, 0.5h, 1h, 1.5h, 2h, 2.5h, 3h, 3.5h, 4h, 6h, 8h, 10h) -Urine collections (0-4 h, 4-8 h, 8-10 h) -Consume study diet -Instructions for urine and fasting overnight	12 hrs
Day 1 Overnight	-Urine Collection at Home (10-24 h)	
Day 2, *24-hours post-dose*	-Arrive fasting Weight, vitals (cycle 2 only) -Submit urine kit -Blood collection (24-hr post-dose) -Urine collection (if necessary) -Eat a standard breakfast	1 hr
Washout (5-14 Days) between Cycle 1 and 2	-No visit required -Resume usual diet -Complete two food diaries	

For Part 1, blood will be collected pre-dose as well as at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12, and 24 hours after the start of dosing for each cycle. For Part 2, blood will be collected pre-dose as well as at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, and 24 hours after the start of dosing for each cycle. Subjects will be asked to collect all urine samples during their stay in intervals of 0-4, 4-8, 8-12, and 12-24 (home kit) hours for Part 1 and 0-4, 4-8, 8-10, and 10-24 (home kit) hours for Part 2 respectively.

It is estimated that it will take approximately 1.5 to 2 years to complete this study.

5.2 Procedures

- Is there a placebo control arm?

☐ **Yes** ☒ **No**

Subjects will be screened using a screening questionnaire as well as by providing a blood and urine sample to determine eligibility. A screening informed consent will be completed at the beginning of the screen visit. Both the questionnaire and samples collected at screening will help monitor subjects for safety by identifying any risks before enrolling the subject into the study. Both drugs used in this study are FDA-approved. Metformin is an orally prescribed drug that is used as a first line of therapy for Type II diabetes patients and trimethoprim is a synthetic antibacterial that is commonly prescribed for urinary tract infections. All information will be collected on screening questionnaires and case report forms (CRFs).

Pregnancy tests will be conducted at screening and at each visit for women of reproductive potential. Women of reproductive potential will be instructed to use a birth control method of their choosing including but not limited to oral contraceptives, condoms, and IUDs. Men of reproductive potential will also be instructed to use a birth control method of their choosing including but not limited to condoms.

All procedures are being performed for research purposes and are not part of the usual standard of care at Tufts.

5.3 Evaluations

Will you perform any laboratory tests for this study?

☒ **Yes** ☐ **No**

SCHEDULE OF STUDY OUTCOMES

	SCREENING (Before Cycle 1 Day 1)	VISIT 1 (Cycle 1 Days 0 - 2)	VISIT 2 (Cycle 2 Days 0 - 2)	VISIT 3 (Cycle 3 Days 0 - 2)*
Informed Consent	X			
Health History Questionnaire	X			
CBC, metabolic panel	X			
Height	X			
Weight	X	X		
Vital Signs[a]	X	X	X	X
Pharmacokinetic blood sampling[b]		X	X	X
Pharmacokinetic urine collection[c]		X	X	X
Urine collection/Urinalysis	X	X	X	X
Pregnancy Test (Serum)	X			
Pregnancy Test (Urine)		X	X	X
Administration of Study Drug[d]		X	X	X
Initiate Subject Food Diary		X	X	
Subject Food Diary Collection			X	X
Concomitant Medication Review	X	X	X	X
Adverse Events		X	X	X

*Visit 3 is only included in Part 1 of the study.
[a] Weight will be assessed only on Visit 1 of Cycle 1.
[b] Pharmacokinetic blood samples will be collected at pre-dose as well as at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12, and 24 hours post-dose at Visits 1, 2, and 3 for Part 1. Pharmacokinetic blood samples will be collected at pre-dose as well as at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, and 24 hours post-dose at Visits 1 and 2 for Part 2.
[c] Pharmacokinetic urine samples will be collected in intervals of 0-4, 4-8, 8-12, and 12-24 hours at each visit/cycle for Part 1. Pharmacokinetic urine samples will be collected in intervals of 0-4, 4-8, 8-10, and 10-24 hours at each visit/cycle for Part 2.
[d] Administration of study drug will be dependent on which group the subject is enrolled in and will be either thiamine only, thiamine + metformin combination, and/or thiamine + trimethoprim combination depending on the cycle/visit/part of the study.

Subjects will be sampled at screening and during Part 1 & 2 at Cycle 1, 2, & 3 (for Part 1) on both Days 1 and 2. All laboratory tests that will be performed comply with the Clinical Laboratory Improvement Amendments of 1988. Details of the analyses are described below.

Screening Laboratory Tests:
During the screening visit, 20 mL of blood will be collected for standard health screen, complete blood count with differential and routine clinical analysis by the Nutrition Evaluation Laboratory (NEL) at the HNRCA. A clean catch urine specimen will also be collected for a standard dipstick urinalysis. Pregnancy testing will be conducted for women of childbearing age.

Intra-Study Laboratory Tests and Procedures:
Blood specimens will be collected during Parts 1 and 2 of the study on Days 1-2 of each of the cycles at pre- and post-dose of the intervention. All specimens will be sent to the NEL for specimen processing. Approximately 10 mL of whole blood will be collected at baseline, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12, and 24 hours post dose during Cycle 1, 2 and 3 for Part 1 and for Part 2 at baseline, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, and 24 hours post dose during Cycle 1 and 2 for measures of Thiamine, TMP, TPP, Metformin and Trimethoprim in plasma and/or RBC at UC Davis.

Urine specimens will be collected at pre-dose void, 0-4, 4-8, 8-12 and 12-24 (home kit) hours for Part 1 and at pre-dose void, 0-4, 4-8, 8-10 and 10-24 (home kit) hours for Part 2 and will be processed by the NEL. Three aliquots will be sent to UCSF and/or UC Davis for measure of Thiamine, TMP, TPP, Metformin and Trimethoprim.

All specimens transferred to UCSF and/or UC Davis will be stripped of all HIPPA identifiers and coded.

- **Collection and Storage of Human Biological Specimens (Tissue Banking)**

Will biological specimens be stored for **future, unspecified**, research?

☒ **Yes** ☐ **No**

Optional participation in tissue banking is offered during the informed consent process and an additional signature will be required to consent to tissue banking. Blood and urine samples will be collected during Parts 1 and 2 of the study on Days 1-2 of each of the cycles at pre and post-dose of the intervention. Tissue Banking will not require an additional blood draw or collection from subjects from that in the main study. The participant will also be informed on how he/she can withdraw his/her sample from the tissue bank if he/she so desires to do so at any time. Participants will be asked to take the time to think about if they wanted to do this, if more time is needed, and will be encouraged to ask any questions or mention any concerns they may have about having their samples stored. All questions will be answered to the participants' satisfaction and any concerns will be addressed accordingly so as to allow participants to give informed consent.

Types of biological materials to be stored and processed
Following signing of optional tissue banking consent form, blood samples will be collected according to IRB approved protocol. Tissue banking samples will be stored for an indefinite period for future, unspecified research in a -80C Freezer located in laboratory of the PI. They will be stripped of all HIPPA identifiers and coded. If it is determined that additional analysis of the samples is warranted we will submit the appropriate documents to the IRB. These analyses may include, but are not limited to, genome wide association studies and characterization of metabolites. For these, samples may be distributed to the Nutritional Evaluation Lab, UCSF, UC Davis, or any other collaborators deemed by the principal investigator.

Measures taken to guard against disclosure of confidential information about the source individual
a. The biological materials will be transferred to the tissue bank after they have been de-identified and provided with a unique subject code. Subject codes may be broken at study completion for data analysis or if study subject elects to withdraw from the study.
b. The master list of the codes will be kept secured electronically (by using a password) and will be filed and locked in a cabinet.
c. A single designated research technician for each site will be solely responsible for all information related to the de-identification and re-coding of the biological material to be banked. The information will then be transferred to the PI and secured electronically (by using a password) and filed and locked in a cabinet.
d. If the PI turns the study over to a new PI, the key codes will be transferred to the new PI.
e. Banked biological material ready to be processed on-site will remain de-identified and coded.
f. When no further use of the banked biological materials has been established the stored samples will be discarded and the key code destroyed.
g. We will not go back to the medical record or study volunteer for additional health information. Their contact information at this time is totally useless.

Withdrawal Consent to banking of biological materials
At any time after tissue banking permission has been granted. If the study participant wishes to withdraw consent to the research use of biological materials for future studies, after the code has been broken, the samples will be retrieved and destroyed.

6 Ethics and Protection of Human Subjects

6.1 Informed Consent Process

Will subjects be required to provide informed consent?

☑ **Yes** ☐ **No**

If **Yes**, describe the following*:*

- The consent process will take place in a quiet room of the HNRCA nursing floor. Interested subjects will have as much time as they need to review the informed consent, and will be encouraged to ask any questions. They may also go home and discuss participation with peers or loved ones to ensure they are interested. Screening ICFs will be mailed home prior to their visit to the HNRCA, giving additional time to review it prior to their visit should they choose. They are not expected to bring this consent with them, but rather use it as a reference to prepare for the visit.
- The investigators will follow the SOP: Informed Consent Process for Research (HRP-090).
- Illiterate and non-English speaking subjects will not be enrolled because there are not funds to hire a translator to review the ICF and to translate any safety concerns during the study period. Instructions for the menus and urine collections and food logs would also need to be translated. No direct benefit is expected to come from participation in this study, therefore we do not feel that we are discriminating against illiterate or non-English speakers.

6.2 Waiver or Alteration of Consent Process

N/A

6.3 International Research

N/A

6.4 Confidentiality

Procedures to protect subject confidentiality:

Personal information of subjects who consent to participate in the study will not be given to anyone without permission unless the law requires it. Every effort will be made to keep subjects' information private, but this cannot be totally guaranteed. The Tufts Medical Review Board

(IRB), the HNRCA, the National Institute of Health or study sponsor may check records that identify subjects. These records may include medical or research records and the signed consent form, which will be locked in a locked medical records room at in the HNRCA Volunteer Services Department. Electronic research records will be maintained in a secure, HIPAA compliant REDCap database maintained by the HNRCA Scientific Computing Department with access restricted to essential study personnel only.

All records of subjects that have signed a screening consent, including records of ineligible subjects will be stored for a minimum of 10 years. Only authorized personnel may access the record.

The HNRCA Volunteer Services Department is staffed during normal working hours and is locked during non-working hours. If it is necessary for a medical record to leave the admissions office, it must be signed out only by an HNRCA employee (who is identified by Tufts ID) in a log of the HNRCA ID, name of employee taking possession of the chart, and the date. The records of this study might also be reviewed to make sure all rules and guidelines were followed.

The HNRCA maintains a computerized database of participant information referred to as Protocol Manager (PM), which is a secure Oracle database that is backed up nightly. The database is centralized under the Volunteer Services Department (IRB approved protocol # 6701). Computer access to the database is provided through the Protocol Manager User Interface which was designed as an internal website ("intranet") in order to enable utilization by both PC and Mac users. An electronic gatekeeper (known as a "firewall") blocks all terminals outside of the HNRCA from gaining access. The Scientific Computing Department subscribes to Microsoft's security bulletins and appropriate security updates are applied on an ongoing basis.

If a subject withdraws or is terminated from the study, the study data generated so far will be retained as part of the study database.

Coded blood sample vials collected during the study will be stored without personal identifiers (names, addresses, etc.) at the Nutrition Center until the analyses are completed. Any tissue banking samples will be stored indefinitely at the University of California, San Francisco. Subjects may withdraw consent for DNA storage at any time during or after the study by writing a letter to Dr. Giacomini (School of Pharmacy, Dept. of Bioengineering and Therapeutic Sciences, 1550 4th Street, Box 0446, UCSF, San Francisco, CA 94143). All paper study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Only Dr. Giacomini and appointed research staff will have access to this code. Statistical summaries and reports generated for distribution will not contain personal identifiers (names, addresses, etc.)

6.5 Provisions to Protect the Privacy Interests of Subjects

To protect the privacy interest of participants, study procedures (except for meals and blood draws) will be conducted in private testing areas where they can confidentially interact with study personnel. Following informed consent, participants will each be given a unique participant identification number and will never be asked to list their personally identify able information on study materials or testing software. Access to personally identifiable information (PII) by experimenters will be limited to participants' phone numbers for the purpose of contacting the participant to track compliance and to notify participants of their group assignment at the conclusion of the study, when the randomization code is broken, and participants' addresses for the purpose of mailing compensation. All study data will be coded to avoid the need for further storage or access of PII.

6.6 Provisions to Monitor the Study to Ensure the Safety of Subjects

The data will be reviewed, including safety data, adverse events, and efficacy data by the study physician. Research nurses will check in with volunteers at each visit to ensure no changes in health status have occurred. Safety data collection starts at screening and concludes with the final visit in which we reevaluate health status. A data and safety monitoring board will also be put together to assess the study and monitor adverse events as well as safety data periodically.

6.7 Compensation for Research-Related Injury

Does the research involve greater than minimal risk to subjects? *(or if minimal risk, is there potential risk of research-related injury?)*:

☒ **Yes** ☐ **No**

There is no plan to compensate volunteers for any injury incurred as a result of participation in this study. Volunteers and/or their insurance companies will be responsible for any charges for treatment of research-related injuries, and will have reviewed and consented to this in the screening and main ICFs.

6.8 Economic Burden to Subjects

Does the research involve any costs to subjects?

☐ **Yes** ☒ **No**

6.10 Vulnerable Populations

If the research involves individuals who are vulnerable to coercion or undue influence, describe the rationale for their inclusion and the additional safeguards included to protect their rights and welfare.

Will pregnant women be enrolled?

☐ **Yes** ☒ **No**

Will the research involve neonates of uncertain viability or non-viable neonates?

☐ **Yes** ☒ **No**

•

Will subjects who are not yet adults (neonates, children, teenagers) be enrolled?

☐ **Yes** ☒ **No**

- Will minors who are:
  - i) married, widowed, divorced; or
  - ii) the parent of a child; or
  - iii) a member of any of the armed forces; or
  - iv) pregnant or believes herself to be pregnant; or
  - v) living separate and apart from his/her parent or legal guardian, and is managing his/her own financial affairs

be approached for study participation for either themselves or their child?

☐ **Yes** ☒ **No**

Will wards of the state and/or children at risk of becoming wards of the state be enrolled (this includes foster children or any child that is in state custody)?

☐ **Yes** ☒ **No**

- Will cognitively impaired adults (adults with impaired-decision making capacity) or adults who may lose the capacity to consent be enrolled?

☐ **Yes** ☒ **No**

Will prisoners be enrolled?

☐ **Yes** ☒ **No**

Will students and/or employees be enrolled in this research?

☒ **Yes** ☐ **No**

Although students and employees will not be targeted for recruitment and enrollment, employees of Tufts University and/or the HNRCA (employee-subjects) who voluntarily want to participate in the study will be eligible for screening and enrollment. In order to qualify for the study, employee-subjects must respond to IRB-approval advertisement of their own accord and will not be directly approached by any person seeking to recruit them for participation in the study. Members of the research team, as direct-report subordinates of the PI and anyone who is direct-report subordinate to any of the research team members in any other capacity and staff and scientists who are primarily trained in nutrition will not be eligible to participate in the study. If employee-subjects qualify to participate in the study, they cannot participate as volunteers during hours in which they are being compensated by Tufts University for their regular work. This includes use of vacation, personal days and sick time.

7 Adverse Event Monitoring

7.1 Definitions

An adverse event (AE) is any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents for both Parts 1 and 2. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in

Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	There is a reasonable possibility that the adverse event, incident, experience or outcome may have been caused by the procedures involved in the research.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

7.2 Reporting Procedures

Serious adverse events (SAE) and unanticipated events (UE) will be reported by participants as soon as practically possible and investigators will report these to the IRB within 24 hours of study team becoming

aware of event. The definition and reporting requirements for SAEs and UEs will adhere to Tufts University policies. All SAE and UE will be reviewed by the study physician and an event evaluation form will be completed that will include a description of the event, assessment of potential relationship to the intervention, assessment of need for change in the consent or the study activities, event outcome and a classification of the main organ system involved. Raw data will be available for review by the Study Physician if requested.
An adverse event is defined as: "Any untoward or unfavorable medical occurrence in a human research participant, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the individual's participation in the research, whether or not considered related to the individual's participation in the research."

Reporting timeframe for SAEs and AEs:
Investigators will promptly report unexpected adverse events, serious adverse events, unanticipated problems involving risk to subjects or others, and deviations to IRB within five business days using the Reportable New Information (HRP-214) form. The study physician will be notified of potential unexpected study-related events, and serious adverse events that may possibly be related to the study for further classification. All other adverse events will be reported to the Tufts Institutional Review Board at the time of continuing review. If adverse event monitoring suggests that events are occurring with greater frequency or severity than initially expected, the PI will submit a report of an Unanticipated Problem to the IRB, with an amendment if a modification of the study design is indicated.

7.3 Reportable New Information

Reportable new information will be reported to the IRB per the Tufts Health Sciences IRB's Reportable New Information policy.

8 Statistical Considerations

8.1 Study Endpoints

Part 1:

- Primary Endpoint

  To determine whether SLC19A3 inhibitors metformin and/or trimethoprim decreases the AUC, area under the curve, of thiamine between the different treatment groups, thiamine versus thiamine and SLC19A3 inhibitor. This endpoint will be assessed at the end of Part 1 using all data available across all three groups, specifically comparing data from the thiamine only arm against the thiamine and SLC19A3 inhibitor combination arms. A decrease in AUC in the combination treatment will suggest inhibition of thiamine uptake through inhibition of SLC19A3.

- Secondary Endpoint

  Investigate metabolic signatures reflecting the activity of TPP-dependent enzymes after dosing thiamine versus combination of thiamine and SLC19A3 inhibitor. Thiamine pyrophosphate (TPP), one of the major metabolites of thiamine, is a co-factor for multiple metabolic pathways. Thus, it is possible that a decrease in thiamine absorption by metformin could affect the concentration of key metabolites and metabolic ratios of TPP-dependent enzymes and pathways.

Part 2:

- Primary Endpoint

  To confirm whether trimethoprim affects the AUC, area under the curve, of thiamine between the different treatment groups, thiamine versus thiamine and trimethoprim. This endpoint will be assessed at the end of Part 2 using all data available across both groups from both Parts 1 and 2, specifically comparing data from the thiamine only arm against the thiamine and trimethoprim combination arm. A decrease in AUC in the combination treatment will suggest inhibition of thiamine uptake through inhibition of SLC19A3.

- Secondary Endpoint

  Further investigate metabolic signatures reflecting the activity of TPP-dependent enzymes after dosing thiamine versus combination of thiamine and trimethoprim. Thiamine pyrophosphate (TPP), one of the major metabolites of thiamine, is a co-factor for multiple metabolic pathways. Thus, it is possible that a decrease in thiamine absorption by trimethoprim could affect the concentration of key metabolites and metabolic ratios of TPP-dependent enzymes and pathways.

8.2 Statistical Analysis

Analysis of Primary Endpoint

For Part 1 and Part 2, pharmacokinetic parameters including half-life, apparent volume of distribution*, total clearance*, renal clearance, net reabsorptive or secretory clearance, absorption rate constant, K_a, and AUC_{po} of thiamine and the respective SLC19A3 inhibitor will be calculated from plasma and urine drug and vitamin levels by standard pharmacokinetic methods using WinNonlin [55-59] (*these parameters are divided by bioavailability as oral doses are given). We will estimate glomerular filtration rate, GFR, using standard methods described in our previous studies [56, 57, 60, 61]. To assess net secretory or reabsorptive clearance of the compounds, we will subtract fu*GFR (where fu is the unbound fraction of metformin, thiamine, TMP or TPP) from renal clearance of the compounds directly measured from plasma and urine values. Previously we have used these methods to calculate net secretion (or reabsorption) of several drugs [57-59, 62]. In addition, for TMP and TPP, half-life, AUC_{po}, renal clearances, net reabsorptive or secretory clearance, and ratios of AUCs of TMP and TPP to thiamine will be calculated using standard pharmacokinetic methods for metabolites [63].

For statistical analysis, we will use paired-t-tests to compare the pharmacokinetic parameters of thiamine in the absence and presence of an SLC19A3 inhibitor. In the pharmacokinetic analyses, we expect to see a significant influence of the SLC19A3 inhibitor on the oral exposure to thiamine, AUC_{po}. Because SLC19A3 in the proximal tubule mediates reabsorption of thiamine [64, 65], we may also observe an increase in the renal clearance (reduction in reabsorptive clearance) of thiamine when volunteers are dosed with an SLC19A3 inhibitor. Understanding the effect of metformin and/or trimethoprim on renal clearance of thiamine is also of interest and could potentially contribute to even lower plasma thiamine levels.

Analysis of Secondary Endpoint

For the metabolic signatures, results will be processed with pair-wise mean testing, hierarchical cluster analyses, and multivariate discriminate analyses while employing multiple comparison corrections, as appropriate. Basal thiamine status including transketolase activity will be evaluated as a covariate prior to all analyses. Our primary analyses will focus on comparing the levels of the individual metabolites and metabolite ratios after vitamin administration with the baseline levels to determine if there are effects of the vitamin on the metabolite levels and ratios in the plasma. The primary comparison will test differences between treatments using one-way ANOVA with Holm-Sidak post hoc comparison, or Student's t-Tests with the False Discovery Rates adjustment of Benjamini and Hochberg[67]. For Part 1 and Part 2, our second comparison will be to compare the levels of metabolites after thiamine administration with their levels after thiamine plus SLC19A3 inhibitor administration at multiple time points. To identify discriminants of thiamine vs. thiamine + SLC19A3 inhibitor exposure, all variables will be transformed to normality and subjected to orthogonal partial least squares – discriminant analysis (OPLS-DA) and boot strapped models will be built with a 3:1 training/test set splits using the imDEV v 1.4.268. Variables with variable importance in projection (VIP) scores <1 will be removed from further consideration, and models refined to minimize the root-mean-squared-error of prediction (RMSEP) and maximize Q2, a measure of model performance[69]. The retained variable set will be organized using hierarchical cluster analysis. To test for significance of discriminate variables of thiamine or SLC19A3 inhibitor effects, including levels of metabolites or metabolic ratios, we will compare levels of retained metabolites (or ratios) at each time after vitamin or drug administration with baseline values, using paired-t- tests, and among times and treatments using two-way ANOVAs with Holm-Sidak post hoc comparison. To assess covariate behavior among thiamine, TPP, metformin, other metabolites and/or or metabolite ratios, hierarchical cluster

analyses will be performed and Spearman's correlation matrices will be generated. Linear correlation analyses will be conducted, and correlation coefficients will be estimated for each metabolite in each volunteer (in each study arm). To compare time-dependent changes in each study arm, in addition to our pharmacokinetic analysis, plasma thiamine will first be modeled as a continuous variable using partial least squares discriminate analysis, and variables with variable importance in projection scores (VIP) >1 will be selected. The average metabolite value will then be independently calculated in each study arm, and variables will be organized in the self-organizing maps using the Kohonen package in R70. Patterns will be compared between treatment groups by regression analysis of representative variable group members. Additional complex relationships will be examined if the data warrant those, for example, Emax models may be used to characterize the relationship between thiamine levels and levels of metabolites or metabolic ratios.

8.3 Number of Subjects

As many as 30 healthy volunteers will be screened for enrollment in Part 1 and 60 in Part 2. All subjects will be enrolled at the Tufts site since this study is not a multi-site study. Seven subjects are expected to be enrolled at the Tufts site for Part 1 with the goal of having six subjects complete the study. Fourteen subjects are expected to be enrolled at the Tufts site for Part 2 with the goal of having twelve subjects complete the study.

8.4 Data Management

The research data for this clinical trial will mainly consist of questionnaires and clinical measures of blood, serum, plasma and urine related to the proposed interventions. For a complete list of analysis types please refer to 8.2 Statistical Analysis. These data will be captured and managed using a REDCap database designed specifically for this study using the HNRCA's deployment of REDCap which are both HIPAA and 21CFR11 compliant. In addition to the above database, Analytical measures from the Nutrition Evaluation Laboratory will be recorded using the LabWeb LIS system. These measures will then be imported into the REDCap database using the provided API and will undergo external validation checks to ensure that the data has been imported correctly. Additionally, metadata files and data dictionaries/codebooks will be used to provide all information necessary to properly use and understand the data files. The HNRCA data management program records and stores subject identifiers separately from clinical information and results of study analyses. Access to each section is restricted to pertinent team members. A study code number will identify each laboratory specimen for immunological testing as related to investigation of the immune response. The master list which links the identity of the subject with the laboratory specimens will be kept in a locked file cabinet in the office of the PI and on a password protected database on secure Tufts server. For electronic records, other study information is maintained on a secure database that is password- protected and requires user-level authentication with required periodic password changes. Only study personnel will have access to the database to record results as they accrue. While being analyzed, samples will be stored in locked areas in the secured laboratory facility.

To ensure the safe transfer of data and samples to institutions outside of Tufts,a Materials Transfer Agreement (MTA) between University of California and Tufts University is in process and we will submit the finalized agreement in a later amendment.

In accordance with HNRCA policy, we will retain all data in locked file cabinets (paper) and secure databases (electronic) for ten years, after which point it will be destroyed.

8.5 Randomization

Will subjects be randomized?

☒ **Yes** ☐ **No**

9 Drugs or Devices

Will the research involve drugs?

☒ **Yes** ☐ **No**

The study physician and nursing staff onsite will be responsible for all drugs. The compounding pharmacy will send study drugs to UCSF, who will then ship study drugs to Tufts and UC Davis, limiting the number of individuals handling the compounds. A safe or locked refrigerator will be used to store all drugs. All drugs will be safely disposed of via Tufts third party contractor after completion of the study.

UC Davis will have their own drug supply to store and conduct analytical testing for stability throughout the life of this study and will not require remaining drug from Tufts. To obtain the drugs, the compounding pharmacy requires the name of the physician and study protocol to process the order. The compounding pharmacy will then fill the order by ordering pre-formulated tablets directly from the manufacturer or by ordering thiamine powder and capsules. The thiamine powder and capsules will then be prepared with 5mg of thiamine in each capsule. The thiamine capsules will be validated by UC Davis for accuracy.

Thiamine is available over the counter and metformin and trimethoprim are available via prescription. Volunteers taking any other medications will be excluded from this study. All medications will be reviewed by the attending physician during the consent process.

Volunteers will be administered tablets under nursing supervision and will not be receiving any drugs or supplements to take at home. The tablets and capsules are to be swallowed as any other ordinary pill. No additional instruction on administration will be given to the study subjects.

Will the research involve devices?

☐ **Yes** ☒ **No**

10 Study Administration

10.1 Setting

All the procedures will take place at the JM-USDA-HNRCA at Tufts University. Participants will visit the site at the scheduled days (see study scheme) where they will receive the meals. A Data Monitoring and Safety Board will be composed to oversee this study.

10.2 Registration

All the materials used for the pre-screening and screening visits to ensure that a subject is appropriately enrolled or registered in the study prior to receiving any study intervention are included in the IRB package or are part of IRB submission #6701.

10.3 Resources Available

The members of the team include:

Principal Investigator: Andrew Greenberg, MD

Study Physician: Lisa Ceglia MD

Co-Investigators:

Study Coordinator: Kim Vo Trinh

Ryan Piccirillo

Metabolic Research Unit personnel:

Jean McShea

Janice Klian

Mary Weiss

Margaret Y Vilme

Kimberly Dupiton

Helen Rasmussen

Cheryl H Gilhooly

Carmelle St. Victor

Catherine Murphy

Michelle Hallam-Naing

The team has the combined expertise to conduct the proposed study from recruitment to data analysis.

The MRU has demonstrated capacity to successfully conduct the relevant aspects of the study (recruitment, preparation of the diets, etc.)

We anticipate to contact about 300 subjects in order to recruit 60 individuals in order to enroll 14 subjects and have 12 subjects complete the study.

Weekly meetings of the team will take place to revise all the aspects of the study and to resolve any unanticipated problem that may arise.

10.4 IRB Review

The Tufts Health Sciences Institutional Review Board (HS IRB) will review and approve this study.

10.5 Multi-Site Research

Is this a multi-site study where Tufts is the sponsor, primary grant recipient, or coordinating site?

☐ **Yes** ☒ **No**

○

10.6 Community-Based Participatory Research

Note: "Community-based Participatory Research" is a collaborative approach to research that equitably involves all partners in the research process and recognizes the unique strengths that each brings. Community-based Participatory Research begins with a research topic of importance to the community, has the aim of combining knowledge with action and achieving social change to improve health outcomes and eliminate health disparities.

Will this study involve community-based participatory research?

☐ **Yes** ☒ **No**

10.7 Sharing Results with Subjects

Will results (overall study results or individual subject results, such as results of investigational diagnostic tests, genetic tests, or incidental findings) be shared with subjects or others (e.g., the subject's primary care physician or the subject's treating physician)?

☐ **Yes** ☒ **No**

- # References

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