The effect of Vigabatrin on Insulin Sensitivity

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SPECIFIC AIMS

Nonalcoholic fatty liver disease (NAFLD) is a common complication of obesity and is associated with hyperinsulinemia, mutli-organ insulin resistance and an increased risk of developing type 2 diabetes (T2D). The hallmark feature of NAFLD is an increase in intrahepatic triglyceride (IHTG) content. Data from studies conducted in rodent models suggest increased IHTG content can cause insulin resistance through signaling by the hepatic vagal afferent nerve (HVAN). In rodent models of obesity and NAFLD, HVAN activity is reduced leading to impaired insulin sensitivity and glucose control. The reduction in HVAN activity is likely due to increased hepatic release of GABA, an inhibitory neurotransmitter, attributable to increased expression of GABA-Transaminase (GABA-T). In lean rodents GABA-T acts to break down high levels of GABA to produce succinate semialdehyde and glutamate, but in the livers of obese mice, the opposite reaction occurs with increased succinate semialdehyde and glutamate availability driving the GABA-T reaction toward GABA production, rather than breakdown. Pharmacological inhibition of GABA-T in obese mice by treatment with vigabatrin, an irreversible inhibitor of GABA-T that is approved by the FDA to treat seizure disorders, improves glucose tolerance and reduces hyperinsulinemia, hyperglycemia, and insulin resistance. It is not known if vigabatrin can also improve metabolic function in people.

The overall goals of this pilot study are to determine the potential effect of vigabatrin on insulin sensitivity, insulin secretion and glycemic control in people with obesity and NAFLD (IHTG content ≥5.6%). Accordingly, we will conduct a 3-week, single-arm trial to assess the effect size of treatment with vigabatrin on the following specific aims with the larger goal of determining whether a large, randomized controlled trial investigating the effect of vigabatrin is warranted:

Aim 1: Determine the effect of GABA-T inhibition on insulin sensitivity in people with NAFLD. We will perform hyperinsulinemic-euglycemic clamp procedures plus stable isotope tracer infusions to assess skeletal muscle insulin sensitivity before and after treatment.

Aim 2: Determine the effect of GABA-T inhibition on oral glucose tolerance and glucose-stimulated insulin secretion. We will perform oral glucose tolerance tests to simultaneously assess glucose control and oral glucose-stimulated insulin secretion before and after treatment.

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) affects about one-third of the adult population in the United States[1]. Insulin resistance is a key feature of NAFLD central to many of the cardiometabolic complications associated with excess adiposity because insulin is a key regulator of liver glucose production, liver triglyceride (TG) synthesis and plasma TG concentration[2-8]. Accordingly, insulin resistance is implicated in the pathogenesis of T2D, dyslipidemia, hypertension, and coronary heart disease[2-5,7-9]. Moreover, the degree of hepatic lipid accumulation is positively related to the magnitude of hyperinsulinemia and insulin resistance and the severity of T2D[10-15].

Hepatocyte Depolarization Regulates Glucose Homeostasis: Hepatic lipid accumulation depolarizes hepatocytes[16,17]. Expression of ligand-gated ion channels in hepatocytes through use of an adeno-associated virus (AAV), increases hepatocyte depolarization (Fig. 1A), inhibits activity of the HVAN (Fig. 1B) and induces hyperinsulinemia (Fig. 1C). Further, AAV mediated delivery of a plasmid encoding for hepatocyte specific expression of a hyperpolarizing Kir2.1 channel prevents obesity induced hyperinsulinemia and insulin resistance (Fig. 1D and 1E). In addition, hepatic vagotomy decreases insulin-mediated skeletal muscle glucose clearance[18,19] potentially due to skeletal muscle parasympathetic acetylcholine signaling, which limits acetylcholine mediated vasodilation and resulting improved skeletal muscle blood perfusion and increased interstitial insulin and glucose concentrations. These increased insulin and glucose concentrations encourage GLUT4 translocation to the cell surface and glucose uptake. The HVAN also tonically suppresses activity of parasympathetic nerves that project to the pancreas[20]. Severing the HVAN increases activity of these pancreatic projections and acetylcholine release onto β-cells thereby stimulating increased insulin release[20,21]. In turn, stimulation of the HVAN decreases acetylcholine signaling at the β-cell and depresses insulin release[20,21]. Moreover, obese Kir2.1 expressing mice, which prevented hepatocyte depolarization in obesity, did not exhibit hyperinsulinemia despite similar hepatic lipid accumulation as controls (Kir2.1: 94.2±10.6 mg triglycerides/g liver versus eGFP control: 98.4±6.6 mg triglycerides/g liver; P = 0.73). Importantly, a hepatic-

specific vagotomy eliminates signals originating from the liver but does not alter basal signaling in the nucleus tractus solitarii (NTS)[22-24]. Taken together these data suggest hepatocyte depolarization is an important mediator of the relationship between hepatic lipid accumulation and dysregulated glucose homeostasis, and

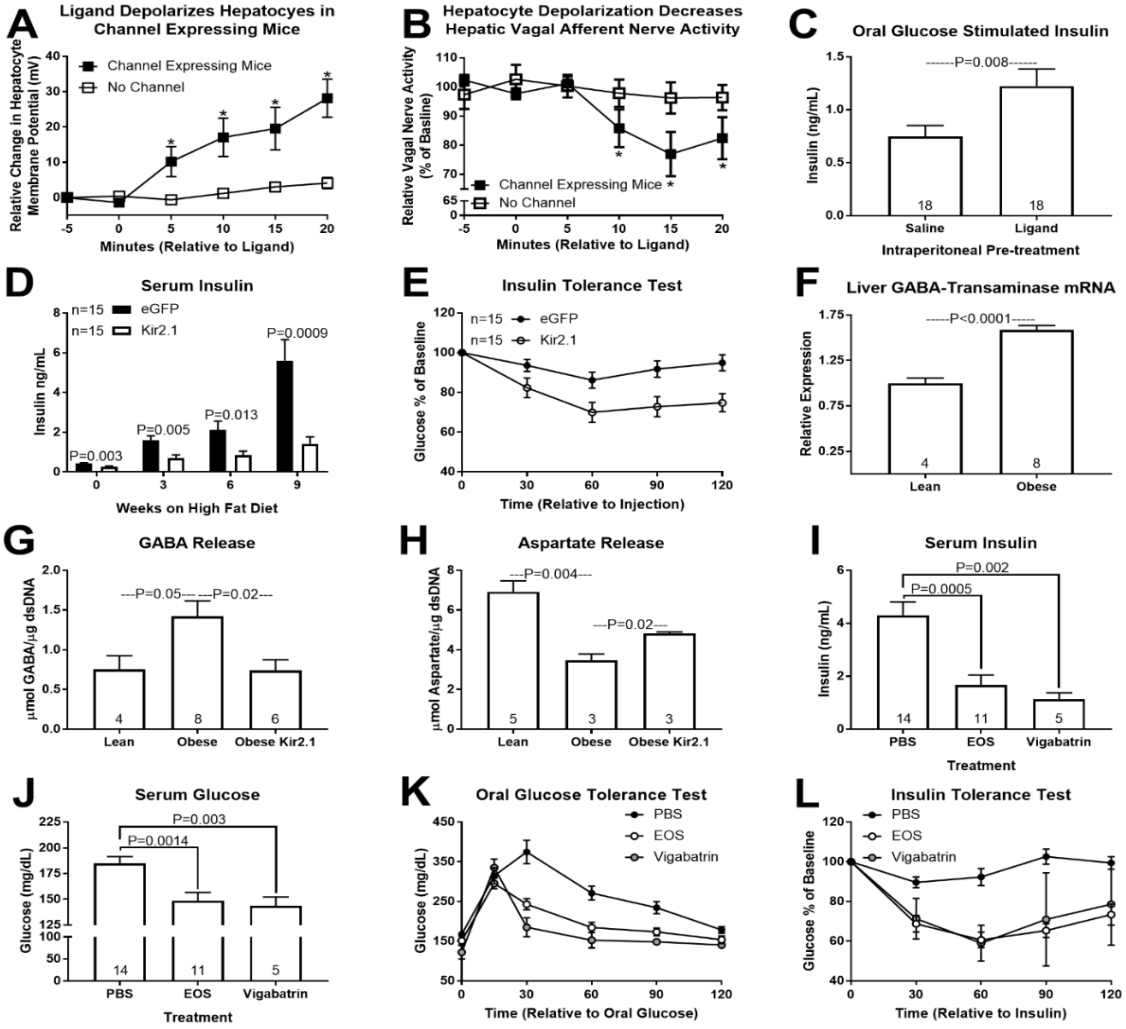

Figure 1. Hepatic Regulation of Glucose Homeostasis. Ligand-induced hepatocyte depolarization (A) decreases hepatic vagal afferent nerve activity (B) and exacerbates glucose stimulated insulin (C). Increased hepatic Kir2.1 channel expression prevents high fat diet-induced hyperinsulinemia (D) and insulin resistance (E). Diet induced obesity increases liver GABA-transaminase (GABA-T) mRNA expression (F), increases hepatic slice GABA (G) release and decreases hepatic slice aspartate (H) release. Kir2.1 channel expression in obese mice decreases hepatic slice GABA (G) release and increases hepatic slice aspartate release (H). The GABA-T inhibitors ethanolamine-O-sulfate (EOS) and Vigabatrin limit diet-induced obesity hyperinsulinemia (I), and hyperglycemia (J), while improving oral glucose tolerance (K) and insulin tolerance (L).

that prevention of NAFLD-induced hepatic depolarization lowers circulating plasma insulin concentrations and improves insulin sensitivity.

Obesity increases hepatic GABA release due to increased GABA-T activity: Gamma aminobutyric acid (GABA) is an inhibitory neurotransmitter released in the liver. In *ex vivo* liver slices from rodents, diet-induced obesity increases release of GABA, and decreases release of aspartate, an excitatory neurotransmitter (Fig. 1G and 1H). Because the liver does not express measurable levels of the glutamate decarboxylase enzymes (i.e. GAD65 and GAD67) that synthesize GABA in the nervous system, Renquist and colleagues sought another mechanism by which GABA could be formed. They found obesity increases the expression of GABA-transaminase (GABA-T) mRNA at the liver (Fig. 1F) and expression of genes involved in ketogenesis and

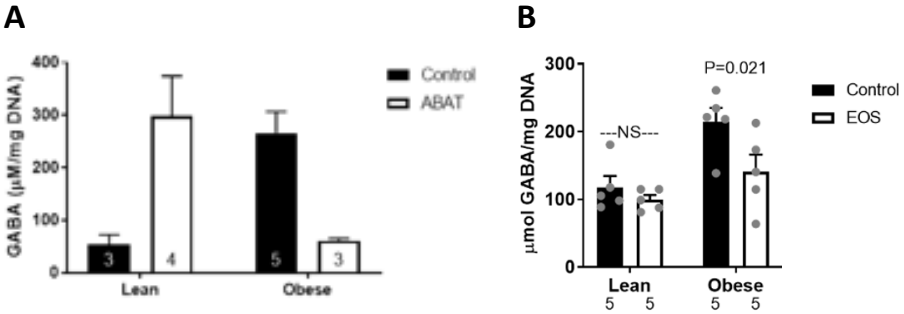

Figure 2. GABA-T inhibition by knockdown of the ABAT gene (A) or pharmacologically with EOS (B) increases GABA levels in lean rodents but decreases GABA levels in obese rodents

gluconeogenesis[25,26]. In lean rodents, GABA-T decreases GABA concentrations by converting GABA and 2-oxoglutarate to produce glutamate and succinate semialdehyde; pharmacological inhibition of GABA-T by ethanolamine-O-sulfate (EOS) or vigabatrin or knockdown of GABA-T (ABAT) gene by an anti-sense oligonucleotide (ASO) increases GABA levels (Figure 2A). In contrast, in obesity increased gluconeogenic flux and an altered hepatic redox state (Figure 3A) inhibits the conversion of succinate to fumarate in the TCA cycle by the enzyme succinate dehydrogenase and instead drives succinate to form succinate semialdehyde by succinic semialdehyde dehydrogenase leading to increase GABA production. Inhibiting succinate dehydrogenase replicates this process resulting in increased GABA production (Figure 3B). Knockdown of the GABA-T gene by ASO and pharmacological inhibition of GABA-T by EOS treatment both reduce GABA levels in in obese mice (Figure 2A and B). This data is supported by the observation that mice which lack phosphoenolpyruvate carboxykinase, a key enzyme in gluconeogenesis, are protected from obesity induced hyperinsulinemia and insulin resistance[27] and lean mice that lack hepatic insulin signaling and have unrestricted hepatic gluconeogenic flux display hyperinsulinemia and insulin resistance[28].

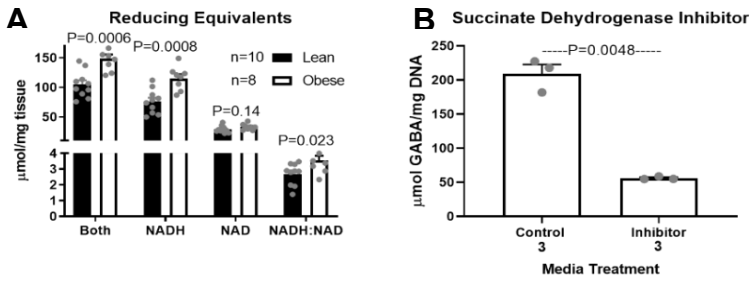

Figure 3. NADH significantly higher in obese livers (A) GABA reduced with succinate dehydrogenase inhibitor (B)

Inhibiting GABA-T activity in obesity improves glucose control: Diet induced obese mice treated with one of two GABA-T inhibitors, ethanolamine-O-sulfate (EOS) or vigabatrin, display lean state serum glucose and insulin concentrations, insulin sensitivity and glucose tolerance after only 3 days (Fig. 1I-1L). In addition, knockdown of the GABA-T gene by ASO, in obese mice, improves glucose and insulin tolerance (Figure 4A and B) and increase whole-body and muscle specific glucose uptake during hyperinsulinemia (Figure 4 C and D). This data supports a role for GABA-T in the development of insulin resistance and hyperinsulinemia in rodents. Whether inhibition of GABA-T improves insulin sensitivity and glucose control in humans with obesity and increased liver fat is not known.

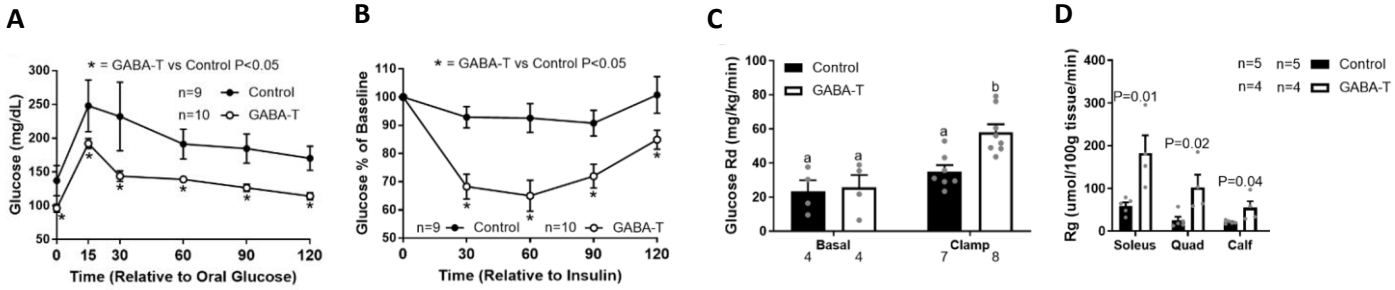

Figure 4. ASO Knockout of GABA-T improves glucose (A) and insulin (B) tolerance and increases whole-body (C) and muscle (D) insulin-stimulated glucose uptake.in obese mice.

Summary: Nonalcoholic fatty liver disease is associated with insulin resistance, and is an important risk factor for the development of T2D and CVD. Studies conducted in mouse models have shown a mechanistic link between NAFLD and insulin resistance; obese animals with NAFLD have an increase in hepatic GABA which decreases HVAN activity which in turn induces insulin resistance. Use of vigabatrin, an FDA-approved drug for seizure disorders, lowers hepatic GABA release and improves glucose tolerance and insulin sensitivity. However, the effect of vigabatrin on glucose tolerance and insulin sensitivity in people with obesity and NAFLD is not known. Accordingly, the goal of this study is to conduct a pilot study to determine whether GABA-T inhibition may improve metabolic function in people with obesity and NAFLD and a larger, randomized controlled trial investigating the effect of vigabatrin is warranted.

APPROACH

Study Participants

A total of 6 adults (18-60 years old) with obesity (BMI 30.0-49.9 kg/m^2), NAFLD (IHTG content ≥5.6%), and insulin resistance (Homeostatic Model of Insulin Resistance (HOMA-IR) Score[29]>2.5), of all races and ethnic groups will be enrolled in this study.

Exclusion criteria will include: 1) previous bariatric surgery; 2) structured exercise ≥250 min per week (e.g., brisk walking); 3) unstable weight (>4% change during the last 2 months before entering the study); 4) significant organ system dysfunction (e.g., diabetes, severe pulmonary, kidney or cardiovascular disease); 5) cancer; 6) polycystic ovary syndrome; 7) major psychiatric illness (including suicidal ideation or previous suicide attempts); 8) conditions that render subject unable to complete all testing procedures (e.g., severe ambulatory impairments, limb amputations, or metal implants that interfere with imaging procedures, coagulation disorders, aversion to needles, metal implants that prevent magnetic resonance imaging); 9) regular use of tobacco products; 10) excessive consumption of alcohol (≥3 drinks/day for men and ≥2 drinks/day for women); 11) use of medications that are known to affect the study outcome measures or increase the risk of study procedures and that cannot be temporarily discontinued for this study; 12) pre-existing visual field deficits; or those at high risk of irreversible vision loss, including patients with retinopathy or glaucoma; 13) pregnant or lactating women; 14) illicit drug use); 15) persons who are unable or unwilling to follow the study protocol; 16) persons who are not able to grant voluntary informed consent; 17) patients at risk for severe anemia (hemoglobin less than 13.5 gram/100 ml in men or 12.0 grams/100 ml in women or hematocrit < 39% in men or < 36% in women); 18) patients with a heart failure or a history of lower limb edema; xxii) patients with mild or more severe renal insufficiency (CLcr <80 mL/min/1.73m^2); 19) patients with existing peripheral neuropathy; 20) and women who have active menstral cycles but are not using birth control (acceptable contraception includes barrier/hormonal/IUD).

Experimental Design

Overview

The testing schedule presented in Table 1. All participants will undergo comprehensive outpatient screening visits, including medical examination, visual field examination, blood tests, EKG, and an MRI scan to assess IHTG content to determine eligibility for the study. Participants who meet the inclusion criteria will be admitted to the inpatient Clinical and Translational Research Unit (CTRU) at Washington University School of Medicine on two separate occasions to complete baseline testing. After completion of baseline testing, subjects will receive vigabatrin to be taken over 30 days. Participants will be instructed to take one 500 mg pill 2X/day for the first 7 days (days 0-6; 1000 mg/day), one pill in the morning and two pills in the evening for days 7-13 (1500 mg/day), and two pills twice a day for days 14-23 (2000 mg/day). On day ~21, participants will again arrive at CTRU an overnight fast for a repeat modified oral glucose tolerance test (mOGTT) with a hyperinsulinemic-euglycemic clamp (HEC) procedure and repeat peripheral vision tests performed on day ~23. Throughout the study, participants and the study team will be blinded to treatments. After completing the HEC, participants will begin stepping down the vigabatrin dose as recommended by the manufacturer, moving to the 1 pill 2X/day dose for days 24-30 before discontinuing treatment. Lastly, participants will be seen for repeat vision testing that occurred in screening approximately 6 months after initiation (5 months after discontinuation) of the drug.

Screening Visits

After informed consent is obtained, participants will undergo three separate screening visits to confirm eligibility.

Screening visit 1. Participants will arrive in the CTRU in the morning after they have fasted for 10h-12h overnight at home where they will undergo a comprehensive screening process, including a medical history, physical examination, standard blood tests and questionnaires to assess alcohol intake, eating habits, sleeping patterns, current level of anxiety, depression (measured with the Beck Depression Inventory-II[30]), quality of life and any potential barriers that could interfere with their ability to complete all study requirements/visits.

Vital signs (blood pressure, heart rate, respirations, temperature) will be collected while patient is seated in a comfortable position. The physical exam will include examination of eyes, ears, nose, throat, heart, and lungs. Laboratory and blood work will include: pregnancy test (for females only-measures hCG horome); urine drug screen; complete blood count with differential (red blood cell count, MCV, MCH, MCVC, platelets, MPV, hemoglobin, hematocrit, leukocyte count, neutrophils, lymphocytes, monocytes, eosinophils, basophils); comprehensive metabolic panel (glucose, Sodium, potassium, carbon dioxide, and chloride, calcium, albumin, total protein, ALT, AST, ALP, bilirubin, BUN, creatinine); lipid panel (triglycerides, total-, HDL-and LDL-cholesterol); Hemoglobin A1C (HbA1C) and Prothrombin time and international normalized ratio (PT/INR). A peripheral vision field test will be performed on all participants.

Screening visit 2. If inclusion criteria for age, body mass index, HOMA-IR score and vision are met, subjects will be scheduled to undergo imaging tests approximately 1 week later. As part of these tests, IHTG content will be measured by magnetic resonance imaging (MRI) to confirm if the participant is eligible for the study. During this visit, MRI will be used to quantify liver volume and abdominal subcutaneous and intra-abdominal adipose tissue volumes with liver stiffness also determined by magnetic resonance elastography (MRE). If participants have any emotional anxiety associated with completing the MRI and MRE procedures (e.g., fear of confined spaces), they will have the option of taking Alprazolom or Lorazepam, prescribed by a study physician, to allow the MR scans to be completed.

Screening visit 3. If IHTG content ≥5.6% at screening visit 2 subects will be subjects will be scheduled to undergo vision testing approximately 1 week later. Vision monitoring will include best corrected distance visual acuity, perimetry and spectral domain optical coherence tomography (sdOCT), sdOCT will include the macula. Perimetry will be automated, threshold testing of the central 60 degree.

Baseline Testing Visit 1

Subjects will be at the CTRU for approximately 6 hours. During this admission, subjects will complete a mOGTT clamp procedure to assess oral glucose tolerance and a dual energy X-ray absorptiometry (DXA) scan to measure whole- body composition.

Whole-body composition analyses. Total body fat mass, fat-free mass and appendicular fat-free mass will be determined using dual energy X-ray absorptiometry (DXA).

Modified 3-hour oral glucose tolerance test (mOGTT). An IV catheter will be inserted into an antecubital vein for blood sampling and kept patent with a continuous infusion of saline. Blood samples to determine plasma glucose, insulin, and C-peptide concentrations will be collected 15, 10 and 5 min before ingesting a 75-gram glucose load and 10, 20, 30, 60, 90, 120, 150, and 180 min after glucose ingestion. The total areas under the curve (AUCs) for glucose, insulin, and C-peptide concentrations will be calculated by using the trapezoid method. Insulin sensitivity will be assessed by using the oral glucose insulin sensitivity (OGIS) index [31]. Indices of β-cell function will be estimated from plasma glucose and C-peptide concentrations by using the oral minimal model of C-peptide secretion and kinetics [32,33]. This model calculates the insulin secretion rate as a function of time and the following indices of β-cell responsivity: (i) dynamic responsivity index ($\Phi d(10^9)$), which is an index of insulin secretion in response to the rate of change in glucose concentration; (ii) static responsivity index ($\Phi s(10^9 min^{-1})$), which is an index of insulin secretion in response to a given glucose concentration; and (iii) overall responsivity index (Φo ($10^9 min^{-1}$)), which is a global sensitivity to glucose index of postprandial insulin secretion.

Baseline Testing Visit 2

Subjects will be admitted to the CTRU at 1800 h for ~24 hours. During this admission, subjects will complete a HEC procedure to assess insulin sensitivity. Subjects will be given a standard meal the evening before the HEC procedure (day 1) and will then fast until the end of the HEC procedure on day 2.

Hyperinsulinemic-euglycemic clamp (HEC). At 0700 h after a 12 hour fast, a primed constant infusion of [6,6-$^2H_2$]glucose will be started through an intravenous catheter inserted into an antecubital vein. An additional catheter will be inserted into a radial artery for blood sampling. 210 min after the start of the tracer infusion (after the basal period is completed), a one-stage HEC procedure will be initiated and continued for an additional 210 min. During the HEC, insulin will be infused at a rate of 50 mU/m^2 body surface area (BSA) after

initiation with a two-step priming dose: 200 mU/m^2 BSA/min for 5 min followed by 100 mU/m^2 BSA/min for 5 min. This will result in plasma insulin concentrations of ~100 mU·l^{-1}. Dextrose, enriched with [6,6,-$^2H_2$]glucose, to minimize changes in plasma glucose isotopic enrichment [34], will be infused at a variable rate to maintain plasma glucose concentration (monitored every 10 min) at ~100 mg/dl during insulin infusion. The [6,6,-$^2H_2$]glucose infusion will be stopped during the clamp because of the expected decrease in hepatic glucose production [35]. The insulin concentration achieved during the clamp reflects postprandial plasma insulin concentrations [36,37] and is ideal to evaluate insulin's effect on glucose disposal [38]. Blood samples for kinetic analyses will be collected immediately before the start of the tracer infusions and then every 6-7 min for a 20 min period at the end of the basal and insulin infusion stages. The data (average values) obtained during these times will be used to determine hormone and substrate concentrations and glucose enrichments to calculate the glucose rate of appearance (Ra) and disappearance (Rd) from plasma. Endogenous glucose rate of appearance (Ra) in plasma will be calculated by dividing the glucose tracer infusion rate by the average plasma glucose TTR during the last 20 min of the basal stage and at the end of the HEC procedure. Glucose Rd will be calculated as the sum of endogenous glucose Ra and the infusion rate of exogenous glucose. Insulin-induced stimulation of glucose Rd during the HEC procedure will be used as an index of skeletal muscle insulin sensitivity [39,40].

Sample processing

Plasma glucose concentration will be determined by using a YSI analyzer (Yellow Springs Instruments Co., Yellow Springs, OH) and plasma C-peptide and insulin concentrations will be determined by using electrochemiluminescence assays (Elecsys 2010, Roche Diagnostics, Indianapolis, IN). Plasma FFA concentrations will be assessed by gas chromatography [41]. Glucose tracer-to-tracee ratio in plasma will be measured by using gas-chromatography/mass-spectrometry as previously described [41-45].

Intervention

Vigabatrin capsules will be taken by participants daily for 30 days. Drug tablets will be administered orally as provided by the manufacturer and dosage matching the FDA label. Specifically, we will provide each patient with a total dosage of 44.5 g vigabatrin. Subjects will be instructed to take one 500 mg pill 2X/day for the first 7 days (days 0-6; 1000 mg/day), 1 pill in the morning and 2 pills in the evening for days 7-13 (1500 mg/day), and two pills 2X/day for days 14-23 (2000 mg/day). On day 24, participants will initiate a step-down protocol as recommended by the manufacturer, moving to the one pill 2X/day dose for days 24-30, before discontinuing treatment. Study participants will meet with the research coordinator or one of the investigators weekly to ensure compliance with the study protocol and problem-solve any barriers. Regular medical monitoring by the study research coordinator and medical staff will be performed during the entire study period. Subjects will be asked to come in weekly while on the drug. At the visit, a general wellness check (including depression screening with the Beck Depression Inventory-II[30]), vital signs and body weight and a urine drug test will be obtained, and blood samples (7 ml of blood (~0.5 tablespoon)) will be obtained at the weekly visit for the first two weeks of dose escalation to assess routine laboratory tests. Study participants will be instructed to keep their body weight stable (< 2% change) during the treatment period.

Dispensing and accountability

A medication accountability log will be used to document dispensing of the vigabatrin to the participant and the return of unused product by the participant. The medication accountability log will document the participant ID, initials, the amount of medication and the date the drug was dispensed to the participant, and the amount of medication that the study participant returned. Participants will be instructed to return unused medications at every scheduled study visit and study end. The study research coordinator will perform a pill count and document this in the medication accountability log at each study visit.

Repeat studies

The HEC procedure and mOGTT performed at baseline as well as the MRI to determine liver triglyceride content at screening will be repeated after 3 weeks of treatment. In addition, depression measured with the Beck Depression Inventory-II[30] (measured at screening) will be re-assessed at the repeat mOGTT.

Vision testing will be repeated during the clamp visit (day 23). Vision and depression (Beck Depression Inventory-II[30]) will be assessed approximately 6 months after participants initiated the drug.

Table 1. Schedule of tests performed

	SCREENING			BASELINE TESTING		WEEK 3 TESTING		STUDY COMPLETION
Assessment	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 1**	**Visit 2**	**Visit 1**	**Visit 2**	
Informed Consent	X							
Anthropometrics								
Weight	X			X	X	X	X	X
Height	X							
Vital Signs								
Blood Pressure	X			X	X	X	X	X
Heart Rate (Pulse)	X			X	X	X	X	X
Respirations	X			X	X	X	X	X
Temperature	X			X	X	X	X	X
Medical Evaluation								
Medical History	X							
Physical Examination	X							X
12-Lead Electrocardiogram	X							
Depression Screening	X					X		X
Lab/Blood Work								
Urine pregnancy test (females only)	X	X	X	X	X	X	X	
Urine drug test	X	X	X	X	X	X	X	
PT/INR	X							
CBC/diff	X					X		
CMP	X					X		
Lipid panel	X					X		
HbA1C	X					X		
Body composition analysis								
Dual energy X-ray absorptiometry (DXA)				X				
Abdominal MRI		X				X		
Liver volume, TG content and stiffness by MRI/MRE		X				X		
Metabolic function								
Modified 3-hour oral glucose tolerance test (mOGTT)				X		X		
Hyperinsulinemic-euglycemic clamp (HEC)					X		X	
Vision Testing								
Peripheral Vision Tests			X				X	X

Statistical Analyses

Statistical Analysis: The effect size of vigabatrin treatment on insulin sensitivity during the HEC and insulin secretion assessed during the mOGTT will be determined by using Cohen's *d* tests. Using these effects sizes we using G*Power 3.1.9.2 to estimate the number of participants per group that would be required to detect an ~15% improvement in insulin sensitivity between placebo and vigabatrin groups with >90% power using two-sided tests at the $\alpha=0.05$ level if a randomized, controlled trial were to be conducted.

PROTECTION OF HUMAN SUBJECTS

Human Subjects Involvement and Characteristics. A total of 6 men and women of all races and ethnic groups will be studied. Subjects will be screened carefully with a medical history and physical examination, and blood tests. Exclusion criteria will include: 1) previous bariatric surgery; 2) structured exercise ≥250 min per week (e.g., brisk walking); 3) unstable weight (>4% change during the last 2 months before entering the study); 4) significant organ system dysfunction (e.g., diabetes, severe pulmonary, kidney or cardiovascular disease); 5) cancer; 6) polycystic ovary syndrome; 7) major psychiatric illness (including suicidal ideation or previous suicide attempts); 8) conditions that render subject unable to complete all testing procedures (e.g., severe ambulatory impairments, limb amputations, or metal implants that interfere with imaging procedures; coagulation disorders); 9) regular use of tobacco products; 10) excessive consumption of alcohol (≥3 drinks/day for men and ≥2 drinks/day for women); 11) use of medications that are known to affect the study outcome measures or increase the risk of study procedures and that cannot be temporarily discontinued for this study; 12) pre-existing visual field deficits; or those at high risk of irreversible vision loss, including patients with retinopathy or glaucoma; 13) pregnant or lactating women; 14) conditions that render subject unable to complete all testing procedures (e.g. aversion to needles, metal implants that prevent magnetic resonance imaging); 15) persons who are unable or unwilling to follow the study protocol; 16) persons who are not able to grant voluntary informed consent; 17) patients at risk for severe anemia (hemoglobin less than 13.5 gram/100 ml in men or 12.0 grams/100 ml in women or hematocrit < 39% in men or < 36% in women); 18) patients with a heart failure or a history of lower limb edema; xxii) patients with mild or more severe renal insufficiency (CLcr <80 mL/min/1.73m^2); 19) patients with existing peripheral neuropathy; 20) and women who have active menstral cycles but are not using birth control (acceptable contraception includes barrier/hormonal/IUD).

Sources of Materials. All specimens will be obtained solely for research purposes. Although these generally will be obtained specifically for the purposes of the study, use will be made, where appropriate, of existing records and data obtained as part of routine clinical care. Measures to be obtained include: 1) detailed medical history, 2) a physical exam, 3) clinical laboratory evaluation consisting of blood specimens to assess complete metabolic panel, lipid profile and complete blood count, 4) DXA scan to evaluate body composition, and 5) MR scans to evaluate intra-abdominal adipose tissue, intrahepatic triglyceride content. All data from individual subjects will be maintained confidentially and their names and identities will not be disclosed in any published document.

Potential Risks. We anticipate no psychological, social or legal risks beyond those of participation in health-related research in general with the exceptions listed below. The potential risks associated with participation in this study are moderate and are listed below. They will be explained to all subjects who desire to participate in this research project. In addition, subjects will be informed that there is a possibility of unforeseeable risk, although from our experience with such studies, we consider this unlikely. The research coordinator, research nurse and/or the PI will ensure understanding of the consent and study procedures, as well as laboratory results; in addition, the research nurse and/or the PI will explain the different procedures and answer any questions the subjects might have before initiating any study-related procedures. Whenever concerns arise, subjects will be informed that they are free to withdraw from the study at any time with no bias or prejudice.

This research involves exposure to radiation from the dual-energy x-ray absorptiometry (DXA) for body composition measurements. The amount of radiation from these procedures, when averaged over the entire human body, is equivalent to a uniform whole-body dose of <1 mrem. This is equivalent to less than 3% of the amount of natural background radiation exposure all people in St. Louis receive each year.

This research involves collection of blood samples, each of which has potential risks associated. Possible side effects of venous catheter insertion are discomfort, bruising, bleeding, and/or infection at the site of needle insertion. Occasionally some people experience dizziness or feel faint. A blood clot can occur at the site of the arterial catheter insertion which could decrease blood flow to the hand and cause tissue damage requiring corrective surgery. However, the risk of this is extremely small because a small size catheter is used. Furthermore, before catheter placement, all subjects are examined to make sure that they have adequate blood flow to the hand from the other major artery that supplies the hand. Subjects who are judged to have insufficient compensatory blood flow will be excluded from the study. Lastly, there is a possibility that potentially adverse medical conditions will be identified as a result of the medical evaluation at screening.

Vigabatrin limits central nervous system GABA breakdown. Study subjects will be advised to not drive a car or operate other complex machinery until they are familiar with the effects of the study drug on their ability to perform these activities. Participants will be warned about possible complications and told to contact their physician and the study physician should they experience any of the side effects listed for vigabatrin (e.g. headache, irritability, dizziness, drowsiness, tiredness, memory problems, intrameylinic edema, weight gain, anemia, swelling, numbness/burning pain/tingly feeling in the hands or feet, loss of coordination, joint pain, cold symptoms (such as stuffy nose, fever, sneezing, sore throat), nausea, vomiting, diarrhea, sleep problems (insomnia), uncontrolled back and forth eye movements, tremors, pale skin, lightheadedness, shortness of breath, rapid heart rate, trouble concentrating, confusion, mood or behavior changes, depression, or thoughts about suicide). Participants will be given a list with suicide prevention resources at the time of Consent.

According to vigabatrin FDA label: In U.S. and primary non-U.S. clinical studies of 4,079 SABRIL treated patients, the most common (≥5%) adverse reactions associated with the use of SABRIL in combination with other AEDs were headache, somnolence, fatigue, dizziness, convulsion, nasopharyngitis, weight gain, upper respiratory tract infection, visual field defect, depression, tremor, nystagmus, nausea, diarrhea, memory impairment, insomnia, irritability, abnormal coordination, blurred vision, diplopia, vomiting, influenza, pyrexia, and rash. The adverse reactions most commonly associated with SABRIL treatment discontinuation in ≥1% of patients were convulsion and depression. There are potential rare side effects including constriction of the visual field which could potentially lead to vision loss. There is no evidence that a cumulative dose <1 kg affects vision. However there is 4% increase in visual field changes in patients that have taken a cumulative dose of > 1 kg[48]. Vigabatrin is FDA approved for use in people with epilepsy and there have been limited studies looking at the effects of vigabatrin in people without neuronal disturbance. One randomized controlled trial was conducted to investigate the effect of vigabatrin (total dose 218 grams) on cocaine addiction[49]. Headaches were more commonly reported in the placebo group as compared to vigabatrin, but there were no other differences in side effects between the vigabatrin and control groups[49]. Compliance was only 40-60% for subjects on vigabatrin, which could impact experience of side effects[49]. Importantly, in a secondary analysis, the investigators found no significant differences in vision between the placebo and the compliant vigabatrin subjects[50]. Similarly, no visual field defects were reported with short term (6 weeks) use of vigabatrin for treatment cocaine dependence (total dose 137 grams)[51]. For this study participants will be taking total dosage of 44.5 grams. Vision tests will be performed prior to starting vigabatrin, at 3 weeks and 6 months after drug initiation to ensure no changes.

Adequacy of protection against risks

Recruitment and Informed Consent. Potential subjects will be recruited from a pool of subjects in the Center Human Nutrition database and from the community-at-large. The objectives of the project, all experimental procedures, all of the requirements for participation, and any possible discomforts and risks and benefits of participation will be clearly explained in writing and orally, in lay terms, to the subject by the PI or research coordinator. After all questions have been answered, and the subjects have been informed orally and in writing that they are free to withdraw from the study at any time with no bias or prejudice, and agree to participate, written informed consent, approved by the Washington University School of Medicine Institutional Review Board, will be obtained. The consenting procedure will be conducted in the CTRU at Washington University School of Medicine before initiating any study-related procedures (screening tests, body composition analyses, etc.).

Confidentiality. All personnel involved in the design and conduct of this research project will receive the required education on the protection of human research participants prior to the start of this project. All specimens will be obtained solely for research purposes. Study samples and data sheets will be coded with an identification number for each subject. All data will be treated confidentially, and the subjects' names and identities will not be disclosed in any published reports.

Medical risks related to this study (described above). Complete physical examinations will be performed to screen out subjects who may be at increased risk. All procedures will be performed by qualified and experienced personnel and subjects will be carefully monitored during all procedures and during the intervention. Intravenous tracer solutions will be prepared in a designated, sterile mixing room. Careful aseptic techniques will be used when inserting the catheters and when obtaining blood samples to decrease the risk of

infection. The total amount of blood collected during the full set of research procedures will be ~500 mL including all screening, baseline, post-treatment and medical monitoring visits . If a participant develops a health problem or potential health problem, the PI/study physician and, if necessary a medical consultant who is not part of the research team, will decide whether the participant should be continued in the study, and/or what further steps regarding medical evaluation/treatment should be performed. The CTRU and hospital facilities used for the study are equipped with defibrillators and all appropriate emergency medications. Should the need for medical treatment arise, the PI/study physician will arrange for medical evaluation at the Washington University School of Medicine, which provides all the resources of a large teaching hospital for subject evaluation and treatment. The subject and the primary care physicians will be made aware of any abnormal findings while participating in the study. The participants will be given the phone numbers of the members of the research team (including an emergency phone number) and told to call a member of the research team immediately if they develop any unusual signs or symptoms. Subjects will be screened for depressive symptoms before starting treatment, while on treatment and at study completion. If the subject reports significant increase in depression or thoughts of suicidal ideation, the subject will be removed from the study and immediately be sent for psychiatric evaluation. Vision testing will be completed in all subjects at baseline, after a week at the maximum dose and then 6 months after drug initiation. If vision changes are noted, subjects will be referred for further testing and evaluation by an ophthalmologist.

Potential benefits of the research to the participants
Potential benefits to the participants include obtaining information regarding their body composition and a medical examination. Participants' basal measures of glucose homeostasis may improve with this treatment. These results could support the findings from studies conducted in rodent models and lead to a new approach for treating metabolic abnormalities in people.

Importance of the knowledge to be gained
The information gained from this work has important public health implications in the potential of development of a new drug for treatment or prevention of NAFLD.

Data and safety monitoring plan

Data from the study will be monitored on a continual basis by the PI. All serious adverse events (SAEs), adverse events (AEs), and laboratory values will be reviewed by the PI on an ongoing basis. The PI will be responsible for annual reports to Washington University School of Medicine's IRB and for reporting any adverse events to the IRB.

The IRB at Washington University School of Medicine defines unanticipated problems (adverse events) as involving risks to participants or others which are: 1) unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the study-related documents, such as the IRB-approved research study and informed consent document; and (b) the characteristics of the subject population being studied; and 2) are related or possibly related to participation in the research; and 3) suggest that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Adverse events will include vision loss, suicidal behavior, anemia, peripheral neuropathy, edema, ear disorders: deafness, gastrointestinal disorders, hepatobiliary disorders (cholestasis), nervous system disorders (dystonia, encephalopathy, hypertonia, hypotonia, muscle spasticity, myoclonus, optic neuritis), psychiatric disorders (acute psychosis, apathy, delirium, hypomania, neonatal agitation, psychotic disorders), respiratory disorders (laryngeal edema, pulmonary embolism, respiratory failure, stridor skin), and/or subcutaneous tissue disorders (angioedema, maculo-papular rash, pruritus, Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN))

Adverse Event Reporting: All AEs will be graded according to the following scale:

0 = No adverse events or within normal limits
1 = Mild; did not require treatment
2 = Moderate; resolved without treatment
3 = Severe; required professional medical attention
4 = Life-threatening or disabling

5 = Death

The PI will review AEs and notify the IRB of any changes needed to the protocol. The IRB will be notified within 24 hours of any SAE (grade 4 or 5) occurring at this site via their online system for reporting SAEs, and additional information will be forwarded to the IRB as it becomes available. AE's (grades 2-3) will be reported by the PI to the IRB within 10 working days. Mild AE's (grade 1) will be reported at the annual continuing review. In addition, all laboratory values outside of the normal range will be discussed with the study participant, and appropriate arrangements will be made for treatment, if necessary. The annual summary of all adverse events and any audit reports will be sent to the IRB at the time of continuing review.

If the data received during the course of the study on a study participant indicate they he or she may benefit from (other) medical intervention, then the appropriate recommendation(s) and/or consultation(s) will be made.

A Data and Safety Monitoring Board, chaired by David Carr MD (Professor of Medicine), and including Dominic Reeds, MD (Associate Professor of Medicine) will meet every 12 months with the PI, Co-Investigators, and the study research coordinator, to review study data, discuss any safety issues, and ensure compliance with the protocol.

Study Withdrawal/Discontinuation

Participants are free to withdraw at any time. Female participants who become pregnant will be removed from the study. If the participant's doctor decides that staying in the study places the participant at risk, she/he will be withdrawn. Any condition deemed by the study personnel to interfere with the participant's ability to complete the study will be cause for withdrawal. Subjects will be removed from study if they no longer meet the study criteria, if an SAE occurs, if they do not show up to study visits, or if the study is terminated.

Subjects that are removed or withdraw from the study will initiate the drug step-down protocol by decreasing their drug dose by 1000 mg for one week prior to stopping the drug completely.

No patient will be withdrawn from one part of the study without being removed from all study parts. Blood specimen collected from these subjects will be kept as the samples does not contain any personal identifier. The data and specimen collected until withdrawal from the study will be kept for future as these samples and data might still be useful. All data and samples will have an assigned identification number given to them which are NOT subject's personal identification. This will be explained at the time subjects sign the informed consent form.

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