UNIVERSITY OF MISSOURI IRB PROTOCOL: Project 2006432

PROJECT TITLE: "Evaluation of TVB-2640, a FASN Inhibitor, to reduce de novo lipogenesis in subjects with characteristics of the metabolic syndrome"

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Purpose
The present study is designed to determine whether the drug, TVB-2640 will significantly reduce liver fat synthesis in male subjects with characteristics of the metabolic syndrome.

Background
The continuum of metabolic syndrome, nonalcoholic fatty liver disease (NAFLD) and the more advanced disease of non-alcoholic steatohepatitis (NASH) can progress to significant liver diseases including cirrhosis and hepatocellular carcinoma. Over the next 5 years, NASH will overtake hepatitis C as the most prevalent liver disease in the U.S. In addition, NAFLD is associated with increased comorbidities including cardiovascular diseases and diabetes. Obesity and the metabolic syndrome [4] are two key risk factors for NAFLD which are characterized as an imbalance in energy utilization and storage. This imbalance leads to dysregulated metabolic pathways and inflammatory responses that drive further changes leading to liver damage and comorbid conditions. There are currently no approved drugs to treat metabolic syndrome or NAFLD. The synthesis of fatty acids in the liver, a pathway termed hepatic de novo lipogenesis (DNL), is increased in subjects with metabolic syndrome and NAFLD. The DNL pathway not only produces fatty acids that contribute to elevated liver stores of triglycerides, but the fatty acids that are produced are saturated fatty acid species, primarily palmitate (C16:0), which contribute to signaling events that increase liver inflammation. Therefore DNL is an important pathway for therapeutic intervention to reduce the consequences associated with metabolic syndrome and NAFLD. One of the key enzymes in DNL is fatty acid synthase (FASN); this enzyme is solely responsible for synthesizing palmitate. Normal responses to feeding carbohydrate in meals include a transient increase in DNL which is dependent on the enzyme FASN. Using isotopic tracers, it is clear that subjects with NAFLD have higher rates of hepatic DNL compared to subjects without fatty liver. Additionally, NAFLD subjects do not suppress DNL upon fasting unlike the subjects without fatty liver. This observation forms the basis for evaluating the inhibition of FASN, thereby reducing DNL, as a potential therapeutic target to reduce fatty liver disease.

The drug, TVB-2640 has been tested in previous subjects with cancer because the lipogenesis pathway is important to the control of some cancer progression. The drug was shown to have a low side effect profile and the side effects that did occur (xeroderma, some hair loss, dry eyes), appeared after 14 days of treatment at doses ranging from 150-450 mg per day. Therefore, the present study will use a shorter duration of treatment (10 days), and choose a starting dose at the lower end of this treatment range (50 mg/d).

Specifically, as described in the attached IND application, in 127 cancer patients, at doses of 200 mg, side effects included dry eyes, dyspepsia, hair thinning, dry skin, particularly on the hands and feet. By contrast, at doses of 400-600 mg/d, *for durations longer than 10 days*, corneal edema (n=3) and palmar-plantar erythrodysesthesia (PPE, n=3) were also observed. The corneal edema and PPE were thought to be related to the action of the drug and extended dry eye. All the side effects described above resolved after discontinuation of drug.

It should be noted that, because previous use of this drug occurred in cancer patients on concurrent treatment with paclitaxel, side effects common to paclitaxel were also observed during co-treatment. These effects, including uveitis, fatigue, decreased appetite, and peripheral neuropathy, were not unexpected.

During these studies, a fatty acid biomarker of lipogenesis, found in blood triglyceride (TG) was found to be reduced significantly. The present study will test a lower dose (50 mg/d) in men (no women) with characteristics of the metabolic syndrome, who are otherwise healthy. The focus on subjects with metabolic syndrome is based on the fact that the future use of the drug will be in patients with non-alcoholic fatty liver disease who will likely have metabolic syndrome characteristics.

The Parks lab has developed an oral sugars tolerance test (OSTT) to determine the magnitude of liver stimulation of lipogenesis when an individual consumes an oral bolus of sugars [1]. In the present study, we will use this protocol to determine whether 10 days of drug treatment will significantly reduce fasting and sugar-stimulated lipogenesis. The study is divided into 3 parts (described below) which will allow us to make minor adjustments in the dose of drug after the first six subjects' results are available to optimize the suppression of lipogenesis, while also minimizing any side effects the drug might have.

The overall study design is shown in **Figure 1** and the timing of the procedures is identical for all three parts of the study. The study design is a repeated-measures, with each subject serving as his own control. The study will be unblinded with respect to the research staff working directly with the subjects. However, laboratory personal who will be running the biochemical analyses will be blinded to whether they are analyzing baseline or post-treatment samples.

Summary of study objectives

This study's goal is to identify a drug dose that will effectively lower lipogenesis, while minimizing any side effects. We will start studying two subjects at a dose of 50 mg/day, and continue at 50 mg/day for the following 4 subjects, closely monitoring side effects and drug activity.. Depending on the degree of side effects, effectiveness of the dose in reducing lipogenesis, and PK activity for the initial 6 subjects, the dose for the last 6 subjects may be adjusted to a targeted dose that will provide the most significant reduction in lipogenesis with the least amount of side effects, if any. A decision tree to serve as the basis for these decisions is presented as **Supplementary Figure 1** in the Appendix

We will accomplish the following **specific aims**:

Aim 1 (Part 1, subjects 1 and 2). First, treat two subjects with 50 mg/d of drug for 10 days to preliminarily determine the effects of the drug to lower lipogenesis. Measure fasting and sugar-stimulated lipogenesis before and after treatment, while monitoring for side effects. **Hypothesis 1**: *A 10d duration of 50 mg/d treatment will lower acute lipogenesis stimulated by a bolus of sugars by at least 2% and result in no, or minimal side effects.*

Aim 2 (Part 2, subjects 3-6): Test an additional 4 subjects according to the results of Part 1. **Hypothesis 2***: Additional subjects treated at the same dose will refine the statistical confidence in the observations of Objective 1.*

Aim 3 (Part 3, subjects 7-12): Using the final targeted dose of drug, study 6 subjects using the same in-patient protocol as in the other aims. **Hypothesis 3:** *Using the final dosing scheme, the last six subjects will demonstrate a significant reduction in fasting and stimulated lipogenesis, with the lowest side effects, if any.*

Study Details

The sources of research materials will include following items.
Screening: Research subject and family medical history, demographic data, vitals, height, weight, BMI calculated, and screening laboratory tests including a CMP, hepatitis panel, CBC with differential, concentration of insulin, a lipid panel, apolipoproteins B and A1, ECG, and HbA1c. Demographic data (plus other related data, e.g., emergency contact person), smoking history are collected, along with lifestyle information including food/beverage/alcohol intake and physical activity will be obtained via questionnaire and a urine sample is used for a general drug screen.

Inpatient CRC studies: Blood is processed to obtain plasma and the sample used in the isolation and measurement of blood metabolites and hormones including glucose, fructose, TG, FFA, ketones, insulin, and for the VLDL-lipoproteins, apolipoprotein B100, VLDL-TG concentration, and TG-palmitate lipogenesis. Once the samples have been processed for mass spectrometry and the data acquired, the processed samples will be kept until at least 10 years after publication (the stable isotope does not decay and the samples retain their value). DEXA is used to measure total and regional fat mass and fat-free mass, a FibroScan® ultrasound is performed to measure liver elastography (liver stiffness due to fibrosis), and MRI is used to assess liver fat. Indirect calorimetry is performed to measure energy expenditure, and skin oil collected (Sebutape®) to measure fatty acid composition. **Table 1** shows how the timeframe over which the 324 ml of blood is donated by the subject.

Table 1. Accounting of Blood Usage

Procedure	Volume of whole blood (mls)
Screening blood chemistries	40
V1. Baseline inpatient study	100
V2. D4 safety blood draw	10
V3. D6 safety blood draw	10
V4. D9 safety blood draw	14
V5. Follow-up inpatient study	140
V6. Follow-up safety blood draw	10
Total for 12 subjects (mls)	**324**

Schedule of Events

Telephone Screening: The subject will respond to the recruiting flyer or advertisement and answer the waiver of written consent and screening questions. These questions are for preliminary screening only and are not used as study data since the data represent self-report. The questions include queries about health history/habits, age, gender, height, weight, tobacco use, medications/supplements, over-the-counter drugs use, illness, and chronic conditions. The study design is described in general terms to subjects, with mention of three factors most likely to impact subject interest in participating. These factors include having an IV line, that there are two overnight stays in a hospital research unit, and that the drugs may have side effects. If the subject is interested in being screened for participation, research staff then makes an appointment for the subject to come into the CRC for formal consenting, and screening procedures. The length between the screening visit and beginning study procedures may vary according to subject's availability but will not be longer than 4 weeks. For each of the visits shown in **Figure 1**, the procedures as are described below.

Screening and Consenting Visit: The first visit to the CRC will include a written formal consent. We estimate that we will have to screen 100 people to find the 12 subjects to do the study. After signing the consent form, medical information is obtained by the nurse including DOB, gender, ethnic/racial category, height, body weight (history of body weight), vitals, and medical history (medications use, smoking history and a fasting blood draw (40 mL) is taken to test for biochemistries). An ECG will be performed and read by Dr. Mary Dohrmann, Cardiology.

As soon as the blood biochemistry results are available, the subject is contacted, provided with a copy of the results, told whether he is eligible to continue, and asked if they want to participate further. Staff also ask the subject to describe, in their own words, what the baseline visit will be like. If they agree to participate, an appointment is made for the subject to come to the CRC for their overnight baseline visit.

Three days before visit 1, the subject is provided with food and beverages to meet his total energy needs for weight stability. No alcohol will be consumed during this time. The meals are made up of commercially available foods and the composition is based in foods the subject normally consumes.

Baseline Visit 1: Before arriving to the CRC, the subject will go to Fairview Digestive Health Clinic for a 15 minute FibroScan. If a FibroScan becomes available to be housed in the hospital, then this trip to Fairview would not be necessary and the measurement would be made during the inpatient study. The CRC will begin at 4:00 PM and take 24 hours and the subject will undergo several procedures. The first procedure is MRI to assess liver fat content. Next, after returning to the CRC, an IV line will be placed in one of the arms and an isotope ($^{13}C_1$-acetate, 10g/1200 mL half-normal saline) will be infused through that IV. Afterward, the subject will be fed dinner and then undergo a skin test, where a piece of tape will be placed on the forehead for 30 minutes to collect oils produced by the skin. The subject will sleep overnight at the CRC. At 6:00 AM the next morning, the subject will undergo calorimetry and a second IV line will be placed in the other arm. This IV will be used to draw blood. Following this, he will be asked to consume a sugary drink to begin the oral sugar tolerance test (OSTT). The calorimetry test will be performed again at 9:30 AM and blood will be drawn intermittently until 2:00 PM. The total amount of blood drawn will be 100 mls. After the final blood draw, the subject will be fed lunch and then body composition will be measured by DEXA. After this, he can leave the CRC. If all

baseline procedures are successful, the subject will be given 10 days of drug to begin taking the first dose that evening. Each day, he will take a single pill with water before 10:30 PM (or at bedtime if before 10:30 PM). The study physician, Camila Manrique, MD, will oversee the prescribed dosage the subject will take. This dosage will be determined in consult with the Safety Officer, Ghassan Hammoud, MD, according to the attached Dosing Decision Tree.

The OSTT is a combination of glucose and fructose consumed in 180 ml of water. Specifically, over a period of 15 min, the subject will ingest a "Kool-Aid like" solution of 0.9g/kg fructose and 0.3g/k glucose in water. After the baseline test is completed, the subject takes home all food/beverages that he will consume until he returns for visit 2.

Safety Blood Draw Visits 2 and 3: As shown in **Figure 1**, the subject will return to the CRC for a single blood draw in the morning, 2 days following the baseline visit. The subject is instructed not to exercise on this morning before this blood draw. For 12 hours before these visits, he is asked to not eat or drink anything besides water. Blood will be taken (10 ml) and vitals will be checked. This visit will take about 30 minutes. On day 4 following the baseline visit, the subject will return for safety blood draw visit 3 and the procedures will be the same as safety blood draw visit 2. At the end of visit 3, the subject takes home food to eat until visit 4.

Safety Blood Draw Visit 4: The subject will return to the CRC for a third safety blood draw and all procedures done at the previous safety blood draws will be repeated, with the addition of the skin test. A small amount of blood will be drawn (14 mls). The subject is asked to not exercise on this morning before this blood draw. For 12 hours before he comes to the visit, the subject is told to not eat or drink anything besides water. The subject is told not to apply any lotion or skin products to the face on the morning of this visit. This visit will take about 45 minutes. At the end of visit 4, the subject takes food home to keep him weight stable until Follow-up visit 5.

Follow-Up Visit 5: On the day of the subject's last dose of medication, he will return to the CRC for the follow-up visit. This visit is exactly the same as the baseline visit, however, blood will also be drawn four times during the night to assess drug pharmacokinetics. The subject will come to the CRC at 4:00 PM and stay for 24 hours. The total blood draw will be 140 mls. Following this visit, the treatment portion of this study is over and the subject will discontinue taking the drug.

Safety Blood Draw Visit 6: Approximately 5-7 days following visit 5, the subject will come into the CRC for a final safety blood draw. The subject is asked to not exercise on this morning before this blood draw. For 12 hours before he comes to the visit, the subject is told to not eat or drink anything besides water. A small amount of blood will be taken (10 mls). This visit will take about 30 minutes.

Human Subjects Involvement and Characteristics

Recruitment and informed consent: The study population includes overweight and obese male subjects, aged 35-60y. The recruitment process will include the typical flyers and notices put in public spaces. The recruitment material will be posted around University of Missouri campus, as well as other public places such as swimming pools, churches, and grocery stores. We also plan to use the service called Studykik (https://studykik.com/). All advertisements will state the purpose of the study, eligibility criteria and will tell subjects to call into our laboratory for more information. During these calls, the study will be described briefly. A checklist will be phone through to perform initial screening for age, gender, health status. Subjects who are eligible by phone screen will be scheduled for a screening visit.

The consent form states the purpose of the study, risks, inconveniences, discomforts, and other important information about the study and has been prepared in lay terms so that it is easy to understand. During the screening visit the form is reviewed comprehensively. The subject will be instructed to ask questions about any words or information that they do not clearly understand and questions will be answered by the PI and her staff. This will enable us to determine the subject's comprehension of the study. The consent form will be signed before any study procedures are initiated.

Criteria for inclusion

1. Men with characteristics of metabolic syndrome
   a) Waist circumference greater than 40 in (102 cm)
   b) Plasma TG greater than 150 mg/dL
   c) HDL cholesterol less than 40 mg/dL
   d) Blood pressure greater than or equal to 130/85 mmHg
   e) Fasting plasma glucose greater than 100 mg/dL but less than 126 mg/dL
   f) Fasting insulin great than 10 uU/mL
2. 35-60 years of age
3. Overweight/obese subjects with BMI 27.1 - 45.0 kg/m2
4. Family history of CVD or diabetes
5. Habitual diets containing ≥ 5.0% of energy from added sugars
6. Creatinine clearance of ≥80 mL/min

Criteria for exclusion

1. Diagnosed CVD (unstable angina, NYHA angina > Grade 2), abnormal thyroid function or liver/kidney disease, renal dysfunction (defined by GFR <80 mL/min)
2. Signs of ascites and signs of hepatic encephalopathy (a score greater than 7 on the Child-Pugh scale, that would place them in either class B or C)
3. Chronic skin disorder or treatment for acne
4. History of clinically significant dry eye or eye diseases such as glaucoma
5. Diabetes defined as fasting glucose ≥ 125 mg/dL or HbA1c ≥ 6.5%
6. Habitual diets with low content of added sugars (<5% of total energy)
7. Any tobacco use
8. Elevated liver enzymes ≥ 3x normal (regional norms ALT <42 U/L, AST <40 U/L, and GGT 8-61 U/L) and extended prothrombin time (PT >11.5 seconds)
9. Contraindications of MRI
10. Alcohol intake weekly greater than 56 g/week (4 standard drinks/wk).
11. Major surgery or donation of blood of >500 mL within the past 8 wks.
12. Patients with uncontrolled hypertension, i.e. ≥160/95 mmHg.
13. Patients with known cardiac abnormalities such as:
- Congenital long QT syndrome
- QTc interval > 480 milliseconds
- Any cardiac arrhythmia requiring anti-arrhythmic medication.
14. Patients who have had a myocardial infarction within 12 months of study entry.
15. Patients who have a history of coronary artery disease (CAD) e.g. angina Canadian class II to IV [10]. In any subject in whom there is doubt, the subject will be excluded.
16. Patients with an ECG recorded at screening showing evidence of cardiac ischemia (ST depression of ≥2 mm). If in any doubt, the patient should have a stress imaging study and exercise ECG and, if abnormal, angiography to define whether or not CAD is present.
17. Patients with congestive heart failure that meets New York Heart Association class II to IV definitions and/or ejection fraction <40% by multiple gated acquisition (MUGA) scan or <50% by echocardiogram and/or magnetic resonance imaging (MRI)
18. Patients with a history of sustained ventricular tachycardia (VT), ventricular fibrillation (VF), Torsade de Pointes, or cardiac arrest, unless currently addressed with an automatic implantable cardioverter defibrillator (AICD).

19. Patients with hypertrophic cardiomegaly or restrictive cardiomyopathy from prior treatment or other causes (if in doubt, see ejection fraction criteria above).
20. Patients with a history of pneumonitis.
21. Individuals taking drugs that are substrates for CYP3A or CYP2C9 and have a narrow therapeutic index. Examples of these drugs include statins (atorvastatin, simvastatin), diuretics (conivaptan, naloxego, eplerenone, tolvaptan), anti-hypertensives (nisoldipine, felodipine), anti-inflammatories (celecoxib, ebastine), narcotics and sedatives (alfentanil, midazolam, triazolam), antipsychotics (buspirone, lurasidone, quetiapine), other lipid-lowering agents (lomitapide) and erectile dysfunction medications (avanafil, vardenafil, sildenafil). Other drugs that would make potential subjects ineligible include HIV anti-retrovirals, immunosuppressants and chemotherapeutic agents.
22. Individuals who are taking drugs that are strong inhibitors or reducers of CYP3A. Examples of these drugs include, diuretics (conivaptan), antifungals agents and antibiotics (rifampin, itraconazole, ketoconazole, troleandomycin, voriconazole, clarithromycin), antidepressants (nefazodone, St. Johns Wort) and antihypertensives (diltiazem).

Subject compensation

Subjects will be compensated based on the amount of their participation in the study as follows:
$150.00 for consuming the 3 day pretest diet and for baseline visit 1
$75.00 for participating in safety blood draw visit 2
$100.00 for participating in safety blood draw visit 3
$150.00 for participating in safety blood draw visit 4
$225.00 for consuming the 10-day diet and for participating in follow up visit 5
$500.00 for participating in safety blood draw visit 6
Thus, if subjects complete the study, they will be compensated a total of $1,200.00.

Subjects who live 1-2 hours away from Columbia by car will be reimbursed $25 for the screening visit.

Potential risks

Physical risks may include:

1. Intravenous catheterization: The tests in the present protocol require one IV line to be in for roughly 24 hrs and another to be in for roughly 8 hrs. The standard rule of thumb for hospital infection control is for an IV line to be replaced every 48 hrs. The duration of the IVs in the present protocol is less than this time. Placement of IV lines may cause bleeding, bruising, infection, clotting, pain at the needle site, and/or fainting. Extravasation of study substances could cause swelling and local irritation at the intravenous site. A black and blue mark may result from placement of and IV. This can be painful, but it carries no significant risks. Infections from IVs rarely occur.
2. Blood drawing: During the screening and safety blood draw visits, blood will be drawn through a needle. The risk of blood drawing may include temporary discomfort from the needle stick, bruising, and rarely, infection.
3. The total blood loss for the entire study is 324 ml.
4. Indirect calorimetry: The inpatient stays include 2 calorimetry tests, which are painless. However, persons who are uncomfortable in confined spaces may find this test slightly stressful.
5. Stable isotopes: The stable isotopes received in the IV contain no radioactivity, and have no recognized harmful effects. These compounds are commonplace variants of naturally-occurring compounds (fatty acids, amino acids, etc.). The isotope solution is made by the Investigational Pharmacy and used within 48 hours.
6. DEXA: The radiation during DEXA is equivalent to about 2% of the average radiation dose from all sources (natural background radiation, consumer appliances, radon gas, medical tests, etc.) that a person in the United States receives each year.
7. Magnetic resonance imaging: The risks of undergoing an MRI include some psychological stress from a loud, banging noise during the scan. Subjects may experience nervousness from confinement in a tight space (claustrophobia). Magnetic fields to be used in this study present no known hazards to human subjects whose bodies do not contain any para-magnetic metals.

8. FibroScan: This ultrasound measurement of the liver involves a probe being placed on the skin above the liver. There is no radioactivity associated with this procedure. A FibroScan test is painless and lasts 15 min. The subject will need to lie very still.
9. Intake of TVB-2640: This is an experiment drug which has been shown to have some side effects. Of the people who have taken the drug for 10 days, some have reported, dry skin and dry eyes, sometimes serious enough to stop their participation. A few subjects have reported hair loss or upset stomach. These side effects have disappeared after subjects have stopped taking the drug. Our study staff will be in contact with subjects by phone and email while they are taking the drug to determine if they have any side effects. We will see them personally three times while they are taking the drug to monitor them carefully. One test we will administer is a skin test, to check for the ability of their skin to produce oil.
10. Sugar test: During the baseline and follow up visit, subjects will be asked to consume a bolus of sugars. Risks could include upset stomach.
11. Hospital Stays: During the visits and study, the subject will be restricted to the CRC. Although quiet leisure activities can be provided, confinement to the CRC may be boring or the subject may feel hungry. Some may find it hard to be confined to a hospital bed for the duration of the test.
12. Sebutape: This is a piece of tape that is place on the forehead to collect skin oil. The procedure is noninvasive but the tape tugs slightly on the skin when it is removed.
13. Psychological stress: The psychological stress from participation in this study is minimal. However, some of the questions about food intake and physical activity may make the subjects feel uncomfortable. The indirect calorimetry test is painless; however, persons who are uncomfortable in confined spaces may find this test stressful.
14. Loss of confidentiality: Any time information is collected, there is a potential risk for loss of confidentiality. Every effort will be made to keep subject's information confidential; however, this cannot be guaranteed. The information collected includes: age, gender, height, body weight, blood pressure, and blood tests results (e.g. glucose, blood fats), hepatitis status, urine drug test results, and hormones (insulin). Finally, the investigator will report information to authorities in order to prevent serious harm to the subjects or to others. If the Investigators suspect child, elder or disabled person abuse, they will report such concerns to proper authorities as required by law.
15. Risks to sperm: The effects of the DEXA on the male reproductive system are unknown but could cause harm.
16. Other risks: There may possibly be other side effects that are unknown at this time. The complete history of experience with this drug in humans is described in the Investigators Brochure which is part of the attached IND application.

Protection against risk

1. Infections from IVs rarely occur. However, if any signs of infection appear, the IV will be removed and the area treated. Subjects will be counseled to not donate blood for 8 weeks after the study.
2. Subjects who have donated blood for any other purpose in a short time will be excluded from participation. The red blood cell count (hematocrit) will be measured during screening to rule out anemia and again before the follow-up visit.
3. A doctor, nurse, or licensed technician will be the one to perform the blood draws.
4. The protocols that the CRC nurses will follow to reduce risk of infection include: cleaning the skin thoroughly before inserting the IV, securing the IV with tape, covering the site with the clean gauze, and protecting the site using an elastic sleeve. The site is inspected every time blood is drawn with particular attention to any changes in the surrounding skin with respect to edema, color, and temperature.
5. The foods to be consumed will be made of commercially-available ingredients and pose no foreseeable risk.
6. Subjects who participated in any other research study or medical procedure involving ionizing radiation exposure greater than a chest X-ray in the past 12 months will be excluded.
7. Subjects will be told that they may refuse to answer any questions, take a break, or stop their participation at any time.
8. Subjects will be allowed to practice wearing the plastic hood used during the calorimetry test during the screening visit.

Subject safety procedures

These procedures are divided into two parts: 1) Determination of eligibility and 2) on-study safety monitoring. After screening, subject eligibility will be determined by Drs. Hammoud and Manrique, in consult with Dr. Parks. Once subjects are entered into the study, the clinical trial manager will coordinate the safety visits and send the blood chemistry

results by email to Dr. Manrique, who will review them and document her response by email to the coordinator. If she has any question, she may also consult Dr. Hammoud to discuss a result.

Procedures to maintain confidentiality

The health history form and all subject screening and experimental data and pertinent medical paper records will be placed in individual files and coded for de-identification. This information will only be accessible to the principal investigator, co-investigator, or approved research personnel. All records will be kept in a locked filing cabinet, which only the research personnel have access to. Computerized records of experimental data will be similarly coded and will be maintained on a password secure system. The only confidential information to be disclosed would relate to the subject's medical history. The purpose of obtaining a careful medical history is to verify that the patient meets the inclusion/exclusion criteria. All medical and biographical information will be held in strict confidentiality and no disclosures of personal identity will be made unless specifically requested by the subject. Copies of signed consent forms, as well as the experimental log book, are kept in a locked file cabinet in the laboratory. Only subject ID numbers are used to also insure confidentiality. Participants will not be individually identified in any publications. The participants' right to privacy will be protected to the highest extent possible.

Protection of materials, and privacy

All sample tubes are coded to conceal the subjects' identities and no identifying information is present on the tubes. They are labeled with the subject's three-letter code identifier and the date. No tools (e.g., cell lines, probes, etc) will be generated from any of this material. The study will be conducted in accordance with Good Clinical Practice and HIPAA guidelines. Only the Investigators and study coordinator will have access to the codes. Aside from the sample sent to the Sponsor (3V Biosciences) the samples will not be shared with other investigators within the University of Missouri or outside the University. No identifying information will be shared or reported in any publication.

Potential benefits to human subjects and others

The research plan tests whether the drug will lower liver fat in men with risks for future nonalcoholic fatty liver disease. Development of such a drug would aid the millions of U.S. citizens who suffer from this condition. There is no direct benefit for the subjects and all study procedures are voluntary.

Reducing risk in participants

The PI and Drs. Camilla Manrique will monitor all procedures and the study results. The PI will report adverse events to the IRB. Dr. Ghassan Hammoud is the safety officer. The special precautions are as follows:

1. The subjects will be closely monitored at the CRC by nurses.
2. Standard aseptic technique, frequent monitoring of vital signs, and visual inspection of the IV line sites will be followed during IV line placement and blood sampling.
3. The isotopes used are purchased to be consumed and prepared as sterile for administration via IV. The isotope solution is made by the Investigational Pharmacy and used within 48 hours.
4. The tests in this study have been designed for research, not for medical purposes. The subjects will be informed in the event that the PI or Drs. Camila Manrique or Ghassan Hammoud discovers a possible abnormality; the subject will be given a copy of the screening results and advised to consult with their primary care physician to discuss further tests and/or follow-up.
5. The procedures to maintain confidentiality have been discussed above in the section "privacy."
6. The results of any test or procedure done in this study will follow internal laboratory controls.
7. This study does not involve vulnerable populations.
8. To minimize any risks, BLS certified personnel will be present during all experiments. Venous cannulation will be performed under sterile conditions only by research personnel with required training and experience with these techniques. In case of emergency, during study visits and experimental measurements, a physician investigator and/or research nurse will be present: the University Hospital has in place a system to allow for a code Blue (i.e., cardiac arrest) by dialing 882-7979 in which trained hospital personnel will respond.

Sample size

The present project is a pilot study to allow determination of an appropriate dose of drug for a future study. We will also use the variability observed here to calculate a future sample size. The mass spectrometry lipogenesis measurement produces final data that are in units of percentage, reflecting the fraction of palmitate secreted by the liver in TG that are derived from sugars. Our past research has shown that the lipogenesis measure is highly reproducible within a subject with a relative 1-2% variance between days. For example, one subject's fasting lipogenesis was measured on 3 different occasions and found to be 7.8%, 9.6%, and 10.1% (mean 9.2%, SD 1.2%). In the present study, with 12 subjects we should be able to detect as little of an absolute treatment difference of 2% between baseline (e.g. 9.2%) and follow-up lipogenesis (down to 7.2%), with a SD of that differences as small as 2%, SD 1.4% (α=90%) or the same effect of the drug of 2%, with a SD of 1.4% with 8 subjects (α=80%).

Adverse events and emergencies

Any serious adverse events will be reported directly to the IRB. A detailed report of all adverse events will be submitted to the IRB and a determination of related or unrelated event will be made. In case of emergency, the University Hospital has in place a system to allow for a code Blue (i.e., cardiac arrest) by dialing 882-7979 in which trained hospital personnel will respond. The CRC Research Nurses are certified in BLS and ACLS. The nurse in charge of the study will activate the Code Response and initiate Basic Life Support until the Code Blue Team arrives.

Statistical analysis and calculations

StatView®, 5.0.1 software (version 2008) was used when a paired sample t-test was performed. Regression analysis, one-factor analysis of variance (ANOVA), two-factor ANOVA, and Holm-Sidak post hoc analyses were performed using the statistical package for the social sciences (SPSS®, version 24, 2016). Pearson correlation analysis was performed using SPSS® (version 24, 2016). HOMA-IR was calculated as [(glucose in mg/dL * insulin in □U/mL)/405.

Reference

1. Parks EJ, Skokan LE, Timlin MT, Dingfelder CS: Dietary sugars stimulate fatty acid synthesis in adults. 2546703 *J Nutr*, **138**(6):1039-1046, 2008

CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

INVESTIGATORS' NAMES: Camila Manrique, MD, Ghassan Hammoud, MD, and Elizabeth J Parks, PhD, at the University of Missouri in Columbia, MO
STUDY STAFF: Kimberlee Bingham, BS Majid Syed-Abdul, MS Nathan Le, BS
MU PROJECT #: 2006432 CLINICALTRIALS.GOV NUMBER: NCT02948569
STUDY TITLE: "Evaluation of TVB-2640, a FASN Inhibitor, to reduce de novo lipogenesis in subjects with characteristics of the metabolic syndrome"

INTRODUCTION

This consent may contain words that you do not understand. Please ask the investigator or the study staff to explain any words or information that you do not clearly understand. **This project is a research study. Research studies include only people who choose to participate. As a study participant you have the right to know about the procedures that will be used in this research study so that you can make the decision whether or not to participate. The information presented here is simply an effort to make you better informed so that you may give or withhold your consent to participate in this research study. Please take your time to make your decision. This study is being sponsored by the Company, 3V Biosciences, who has developed an investigational drug to treat liver disease in overweight people. This drug is not approved by the FDA at this time. One of the Co-Investigators, Elizabeth Parks, consults for this company and therefore she received financial compensation from the company sponsoring this research. Elizabeth Parks has designed the study and a physician, Dr. Manrique will be conducting it.**

What is this study about? **People who are overweight can begin to store fat in their liver. This fat is made from dietary sugar and can cause the liver to not work well. You are being asked to take part in this study because your body weight increases the likelihood that your liver will make fat. In order to participate in this study, it will be necessary to give your written consent.**

The following definitions may help you understand this study:

- "**Researchers**" means the study doctor and research personnel at the University of Missouri.
- "**Stable isotopes**" are naturally occurring elements used to help us trace sources of fat in the **blood. Stable isotopes are not radioactive.**

WHY IS THIS STUDY BEING DONE AND HOW MANY PEOPLE WILL TAKE PART?

The company 3V Biosciences has developed an investigational drug called TVB-2640. This drug is not approved by the FDA at this time. In preliminary studies, this drug has been shown to lower liver fat production but the ideal dose for its use has not been decided. The purpose of this study is to test the drug in overweight men, to determine the appropriate dose. The information from this study will enable future studies to test the drug's effects in patients with liver fat due to disease. The tests performed are not part of your standard medical care. Up to 100 people will be screened to find the 12 people who will take part in this study.

WHAT IS INVOLVED IN THE STUDY AND HOW LONG WILL I PARTICIPATE?
This research is measuring how the drug may improve liver health. You will be screened to find out if you are eligible to participate. As shown below, over 20-day period this research includes 2 inpatient study visits, 4 safety blood draws, and a daily dose of the drug for 10 days.

STUDY TIMELINE
The following paragraphs describe the schedule for the study screening visit and five study visits.

SCREENING VISIT
To help decide if you qualify to be in this study, the researchers will ask you questions about your health, including medications you take and any surgeries you have had. This visit is located at the Clinical Research Center (CRC). The CRC is on the 5th floor of the University Hospital and has clinic rooms specially designed for research. You will come to the CRC after fasting overnight for 12 hours (no food or drink, except water). This visit will take 1 hour and you are welcome to bring a family member or close friend to this visit.

Research staff will meet with you to review the study procedures, answer questions, sign this consent form and review your medical history. A small amount of blood will be drawn (about 2.5 tablespoons) to check your general health which includes a test for hepatitis as well as a measurement of your fasting blood sugar to ensure you are not diabetic. Your urine will be tested for drugs. Your height, weight, and blood pressure will be measured and you will be given a chance to learn about a procedure called calorimetry, which we use to measure how many calories your body burns. If you enter the study, this technique will be performed four times. Within a week after the screening visit, you will receive the results of all screening tests and someone on the study team will go over the results with you. Since this is not a treatment study, these lab tests are not being used for standard medical care. You will be given a copy of the results which you can share with your primary physician.

If your screening results qualify you to be in the study, and if you are interested in proceeding with the study, you will be scheduled for a second, baseline visit. Again, you will come to the CRC at the hospital and parking will be provided if needed. For 3 days before this baseline visit, you will be provided a 3-day controlled diet which is made fresh and based on foods you normally prefer. You must consume only these meals, all of the food provided, and no other food. You may not consume alcohol between days -2 and 13 of the study.

BASELINE VISIT 1: On the day of your first overnight hospital test, you will come into the CRC at 4:00 PM. This visit will take about 24 hours and you will undergo several procedures. The first procedures include assessments of your liver health. This will include Magnetic Resonance Imaging (MRI) and also, potentially, a procedure called the FibroScan. These, and all the procedures are described in detail below. When you return to the CRC after your scan, an IV line will be placed in a vein in one of your arms. Next, an IV solution is infused. This solution is called an isotopic tracer and it will continue until 2 PM the next day. You will be fed dinner and then undergo a skin test in which a piece of tape will be placed on your forehead for 30 minutes to collect a sample of the oils produced by your skin.

You will then sleep overnight in the CRC. At 6 AM the next morning, you will arise and wash up. Then, a second IV line is placed in your other arm and this one will be used to draw blood. You will undergo a procedure called calorimetry, which measures the amount of calories you burn. You will then consume a

sugary drink and blood will be drawn off and on until 2 PM. You will undergo calorimetry a second time. After the final blood draw, you will be fed lunch and undergo a procedure called a DEXA scan. The DEXA scan measures your body composition. After this you will be discharged and can go home.

If all baseline procedures are successful, you will begin the drug treatment regimen for the next 10 days. You will take a single daily dose of the drug orally before bedtime or 10:30 PM, whichever comes first. The physician assigned to this study will oversee the prescribed dosage you are instructed to take.

SAFETY BLOOD DRAW VISIT 2: The morning of this visit, please do not exercise, and for 12 hours before the visit, do not use caffeine and vitamin supplements. You will come into the CRC after fasting overnight for 12 hours - no food or drink, except water after 7 PM. A small amount of blood will be taken (about 1 tablespoon) and we will check your weight, blood pressure, heart rate, and temperature. This visit will take about 30 minutes.

SAFETY BLOOD DRAW VISITS 3: This visit is the same as visit #2 above. A small amount of blood will be taken (about 1 tablespoon).

SAFETY BLOOD DRAW VISITS 4: This visit is the same as visit #2 above with the addition of a collection of skin oils. A piece of tape will be placed on your head for 30 minutes to collect the oils produced by your skin. The morning of this visit, do not apply any lotion or skin products on your face. A small amount of blood will be taken (about 1 tablespoon). This visit will take about 1 hour.

FOLLOW-UP VISIT 5: Just as you did for the baseline visit, you will come in to the CRC at 4 PM and the procedures will be the same. However, in this visit, blood will also be drawn 4 times during the night. This visit will take 24 hours and the total blood draw will be about 2 oz or about 2 tablespoons. Following this visit, the treatment portion of this study is over and you will discontinue taking the drug.

SAFETY BLOOD DRAW VISIT 6: Approximately 5-7 days following visit 5, you will come into the CRC for final safety blood draw (1 tablespoon). The same procedures will be done at this visit as with safety visits 2 and 3. This visit will take about 45 minutes.

WHAT ARE THE PROCEDURES AND RISKS OF BEING IN THIS STUDY?

STUDY DRUG

The study drug, TVB-2640, has been given in preliminary studies for 3 weeks to 2 months in duration. Side effects were seen in some subjects after 21 days on the drug and included dry and sore eyes, some hair loss, and upset stomach. Also, dry skin on the hands and feet which can also feel sore. All side effects went away after the subjects stopped taking the drug. These side effects were seen at higher doses than will be used here, and when the drug was given for longer durations. In the present study, you will take the drug for 10 days. Even so, you will be monitored closely and blood samples will be taken every couple of days to document the drug's effects. If you notice any side effects such as dry skin, nausea, or eye problems, please notify the study staff immediately. If your skin gets dry you will be provided with lotion to reduce this side effect. Additionally, it is unknown whether this drug has any effects on a man's ability to reproduce. We advise all subjects to use contraception (condoms) during the duration of this study.

STUDY DIET: FOOD AND BEVERAGES

If you participate in the study you will be provided all your food during the 10 days you are taking the drug. This food is commercially available (bought from HyVee) and the meals are made both fresh and also made up of some frozen meals. When you come in for the safety blood draws, you will be given the food in a cooler to take home. If you prefer, you may be provided a couple of cans of soda to drink during this time (no other soda than these can be consumed). Otherwise, only water, tea, and coffee are allowed (cream and any kind of sweetener is allowed). No additional milk shakes, protein shakes, or energy drinks should be consumed during this study.

It is advised that if you have a special event coming up in the next month (wedding, vacation travel, etc.) that you not participate in the study at this time. You should not consciously change your physical activity while you are in the study. In other words, if you would like to join a gym or begin exercising more, we ask that you wait until the study is over before you do this.

CALORIMETRY

This test measures how many calories your body uses. This procedure will be performed at visit 1 and visit 5. Calorimetry requires resting quietly on your back for 20-30 minutes under a large, clear, plastic hood. You will breathe room air normally and your breath goes into an analyzer to measure what you breathe out. The test is painless; however, persons who are uncomfortable in confined spaces may find this slightly stressful. You will get a chance to familiarize yourself with this procedure during screening.

MEASUREMENT OF BODY FAT AND MUSCLE BY DEXA SCAN

This test will be performed at visit 1 and 5. A DEXA (dual energy x-ray absorptiometry) scan is a procedure to measure your body composition - how much fat and muscle your body has. It is a type of x-ray machine with a moving arm. This procedure involves lying on a table for 20-30 minutes while the DEXA machine passes over your body. Although you will need to remain very still and quiet, you will feel nothing and should have no discomfort. If you have participated in any other research study involving ionizing radiation exposure (x-rays) in the past 12 months, discuss this with the Investigator to determine if you are eligible to participate in this study. You will be exposed to a small radiation dose which is about 2% of the average radiation dose from all sources (natural background radiation, consumer appliances, radon gas, medical tests, etc.) that a person receives in the United States receives each year. However, radiation effects add up. If you need an x-ray in the next year, you should inform your doctor of your participation in this study.

MEASUREMENT OF LIVER FAT BY MRI

This test will be performed at visit 1 and 5 at either the department of Radiology at University Hospital or at the Missouri Imaging Center at South Providence Medical Park. Transportation will be provided. MRI (magnetic resonance imaging) is a technique used to measure the amount of fat present in your liver. The risks of undergoing an MRI could include some psychological stress from a banging noise during the scan. Subjects may experience nervousness from confinement in a tight space (claustrophobia). Magnetic fields to be used in this study have no known hazards to human subjects whose bodies do not contain any magnetic metals (see below for protection from risks). If you feel anxious, you can stop the procedure at any time. You may experience some discomfort and fatigue from lying still during scanning. You will be questioned using a standardized interview about whether you have a heart pacemaker, metallic objects, vascular clips, electrodes, cochlear implants, neurostimulators, shunts, heart valve implants, penile implants, vascular filters, rods & screws, post CABG pacer wires, colored contact lens, dental prostheses, limb prostheses, eye prostheses, shrapnel, metal in head, eye, or skin, and

embolization coils. If such devices cannot be removed safely, you will be excluded from the study. We have a mock scanner to allow you to practice the procedure. When the scan is occurring, the radiology technician is in constant contact with you through head phones.

FIBROSCAN

This procedure is designed to measure the stiffness of your liver. It requires that you lie still on your back while a technician places a wand on your skin over your ribs. The test is painless and takes 15 to 30 minutes.

SKIN OIL COLLECTION

One of the side effects of the drug can be dry skin. To monitor this side effect, a small sample of skin oils will be collected from your forehead using a piece of tape that is placed on the skin for 30 minutes. There are no risks associated with this procedure.

RISKS OF BLOOD DRAWING

During screening and at visits 1-5, you will have blood drawn through a needle. Risks associated with drawing blood from your arm include minimal discomfort and/or bruising. Infection, excess bleeding, clotting, and/or fainting also are possible, although unlikely. As a result of your participation in this study you will have donated blood. If you wish to participate in other research after you finish this project, you should let the investigator know that you have given about 6 oz (or about 3/4 cup). Your blood volume will be checked during screening to make sure that it is in safe limits.

INTRAVENOUS CATHETERIZATION

During baseline visit 1 and follow up visit 5, you will have an IV line placed that is identical to the IV that a person receives when admitted to the hospital. While IVs are normally safe and painless, there is always a very small risk of infection, bleeding, or bruising associated with the catheter. Should the IV fall out of the vein, it is possible that some of the liquid will go into the skin rather than the vein. This may result in swelling and pain around the area of the IV, and the IV would have to be placed in a different location on the arm or hand. The IV lines will be in for about 20 hours. The protocols that the CRC nurses follow to reduce risk of infection include: cleaning the skin thoroughly before IV placement, securing the IV with tape, covering the site with clean gauze, and protecting the site using an elastic sleeve. The site is inspected at each blood draw – with particular attention paid to any changes in the surrounding skin.

HOSPITAL STAYS

During your visit you will be restricted to the CRC. Although quiet leisure activities can be provided, confinement to the CRC may be boring or you may feel hungry. You may move about your assigned hospital room and use the facilities as needed, unless the test being performed requires bed-rest. Some people may find it hard to be confined to a hospital bed for the duration of the test. For the reasons stated above the investigator will observe you closely while giving the treatment described and, if you have any worrisome symptoms or symptoms that the investigator or her associates have described to you, notify the investigator immediately. Investigator's telephone number is (573) 529-1141. For more information about risks and side effects, ask the investigator or contact study coordinator, Kimberlee Bingham at 573-884-1708.

WHAT WILL BE MY RESPONSIBILITIES DURING THE STUDY AND ARE THERE BENEFITS?

While you are part of this study, the researchers will follow you closely to determine whether there are problems. It is your responsibility to do the following:

- Ask questions about anything you do not understand.
- Keep your appointments.
- Follow the researchers' instructions, particularly in consuming the medication.
- Let the researchers know if your telephone number or address changes.
- Tell the researchers before you take any new medication, even if it is prescribed by another doctor for a different medical problem, or purchased over the counter.
- Report to the researchers any injuries or illnesses while you are on the study, even if you do not think they are related.

If you agree to take part in this study, there will be no direct medical benefit to you. You may expect to benefit from taking part in this research to the extent that you are contributing to medical knowledge. You will receive the results of all of your tests. We hope the information learned will benefit others in the future.

WHAT ARE THE COSTS?

You will not be charged for any procedures that are part of this research study. Parking will be provided but there is no compensation for travel to our facilities or for childcare during this study.

WILL I BE PAID FOR PARTICIPATING IN THE STUDY?

You will be compensated a total of $1,200 for completing this study as follows: $150 will be given for baseline visit 1; $75 will be given for safety visit 2, $100 for safety visit 3 and $150 for visit 4, $225 for consuming the 14-day diet and completing follow-up visit 5, and $500 for the final safety blood draw.

WHAT OTHER OPTIONS ARE THERE?

You do not have to participate in this study.

WHAT ABOUT CONFIDENTIALITY?

Information produced by this study will be stored in the investigator's file and identified by a code number only. The code key connecting your name to specific information about you will be kept in a separate, secure location. Information contained in your records may not be given to anyone unaffiliated with the study in a form that could identify you without your written consent, except as required by law.

It is possible that your medical and/or research record, including identifying information, may be inspected and/or copied by the study sponsor (and/or its agent), the Food and Drug Administration (FDA), federal or state government agencies, MU Health Sciences IRB, or hospital accrediting agencies, in the course of carrying out their duties. If your record is inspected or copied by the study sponsor (and/or its agents), or by any of these agencies, the University of Missouri will use reasonable efforts to protect your privacy and the confidentiality of your medical information. The results of this study may be published in a medical journal or used for teaching purposes. However, your name or other identifying information will not be used in any publication or teaching materials without your specific permission.

WHAT IF I AM INJURED?

In the event the research results in injury, the Sponsor agrees that it, and not Institution, will be responsible for the costs of diagnosis, care and treatment of any undesirable side effects, adverse reactions, illness or injury. The study staff will coordinate your care, should you need it. In the event you

have suffered injury as the result of participation in this research program, you are to contact the Risk Management Officer, telephone number (573) 882-1181, at the Health Sciences Center, who can review the matter and provide further information.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Participation in this study is voluntary. You do not have to participate in this study. Your present or future care will not be affected should you choose not to participate. If you decide to participate, you can change your mind and drop out of the study at any time without affecting your present or future care at the University of Missouri. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. In addition, the investigator of this study may decide to end your participation in this study at any time after she has explained the reasons for doing so and has helped arrange for your continued care by your own doctor, if needed. You will be informed of any significant new findings discovered during the course of this study that might influence your health, welfare, or willingness to continue participation in this study.

The Data Safety Monitor for this study is Dr. Ghassan Hammoud, a liver expert. He will be reviewing the blood draw values throughout the time you are participating to monitor your safety. Our study staff will tell you about the new information from this or other studies that may affect your health, welfare, or willingness to continue participation in this study. A description of this clinical trial is available on www.ClinicalTrials.gov, as required by U.S. law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

If you have any questions regarding your rights as a participant in this research and/or concerns about the study, or if you feel under any pressure to enroll or to continue to participate in this study, you may contact the University of Missouri Institutional Review Board (which is a group of people who review the research studies to protect participants' rights) at (573) 882-3181. You may also contact the Research Participant Advocate (RPA) at (573) 884-1925 or (888) 280-5002 (toll-free). If you prefer email, you can reach the Advocate at somrpa@missouri.edu.

You may ask more questions about the study at any time. For questions about the study or a research-related side effects or injury, contact Dr. Camila Manrique at (573) 882-2273. A copy of this consent form will be given to you to keep.

SIGNATURES

I confirm that the purpose of the research, the study procedures, the possible risks and discomforts as well as potential benefits that I may experience have been explained to me. Alternatives to my participation in the study also have been discussed. I have read this consent form and my questions have been answered.

My signature below indicates my willingness to participate in this study.

Subject name (print)	Subject signature	Date	Time

Staff obtaining consent (print)	Staff signature	Date	Time