INSULIN STIMULATED LACTATE PRODUCTION

by

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by

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It has been shown that Type 2 diabetic patients who undergo Roux-en-Y Gastric Bypass surgery (RYGB) show a decrease in muscle lactate production, liver glucose production and pancreatic insulin secretion. To explain these changes, it is hypothesized that gastric bypass surgery “removes” a block at pyruvate oxidation in both muscle and liver. A block at pyruvate oxidation prior to RYGB would cause pyruvate to accumulate in the muscle and its only pathway for disposal would be through export as lactate. After RYGB, pyruvate dehydrogenase would be activated, allowing pyruvate to be oxidized. Thus, lactate would not be exported from the muscle, reducing the availability of this gluconeogenic substrate. Elevated lactate levels drive glucose production. The rapid remission of diabetes after RYGB is accompanied by a reduction in blood lactate concentrations and therefore a reduction in fasting glucose concentration. This study was done to compare glucose and lactate levels in subjects 1-3 years post RYGB to age- and BMI-matched control subjects. Our hypothesis was that there will be no significant differences in glucose and lactate levels in subjects post-RYGB and control subjects, suggesting that RYGB causes these levels to return to normal. Each subject underwent an intravenous glucose tolerance test and blood samples were taken at baseline and 29 additional time points over the course of three hours. Each blood sample was analyzed for glucose and lactate values and the results between the two groups were compared.
Our results show that on average, lactate values in RYGB patients are lower than their age- and BMI-matched controls, suggesting that the surgery induces a lasting change in skeletal muscle metabolism, which enables the oxidation of glucose.
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INTRODUCTION

The obesity epidemic is one that has been no secret in the United States over the past decade. Over one third of the entire population of adults in the United States is obese. Obesity can lead to countless health problems such as cardiovascular disease, which is the leading cause of death in the United States, and Type 2 Diabetes.

Type 2 Diabetes is characterized by high glucose levels due to the body’s inability to produce and/or utilize insulin. If not taken care of properly, diabetes has the potential to cause many complications, including blindness, nerve damage, foot problems, and even death. As one of today’s biggest expenses in health care, it is imperative that research be done to find a cure for this disease that affects so many.

Current research supports the idea that gastric bypass surgery is an effective treatment for both obesity and Type 2 Diabetes. However, surgery is costly and opens doors for potential complications. In addition, the success of gastric bypass surgery is dependent upon the patient’s compliance to diet and exercise regimens as well as attending regular post-operative appointments.

The purpose of this research is to identify and understand the mechanisms that are affected during gastric bypass surgery resulting in the remission of Type 2 Diabetes. If these mechanisms can be identified, new medicines can be developed that replicate these changes and therefore cure Type 2 Diabetes without surgery.

This study specifically looks at the differences in glucose and lactate levels during an intravenous glucose tolerance test in subjects who underwent gastric bypass surgery in the past 1-3 years and their age- and BMI-matched controls. The glucose and lactate
levels will show us if the surgery restores insulin sensitivity to normal for 1-3 years post-surgery.

To explain the mechanism of these changes, it is hypothesized that gastric bypass removes a block at pyruvate oxidation in both muscle and liver. A block at pyruvate oxidation prior to gastric bypass surgery, and thus in Type 2 diabetics, would cause pyruvate to accumulate in the muscle and its only pathway for disposal would be through export as lactate. After the surgery, pyruvate dehydrogenase would be activated, allowing pyruvate to be oxidized. Thus, lactate would not be exported from the muscle, reducing the availability of this gluconeogenic substrate. Taking this mechanism into consideration, our hypothesis is that glucose and lactate levels in subjects 1-3 years post-RYGB will be restored to normal, and thus similar to healthy individuals who have never undergone the surgery.
REVIEW OF LITERATURE

Insulin Action in Skeletal Muscle

The hormone insulin has three target tissues in the body: liver, fat, and skeletal muscle\(^1\). For the purposes of this research we will focus on its role in skeletal muscle and how it contributes to the homeostasis of glucose levels in the body as a whole. Skeletal muscle is responsible for about 75% of the body’s disposal of glucose\(^2\). In normal, insulin-sensitive individuals, insulin is secreted from the beta cells of the pancreas in response to elevated blood glucose. As a simplified explanation of the complex molecular process, the insulin attaches to the “doors” of skeletal muscle cells and opens them, allowing glucose into the cell. This reduces the amount of glucose in the blood and increases the amount of glucose in skeletal muscle, which then uses this glucose for energy. A study by Zierath, et al. referred to this process as “insulin-stimulated glucose transport,” while a study by Idris, et al. referred to it as “insulin-mediated glucose uptake”\(^1,3\). Regardless of its name, this process requiring sensitivity to insulin is essential to maintaining appropriate levels of blood glucose and keeping the body at an appropriate balance.

Role in Obesity

It is widely known that there is a strong relationship between obesity and insulin resistance, which subsequently may contribute to the development of Type 2 Diabetes. Insulin resistance can be defined as a decrease in insulin-stimulated glucose transport and can be caused at the level of insulin signaling in various target tissues and adipocytes. It
has been found that in skeletal muscle of obese individuals, the expression of multiple insulin signaling molecules is reduced, although the exact mechanisms have not yet been discovered\textsuperscript{4-6}.

\textit{How Insulin Resistance is altered with Gastric Bypass}

In a study by Cazzo, et al., the majority of obese, insulin-resistant patients who underwent Roux-en-Y Gastric Bypass surgery showed improved insulin resistance and decreased fasting glucose within one year after surgery, which is consistent with the findings of other comparable studies\textsuperscript{7-8}. This improvement may be the result of altered secretion of gastrointestinal peptides in combination with weight loss\textsuperscript{9} but conclusive evidence for the mechanism improving insulin resistance remains unclear.
METHODOLOGY

Subjects

This study compared two different subject groups. The experimental group, also referred to as Group B, consisted of Caucasian females ages 31-58 that had undergone Roux-en-Y Gastric Bypass surgery within the past 1-3 years, were currently weight-stable and were apparently healthy. Potential subjects for this group were found in Vidant Medical Center and ECU Physicians’ Electronic Health Record system. The potential subjects were contacted and informed about the study and its requirements.

Subjects for the control group, also referred to as Group N, consisted of Caucasian females ages 30-53 that had never had any type of weight loss procedure, were weight-stable and were apparently healthy. To recruit these subjects flyers were posted around the community of Greenville in addition to a mass e-mail sent to all East Carolina University faculty and staff. Interested subjects were told to contact a researcher for more information.

If the potential subjects chose to participate it was verified that they met all inclusion criteria and prior to beginning the study they signed a consent form as well as a UMCIRB HIPAA Privacy Authorization form. Subjects were compensated $100 for their time and travel. A total of 10 subjects for group B and 7 subjects for group N completed the study.
**Protocol**

All subjects underwent a minimal model intravenous glucose tolerance test under specific protocol. Upon arrival, the subject’s weight was obtained after voiding. The weight was used to determine the amount of glucose and insulin to be administered during the minimal model with the following formulas:

**Glucose:** mls of 50% solution of glucose = body mass (kg) \( \times 0.3 \)g \( \times 2 \)

**Insulin:** body mass (kg) \( \times 0.025 \) U

A catheter at least 20 gauge was inserted into the brachiocephalic vein. In ideal conditions, one IV was started in each arm of the subject so that one arm was used for drug administration and one arm was used for lab draws. In some cases this was not realistic due to bad veins or poor blood flow. In all cases, however, two different IV’s were used to administer drugs and draw blood. 40 cc of blood were drawn prior to the administration of glucose for the performance of baseline lab assays. The calculated amount of 50% glucose solution was administered over one minute. Lab draws began at 2 minutes after the glucose administration and were repeated at minutes 3, 4, 5, 6, 8, 10, 12, 14, 16, and 19. At 20 minutes, the calculated dose of U-10 regular insulin was administered over one minute. 2 cc’s of blood were then drawn at the following time points: 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes.

After each lab draw, 1.5 mL of whole blood was immediately and gently transferred into a gray top tube (used for glucose and lactate analysis) using a P1000 pipette. The cap was secured and the tube was inverted 8 times and stored on ice. The remaining blood was then transferred into a purple top tube (used for insulin analysis) and the appropriate volume of protease inhibitor cocktail was immediately added.
depending on the volume of blood in the tube. The cap was secured and the tube was inverted 5 times and stored on ice. Every 15 minutes, all tubes were centrifuged at 2500 rpm for 12 minutes at 4 degrees Celsius. After spinning, 300 microliters of the plasmas were aliquoted into cryovials (3 cryovials for each time point) and were immediately placed into a freezer at -80 degrees Celsius where they remained until the data analysis.

Data Analysis

The plasma from the gray top tubes was analyzed for glucose and lactate values at each time point using the Beckman Coulter UniCel DxC 600i. The results were then analyzed using various methods including t-tests, area under the curve analysis, and software called MinMod Millennium.
RESULTS

Figure 1. Glucose vs. Time

![Glucose vs. Time Graph]

- • B Glucose
- △ N Glucose

Figure 2. Net Glucose Area Under the Curve

![Net Glucose Area Under the Curve Graph]
The glucose values and area under the curve analysis show that the surgery group (group B) has slightly higher glucose values throughout the duration of the glucose tolerance test and thus, a greater area under the curve.

**Figure 3. Lactate vs. Time**

![Lactate vs. Time](image)

**Figure 4. Net Lactate Area Under the Curve**

![Net Lactate AUC](image)
The lactate values and area under the curve analysis show that the surgery group (group B) have lower lactate values throughout the duration of the glucose tolerance test and thus, a smaller area under the curve.

Figure 5. Lactate Area Under the Curve Above Baseline

Figure 6. Lactate Area Under the Curve Below Baseline
These graphs show the area under the curve analysis for lactate both above baseline and below baseline. Above baseline, the surgery group (group B) has a smaller area under the curve and below baseline, group B has a larger area under the curve.
DISCUSSION

At fasting, glucose levels in both the surgery group (group B) and the non-surgery group (group N) are stable. This means that glucose production is equal to glucose utilization. Throughout the glucose tolerance test, the relative stability of glucose levels in both groups indicates that both groups utilize glucose at the rate that it is produced. For lactate, however, both groups show a rise in lactate levels followed by decrease to baseline in group N and below baseline in group B. The area under the curve above baseline shows that group N produces more lactate than group B. The area under the curve below baseline shows that group B utilizes more lactate than group N. This excess utilization tells us that group B has a lot less net lactate production compared to group N.
CONCLUSION

These results are consistent with what is seen in RYGB patients one week after surgery, suggesting that the lactate-producing mechanism that is altered during the surgery is still effective 1-3 years post-surgery. In comparison with our hypothesis that lactate levels in RYGB patients 1-3 years post-surgery would be similar to healthy individuals, the results show that the surgery has a lasting effect that improves pyruvate oxidation to the point where it is even more effective than that of healthy individuals. This supports the idea that improved pyruvate oxidation as a result of gastric bypass surgery is one of the mechanisms contributing to the remission of Type 2 Diabetes. To further this research, other mechanisms altered during RYGB should be investigated to determine which alterations contribute to the remission of Type 2 Diabetes and should therefore be considered during the development of medicines to cure the disease. Additionally, more longitudinal studies should be done to further verify conclusions from this study as well as examine factors contributing to the long-term success or failure of RYGB’s remission of Type 2 Diabetes.
REFERENCES


APPENDIX A

Consent Form

Study ID: UMCIRB 11-001363  Date Approved: 5/28/2014  Expiration Date: 5/27/2015

Reversal of Diabetes by Vertical Sleeve Gastrectomy (VSG)

Consent to Participate in Research that is Greater than Minimal Risk Information to Consider Before Taking Part in This Research

Title of Research Study: Reversal of Diabetes by Vertical Sleeve Gastrectomy (VSG)

Principal Investigator: Walter J. Pories, MD
Institution/Department or Division: The Brody School of Medicine at East Carolina University
Address: 600 Moye Blvd. Brody 4W48, Greenville, NC 27834
Telephone #: 252-744-3290

Researchers at East Carolina University (ECU) study diseases, health problems, environmental problems, behavior problems and the human condition. Our goal is to try to find better ways to improve the lives of you and others. To do this, we need the help of people who are willing to take part in research.

The person who is in charge of this research is called the Principal Investigator. The Principal Investigator may have other research staff members who will perform some of the procedures.

The person explaining the research to you may be someone other than the Principal Investigator. The study coordinator may be asking you to take part in this study.

You may have questions that this form does not answer. If you do have questions, feel free to ask the person explaining the study, as you go along. You may have questions later and you should ask those questions, as you think of them. There is no time limit for asking about this research.

You do not have to take part in this research. Take your time and think about the information that is provided. If you want, have a friend or family member go over this form with you before you decide. It is up to you. If you choose to be in the study, then you should sign the form when you are comfortable that you understand the information provided below. If you do not want to take part in the study, you should not sign this form. That decision is yours and it is okay to decide not to volunteer.

This form explains why this research is being done, what will happen during the research, and what you will need to do if you decide to volunteer to take part in this research.

Why is this research being done?
The purpose of this research study is to study the separate effects of food restriction and bypass of GI hormones by surgery. We are asking you to take part in this research. However, the decision is yours to make. By doing this research, we hope to learn whether the reversal of the diabetes is due to the fact that the surgery bypassed a part of the intestines that might be secreting important hormones (GI hormones).
Why am I being invited to take part in this research?

Group A:
You are being invited to take part in this research because you are having a gastric sleeve procedure. If you volunteer to take part in this study, you will be one of about 16 people to do so.

Groups B and C:
Or additionally, you may be asked to take part in this study as part of 2 “control” groups. (for comparison purposes with the original set of subjects)

Group B:
You may be part of a non-surgery group who is weight stable and matched in age and BMI (weight) to the gastric sleeve procedure group. There will be 10 subjects enrolled in this group.

Group C:
Or if you have had gastric bypass surgery 12 months ago, and are weight stable and matched in age and BMI to the gastric sleeve group. There will be 10 subjects enrolled in this group.

Group D:
Or, as a result of information being learned from data collected from the original sleeve surgery group, if you are scheduled for gastric bypass surgery we may ask you to take part in this research. The study plan would ask that you have a minimal model and microdialysis procedure 1 week before surgery and then again 1 week post surgery. There will be 10 subjects enrolled in this group.

With all groups enrolled, you will be one of about 46 subjects enrolled in this study.

Are there reasons I should not take part in this research?
If you are scheduled to have a repeat weight loss surgery we will not want you to participate in this research project. Also, if you are diabetic and are not able to safely withhold your diabetic medication for 48 hours prior to the study testing. The investigator will determine if you cannot safely withhold diabetic medications and will not include you in the study. If you are taking a certain kind of diabetes medication called a “TZD” (thiazolidinediones) we will not include you in the study as this medicine can affect glucose metabolism. If you are taking thiazide diuretics and are not able to safely withhold the medicine for 48 hours prior to the study, we will not include you in the study.

What other choices do I have if I do not take part in this research?
You have the choice of not taking part in this research study.

Where is the research going to take place and how long will it last?
The research procedures for all groups will be conducted at Vidant Medical Center TA-244.

Group A:
For the vertical sleeve surgery group, you will need to come to Vidant Medical Center TA-244 seven times during the study. Each of the visits will take about 3.5 hours. The total amount of time you will be asked to volunteer for this study is approximately 24 1/2 hours over the next 12 (+) months.

Group B:
For the non-surgery weight stable group, you will be asked to come to the Vidant TA-244 for one study visit lasting 3.5 hours. This is the total amount of time you will be asked to volunteer.
Title of Study: Reversal of Diabetes by Vertical Sleeve Gastrostomy (VSG)

Group C:
For the gastric bypass surgery group (post-surgery 12 months or greater) weight stable and matched in age and BMI to the gastric sleeve group, you will be asked to come to the Vidant TA-244 for one study visit lasting 3.5 hours. This is the total amount of time you will be asked to volunteer.

Group D:
For the group scheduled for gastric bypass surgery you will be asked to come to the Vidant TA-244 for 2 study visits, each lasting 5-5 ½ hours for a total of 10-11 hours.

What will I be asked to do?

Group A:
Vertical Sleeve Group:
You will be asked to participate in 7 study visits (in addition to your surgical procedure). These visits will be scheduled as follows:
1) Preoperatively (1-3 weeks before surgery with 2 visits at your convenience, occurring no closer than 2 days apart.
2) The week after surgery with 2 research visits occurring no closer than 2 days apart.
3) 3 months after surgery with 2 research visit, no closer than 2 days apart.
4) 12 months (plus) post-surgery visit.

At each of the first 3 study time points the following 2 tests will be done, an IV glucose Tolerance (minimal model) test lasting 3 hours, and a meal challenge test lasting 3 hours. At the 4th and final visit, a single IV glucose tolerance test will be done lasting 3 hours. The IV Glucose tolerance test is performed to determine how your body responds to insulin. A nurse will put a catheter into your arm vein and glucose and insulin will be administered to you. Blood will be taken from your vein over a 3 hour period while you are resting in bed. The meal challenge test is performed to determine how you respond to a liquid meal taken by mouth. In this test, you will be asked to drink a liquid meal within a 10-15 minute time period. Blood will then be taken from your arm vein over a 3 hour time period while you are resting in bed.

On the evening before your research visits you will be given a liquid meal to drink instead of eating dinner. Then you will have nothing else to eat until you are finished with your testing the next day. You may drink water during this time. All gastric sleeve subjects will have a hemoglobin A1C as part of their initial test or study visit. The result will be used to place you into the correct study grouping. (Diabetic or non-diabetic) This test will require an additional 6 milliliters of blood drawn. This amount is about the same as 1 teaspoon. During the gastric sleeve procedure a portion of the stomach is removed and discarded. For the purposes of this study we will be collecting tissue from this discarded portion of the stomach. Histology (study of tissue structure) and mRNA (a type of molecule important for the manufacturing of proteins) will be performed on the collected stomach tissue.

At 12 plus months post-surgery you will be asked to return to the study center (Vidant TA-244) for a single visit for a minimal model test. This will be the 7th and final visit.

What will I be asked to do?

Group B:
Non-Surgery Weight Stable Group:
For the non-surgery weight stable group, you will be asked to come to the Vidant TA-244 for a single study visit for a minimal model test. This is the only visit.
Title of Study: Reversal of Diabetes by Vertical Sleeve Gastrectomy (VSG)

What will I be asked to do?

Group C:
Gastric Bypass Surgery Group: (post-surgery 12 months or greater)
For the gastric bypass surgery group (post-surgery 12 months or greater) weight stable and matched in age and BMI to the gastric sleeve group, you will be asked to come to the Vidant TA-244 for a single study visit for a minimal model test. This is the only visit.

Group D:
What will I be asked to do?
For the group scheduled for gastric bypass surgery you will be asked to come to the Vidant TA-244 for 2 study visits consisting of a minimal model and a microdialysis test, 1 week prior to surgery and then 1 week post surgery. These are the only 2 visits.
The microdialysis and minimal model procedures will take place the morning after an overnight fast from midnight.

The microdialysis procedure will be performed under local anesthesia by Dr. Robert Hickner, PhD. Dr. Hickner will insert all probes using sterile techniques. The microdialysis probes are thin plastic tubes, 0.5 millimeters wide (the width of a small needle) and 30 millimeters long (just over an inch). A small amount of anesthetic (lidocaine) will be injected under the skin of your thigh to reduce the pain you may experience when the probes are inserted.
Up to three probes will be placed in the muscle of the thigh, and up to three probes will be placed in the abdominal fat just underneath your skin. The probes will be perfused with a small amount of ethanol, as well as compounds (Amplex UltraRed, Horseradish peroxidase and superoxide dismutase) we use to detect substances in your body called reactive oxygen species. This test will take about 5 hours to complete.

The IV Glucose tolerance (minimal model) test is performed to determine how your body responds to insulin. A nurse will put a catheter into your arm vein and glucose and insulin will be administered to you. Blood will be taken from your vein over a 3 hour period while you are resting in bed.

Every study subject’s blood will be stored in a secure freezer until it is analyzed for different gut hormone levels as well as insulin and glucose levels. Any investigator who utilizes this blood will not know your identity but they will be given information about you, such as gender, sex, age, weight, height, race, activity level, disease(s), medication(s), and concentrations of blood substances (such as glucose and insulin).

We ask your permission to store any leftover blood samples in a secure ECU freezer for an indefinite time. Any testing done on this blood would be for research purposes only. The results of these tests would not be sent to you or your doctor, and will not be used in planning your care. You will not be able to find out the results of these tests even if you ask for them. You will not be paid for blood used for banking or additional research testing. Although it is possible that future research done on your blood may help develop something that is commercially valuable, you will not receive payment from any commercial activity that results from research related to your blood sample.

☐ Yes, I will donate my blood without restriction on what type of future research it may be used for.

☐ No, I will not donate my blood for banking and/or future research use.

What possible harms or discomforts might I experience if I take part in the research?
Title of Study: Reversal of Diabetes by Vertical Sleeve Gastrectomy (VSG)

There is the possibility of risks (the chance of harm) when taking part in research. We know about the following risks or discomforts you may experience if you choose to volunteer for this study. These are called side effects. The following side effects are known to occur in some people:
Possible side effects from blood draws and intravenous catheters are fainting, bruising, soreness, and tenderness at the needle site and, on rare occasions, infection. Precautions will be taken to reduce the likelihood of these difficulties. Although your body naturally replaces lost blood when it has adequate iron, temporary mild anemia may occur until your body has replaced blood taken from you. This could cause symptoms of mild fatigue or shortness of breath with exertion. There is a risk for hyperglycemia and hypoglycemia during the performance of these tests.

Possible side effects from glucose infusion may be headache, warmth in the trunk, a funny taste in the back of the mouth, a sensation moving up and burning in the arm.

The approximate amount of blood that will be collected with each glucose tolerance test is 92 milliliters, comparable to 3 ounces of fluid. The approximate amount of blood that will be collected with each meal challenge test is 39 milliliters. This amount is comparable to one ounce of fluid. These amounts are considered safe to withdraw for testing, but we will monitor you for any problems, such as weakness, dizziness or shortness of breath.

All vertical sleeve (pre-surgery) subjects will have a hemoglobin A1C as part of their initial test or study visit. The result will be used to place you into the correct study grouping. (Diabetic or non-diabetic) This test will require an additional 6 milliliters of blood drawn. This amount is about the same as 1 teaspoon.

Also for the pre-surgery vertical sleeve subjects and for the pre-surgery gastric bypass subjects, your hemoglobin and hematocrit (blood counts) will be assessed as part of your standard pre-surgery blood work. If your hematocrit is noted to be less than 35%, the study team will consult your surgeon to discuss his opinion about your enrollment into the study.

If you are a diabetic gastric sleeve subject, you will be asked to withhold your anti-diabetic medication for 48 hours prior to your meal tests; this does not usually pose a problem because your food intake during this period is also limited. Your physician will be asked if it is safe for you to discontinue your diabetic medication for 48 hours prior to the study testing. We will require that you monitor your blood glucose by using your home Accu-Check 4 times daily at meal times and at bedtime. You are to notify the study coordinator, Rita Bowden, via pager number 252-413-5506, for any elevated results over 150 mg/dL. We also want you to report any symptoms of elevated blood glucose such as feeling dizzy, weak, or sweaty. The study coordinator will be in touch with your physician to report any problems you might experience while off diabetic medication.

If you are taking thiazide diuretics we will ask you to withhold them for 48 hours prior to study testing. Withholding this medicine could result in a temporary rise in blood pressure, or an increase in edema (swelling) in the body. We will ask your doctor if he feels it is safe for you to hold the medication.

For the pre- and post gastric bypass surgery group having the microdialysis procedure, the following risks are possible:

Insertion of the microdialysis probe can be associated with mild discomfort, similar to that experienced during an intramuscular injection. You may feel discomfort from the substances (for example, alcohol) pumped through the microdialysis probe. Risks associated with this procedure are small, and include hematoma (swelling and bruising) and infection. In previous studies performed by R. Hickner, involving the use of more than 1000 microdialysis probes, no such complications have arisen. There is also a small risk (2 in 1000) of the microdialysis membrane detaching from the microdialysis probe and remaining in your muscle or fat. This membrane is too small and clear to see after detachment from the probe, and therefore remains in the tissue. The membrane is sterile and compatible with the body tissues.
The risk of probe detachment will be minimized by asking you avoid sudden movements during these experiments that might break the membrane.

We will do everything possible to keep you from being harmed. There may be other risks or side effects that occur which we do not know about at this time.

It is important for you to tell us as quickly as possible if you experience a side effect.

Are there any reasons you might take me out of the research?
During the study, information about this research may become available that would be important to you. This includes information that, once learned, might cause you to change your mind about wanting to be in the study. We will tell you as soon as we can. This might include information about the side effects that are caused by taking part in this study. If that happens, we can tell you about these new side effects and let you decide whether you want to continue to take part in the research.

There may be reasons we will need to take you out of the study, even if you want to stay in. We may find out that it is not safe for you to stay in the study. It may be that the side effects are so severe that we need to stop the study or take you out of the study to reduce your risk of harm. If we find that the research might harm you or that it is not providing enough of a benefit to justify the risks you are taking, we will remove you from the study. We may also find that you cannot come for your study visit(s) as scheduled. If this found to be true, we will need to take you out of the study.

What are the possible benefits I may experience from taking part in this research?
We do not know if you will get any benefits by taking part in this study. This research should help us learn more about diabetes and the gastric sleeve procedure’s role in eliminating the disease.

There may be no personal benefit from your participation but the information gained by doing this research may help others in the future.

Will I be paid for taking part in this research?
We will pay you for the time you volunteer while being in this study:

Group A:
Vertical sleeve subjects will be compensated $100.00 per completed study test (of which there are seven) for a total possible amount of $700.00. In the event you receive more than $600 in compensation within a calendar year, you will receive an IRS 1099 form so that you can report the compensation as earned income.

Group B:
The non-surgery weight stable group will be compensated $100.00 to complete one minimal model.

Group C:
The 12 months post-surgery gastric bypass group will be compensated $100.00 to complete one minimal model.

Group D:
The group scheduled for gastric bypass surgery will be compensated $200.00 to complete the minimal model/microdialysis testing before and after surgery for a total of $400.00.
Title of Study: Reversal of Diabetes by Vertical Sleeve Gastrectomy (VSG)

Dr. Walter J. Pories, the primary investigator, is utilizing research money to compensate you for your participation. Subjects are paid at the end of all testing for each single test that was completed. You will not be compensated for any financial benefits gained from the discoveries made from this study, even though they may be directly derived from your blood samples. There will be no costs to you or your insurance provider for participating in this study.

What will it cost me to take part in this research?

You will not be charged for any of the expenses related to any of the experimental trials. Participation in this project is voluntary. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. If the research is published, your identity will be protected, as well as will be the confidentiality of your medical records. You will not benefit financially from any future potential benefits of ECU.

Who will know that I took part in this research and learn personal information about me?

To do this research, ECU and the people and organizations listed below may know that you took part in this research and may see information about you that is normally kept private. With your permission, these people may use your private information to do this research:

- The research team, including Dr. Walter Pories MD the Principal Investigator, study coordinator, research nurses, and all other research staff.
- The ECU University & Medical Center Institutional Review Board (UMCIRB) and the staff who have responsibility for overseeing your welfare during this research, and other ECU office staff who oversee this research.

How will you keep the information you collect about me secure and how long will you keep it?

Information about you will be used and released in such a way that will protect your identity as much as possible. The individual/agencies who may receive health information about you also agree to keep this information confidential. However, there is always a chance that your information could be shared in a way that would no longer be protected. Therefore, although we take precautions to protect your information, confidentiality cannot be absolutely guaranteed. You will not be informed of any results from these research tests.

We are asking your permission to share your health information related to this study with the scientific investigators who are currently or in the future may be engaged in collaborative research with East Carolina’s Metabolic Institute. Your identity will not be linked with the clinical information. If you want us to stop using your information, your blood specimen will be removed from the ECU research bank, but your standard medical care and benefits will not be affected. You can withdraw your consent at any time by calling the study nurse (Rita Bowden RN/252-744-5249) or the study doctor at 252-744-3290, and then sending a written letter for your study file to: Walter Pories MD 600 Moye Boulevard IL-09, Greenville NC 27834.

If we need to share information with other individuals/agencies, we will ask your permission again in writing. At any time, you can ask us to tell you what information has been shared and with whom. However, you cannot have access to your information until the study is over.

Research information continues to be looked at after the study is finished so it is difficult to say when the information will stop. Currently, there is not an expiration date for the use and disclosure of your information for this study.
Title of Study: Reversal of Diabetes by Vertical Sleeve Gastrectomy (VSG)

What if I decide I do not want to continue this research?
Participating in this study is voluntary. If you decide not to be in this research after it has already started, you may stop at any time. You will not be penalized or criticized for stopping. You will not lose any benefits that you should normally receive. You can withdraw your consent at any time by calling the study nurse (Rita Bowden RN/252-744-5249) or the study doctor at 252-744-3290, and then sending a written letter for your study file to: Walter Pories MD 600 Moye Boulevard IL-09, Greenville NC 27834.

What if I get sick or hurt while I am in this research?
If you need emergency care:
Call 911 for help. It is important that you tell the doctors, the hospital or emergency room staff that you are taking part in a research study and the name of the Principal Investigator. If possible, take a copy of this consent form with you when you go.

Call the principal investigator as soon as you can. He/she needs to know that you are hurt or ill. Call Dr. Walter Pories M.D. at 252-744-3290 or Rita Bowden RN at 252-744-5249.

If you do NOT need emergency care, but have been hurt or get sick:
Contact Dr. Walter Pories at 252-744-3290.
Call the principal investigator as soon as you can. As necessary, go to your regular doctor. It is important that you tell your regular doctor that you are participating in a research study. If possible, take a copy of this consent form with you when you go.

If you are harmed while taking part in this study:
If you believe you have been hurt or if you get sick because of something that is done during the study, you should call Dr. Walter Pories M.D. at 252-744-3290 immediately. There are procedures in place to help attend to your injuries or provide care for you. Costs associated with this care will be billed in the ordinary manner, to you or your insurance company. However, some insurance companies will not pay bills that are related to research costs. You should check with your insurance about this. Medical costs that result from research-related harm may also not qualify for payments through Medicare, or Medicaid. You should talk to the Principal Investigator about this, if you have concerns.

Who should I contact if I have questions?
The people conducting this study will be available to answer any questions concerning this research, now or in the future. You may contact the Principal Investigator, D. Walter Pories MD at 252-744-3290 (days) or 252-236-1709 (nights and weekends).

If you have questions about your rights as someone taking part in research, you may call the ECU Office for Human Research Integrity (OHR) at phone number 252-744-2914 (days). If you would like to report a complaint or concern about this research study, you may call the Director of OHR, at 252-744-1971.

Is there anything else I should know?
If additional information comes available that might affect your decision to participate, we will share this information with you as soon as it becomes available.

I have decided I want to take part in this research. What should I do now?
The person obtaining informed consent will ask you to read the following and if you agree, you should sign this form:

- I have read (or had read to me) all of the above information.
- I have had an opportunity to ask questions about things in this research I did not understand and have received satisfactory answers.
- I understand that I can stop taking part in this study at any time.
- By signing this informed consent form, I am not giving up any of my rights.
- I have been given a copy of this consent document, and it is mine to keep.

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<tr>
<th>Participant's Name (PRINT)</th>
<th>Signature</th>
<th>Date</th>
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**Person Obtaining Informed Consent:** I have conducted the initial informed consent process. I have orally reviewed the contents of the consent document with the person who has signed above, and answered all of the person’s questions about the research.

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<tr>
<th>Person Obtaining Consent (PRINT)</th>
<th>Signature</th>
<th>Date</th>
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<thead>
<tr>
<th>Principal Investigator (PRINT)</th>
<th>Signature</th>
<th>Date</th>
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(If other than person obtaining informed consent.)
APPENDIX B

HIPAA Form

Study ID: UMCIRB 11-001363  Date Approved: 6/13/2012  Does Not Expire.

UMCIRB HIPAA Privacy Authorization

The Brody School of Medicine (BSOM)/Vidant Medical Center (VMC): Research Participant Authorization to Use and Disclose Protected Health Information for Storing Tissues/Samples/Specimens for Future Research

For use only with the research consent form for: UMCIRB#: 11-001363

PI: Walter Pories MD

Study Title: “Reversal of Diabetes by Vertical Sleeve Gastrectomy”

When taking part in research, protected health information (PHI) is collected, used, and shared with others who are involved in the research. Federal laws require that researchers and health care providers protect your PHI. Also, federal laws require that we get your permission to use collected PHI for the research. This permission is called authorization.

In order to complete the secondary research project in which you have decided to take part, we need to collect and use some of your PHI as described below.

What types of protected health information (PHI) about me will be used or disclosed?

[ ] BSOM/VMC Billing records  [ ] VMC medical records (in and out patient)
[ ] BSOM/VMC Mental Health records  [ ] VMC/BSOM lab, pathology and/or radiology results
[ ] BSOM Physician/clinic records  [ ] PHI previously collected for research purposes
[ ] Other:  [ ] Samples/tissue/specimens collected as part of the main study

Who will use or disclose my PHI?

[ ] Principal Investigator
[ ] Other members of the research team
[ ] Other providers involved in your care during research procedures, outpatient/inpatient stays during which research is being performed, or physician office visits during which research is being performed.
Location where research will be conducted

The members of the research team will conduct the research study at:
☐ East Carolina University (ECU) ☐ VMC ☒ ECU & VMC ☐ Other

Who will receive my PHI?

☐ Sponsor or other funding source to provide oversight for entire research project
☒ Research investigators to conduct and oversee the research project
☐ Research team members to participate in the various research activities
☐ FDA or other regulatory agencies to provide regulatory oversight
☐ UMCIRB to provide continuing review of the research project
☐ Institutional officials in connection with duties for monitoring research activity
☐ Researchers at other sites to participate in the research when more than one research site is involved
☒ Other Staff and Faculty at the ECU Metabolic Clinic

We will share only the PHI listed above with the individuals/agencies listed above. If we need to share other PHI or if we need to send PHI to other individuals/agencies not listed above, we will ask for your permission in writing again.

How my PHI may be released to others:

The BSOM and VMC are required under law to protect your PHI. However, those individuals or agencies who receive your PHI may not be required by the Federal privacy laws to protect it and may share your PHI with others without your permission, if permitted by the laws governing them.

What if I do not sign this form?

Your tissue/samples/specimens will not be stored for future research. You can still participate in the main part of the study without agreeing to this use of your tissues/samples/specimens without penalty.

How may I revoke (take back or withdraw) my authorization?

You have the right to stop sharing your PHI. To revoke (or take back) your authorization, you must give the investigator your request to revoke (or take back) your authorization in writing. If you want us to stop collecting your PHI for future research, your samples/tissue/specimens will be removed from storage. This will not affect your ability to receive standard medical care or any other benefits for which you are entitled to receive. PHI used for future research prior to revoking (or taking back) your Authorization will continue to be used for the purposes of that research study. Also, the FDA (if involved with your study) can look at your PHI related to the study even if you withdraw this authorization.
Restrictions on access to my PHI:

You may not be able to see your PHI in your medical record related to this study until the study is complete. If it is necessary for your care, your PHI will be provided to you or your physician.

How long may the PHI about me be used or disclosed for this study?
Research information continues to be looked at after the study is finished so it is difficult to say when use of your PHI will stop. There is not an expiration date for this authorization to use and disclose your PHI for this study.

If you have questions about the sharing of PHI related to this research study, call the principal investigator, Walter Pories MD at phone number 252-744-3290. Also, you may telephone the University and Medical Center Institutional Review Board at 252-744-2914. In addition, if you have concerns about confidentiality and privacy rights, you may phone the Privacy Officer at Vidant Medical Center at 252-847-6545 or the Privacy Officer at East Carolina University at 252-744-5200.

Authorization

To authorize the use and disclosure of your PHI for future research in the way that has been described in this form, please sign below and date when you signed this form. A signed copy of this Authorization will be given to you for your records.

<table>
<thead>
<tr>
<th>Participant’s Name (print)</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Authorized Representative Name (print)</td>
<td>Relationship</td>
<td>Signature</td>
</tr>
<tr>
<td>Person Obtaining Authorization</td>
<td>Signature</td>
<td>Date</td>
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APPENDIX C

Non-Surgery Group Flyer

The Bariatric Surgery Research Group at East Carolina University (Brody School of Medicine) is conducting a research study to better understand diabetes.

If you are a Caucasian woman 40-55 years of age, with a BMI ranging from 22 to 32, at a stable weight, and have never had a weight loss surgery procedure, please call our research coordinator for more information.

Enrolled participants will be reimbursed for time and travel.

Research Coordinator:

Jordan Griffin: griffinj11@students.ecu.edu; 252-813-4860