HRP-503B – BIOMEDICAL RESEARCH
PROTOCOL(2017-1)

Protocol Title: The effect of lactation on insulin sensitivity and lipolysis in women

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Clinicaltrials.gov Registration #: NCT04146909

SECTION I: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested.
   Aim 1: Does lactation improve insulin sensitivity and increase lipolysis in women?
   In this Aim, we will assess whether lactation amongst women with a history of GDM will improve whole body insulin sensitivity as well as increase whole body lipid turnover by using hyperinsulinemic-euglycemic clamps combined with [6,6-2H]-glucose and [2H5]-glycerol infusions. These stable, non-radiating isotopes have been used extensively and safely to study the mechanisms of insulin resistance amongst non-pregnant as well as pregnant humans; however, these techniques have not previously been applied to the study of insulin sensitivity during lactation. Our goals will be two-fold: 1) to determine whether lactation induces similar changes in insulin sensitivity in humans as in mice and 2) to pilot the feasibility of performing larger, more detailed metabolic studies in this population of women post-partum.

   Aim 2: Retrospective chart review of women with a history of GDM who had an OGTT 6-8 weeks post-partum as part of standard of care at Yale Medicine's Maternal-Fetal Medicine Clinic.

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities.
   5 years

3. **Background:** Describe the background information that led to the plan for this project. Provide references to support the expectation of obtaining useful scientific data.
   Our premise is that lactation protects against the development of Type 2 Diabetes Mellitus (T2D) by mobilizing tissue triglycerides (TGs) and fully reversing the insulin resistance (IR) of pregnancy. A growing body of epidemiological data has indicated that nursing protects against T2D in all women but particularly amongst women with gestational diabetes mellitus (GDM) (1-8). However, no studies have investigated the detailed molecular mechanisms underlying these beneficial effects of lactation. Many studies have

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demonstrated the importance of intracellular tissue TGs in the pathophysiology of IR and T2D (9). Our preliminary animal studies suggest that lactation lowers liver TG content in post-partum mice. Therefore, we hypothesize that lactation mobilizes tissue lipids post-pregnancy, which, in turn, improves insulin sensitivity. The goal of this study will be to investigate the mechanisms by which lactation prevents diabetes. Given the continued increase in T2D in the US and the world (10-12), understanding how breastfeeding improves the metabolic health of women will have potentially wide-ranging health benefits.

Nursing and Diabetes Risk. A growing number of epidemiological studies have reported a strong inverse relationship between lactation and the risk of developing T2D (1-8,13-16). The protective effects of lactation hold for all women but are particularly striking in those with a prior history of GDM (2,3,6,8,14). Several large cohort studies have shown that lactation reduced the risk of developing metabolic syndrome and/or T2D and that the degree of risk reduction increased with longer durations of breastfeeding (15,16). These initial cross-sectional studies relied on a self-report of diabetes and did not correct for potentially confounding differences in pre-pregnancy metabolic parameters, weight changes or life-style that might co-segregate with diabetes risk as well as the likelihood of successfully nursing ("reverse causation"). However, more recent prospective studies have addressed these concerns. The SWIFT study demonstrated that increasing lactation intensity and duration decreased the risk of subsequent biochemically defined diabetes by up to 57% over two years post-partum in women with previous GDM (4). Similarly, the 30-year follow up of the Cardia study demonstrated that lactation duration/intensity was associated a graded 25%-47% reduction in subsequent long-term risk of biochemically defined T2D in all women (5). Importantly, both of these studies controlled for confounding factors such as antecedent metabolic or life-style differences and both found that T2D risk reduction was independent of weight loss. Finally, Bajaj and colleagues followed a cohort of women over 3 years and showed that nursing for 12 months or more reduced the risk of prediabetes or T2D by 67% and correlated with persistent improvements in maternal insulin sensitivity (13). Therefore, lactation confers strong, independent, dose-dependent and long-lasting protection against T2D in parous women.

Maternal Metabolism during Pregnancy and Lactation. Pregnancy is associated with alterations in fuel metabolism that promote energy storage as fat. In early pregnancy, enhanced tissue insulin sensitivity coupled with elevated circulating insulin levels promotes lipogenesis and adipocyte fat storage (17-19). Pregnancy also stimulates hyperphagia and increased food intake contributes to maternal fat accumulation (20). As pregnancy advances, the initial insulin sensitivity transitions into increasing peripheral IR, which shunts nutrients and energy away from maternal tissues to the fetus. Increased maternal IR leads to increased fasting and post-prandial glucose levels, elevated TGs, increased hepatic gluconeogenesis, elevated rates of systemic lipolysis, and increased insulin secretion (21). During the latter part of pregnancy, decreased adipocyte lipoprotein lipase activity has been shown to reduce the uptake of TGs into maternal fat (20). Finally, as breast epithelial cells proliferate and differentiate during mid-pregnancy, they develop prominent cytoplasmic lipid droplets (22), resulting in significant storage of TGs in the mammary gland.
The onset of lactation represents a shift to energy export via milk production. Milk fat, in particular, is an important source of energy for neonatal growth and lactating mice export up to 32 grams of TGs (approximately the weight of a female mouse) during 20 days of lactation (23). Three sources contribute to milk lipid: dietary fat, fatty acids mobilized from maternal stores, and de novo lipogenesis by mammary epithelial cells (23,24). As a result, lactating mothers respond to the metabolic demands of milk fat production through a combination of increased food consumption, the mobilization of stored TGs, and increased hepatic glucose production to supply substrates for de novo TG synthesis by mammary epithelial cells.

Insulin Resistance and Type 2 Diabetes Mellitus. In addition to pregnancy, IR is the earliest abnormality defined in patients at risk of T2D, typically preceding the onset of diabetes, sometimes for long periods of

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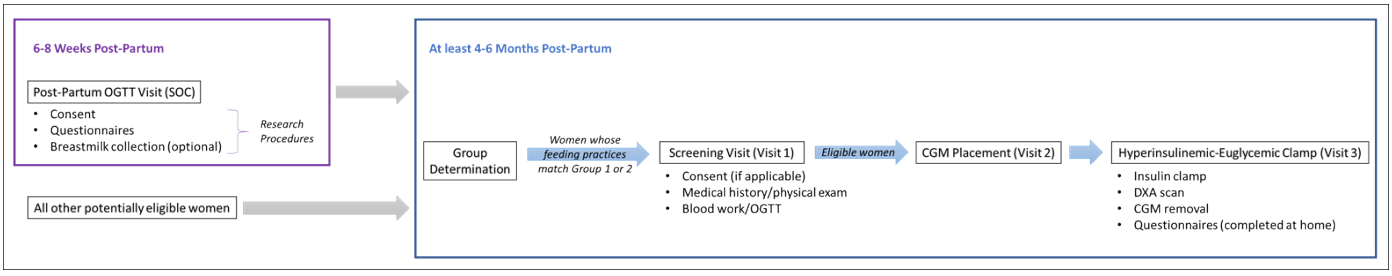
time as part of a constellation of abnormalities known as "the metabolic syndrome" (25-29). Hyperglycemia only occurs when pancreatic beta-cells can no longer sustain the level of hyperinsulinemia required to offset the defects in insulin action that define IR (9). One of the major risk factors for T2D is overnutrition and obesity, (especially increased visceral adipose tissue), which is associated with elevated levels of circulating TGs and non-esterified free fatty acids (NEFAs) as well as the accumulation of TGs within hepatocytes and myocytes (9,26,27). Several studies have shown that intrahepatic and intramyocyte lipid accumulation is more predictive of IR than total body or visceral adiposity (9,29-35). Intracellular TG accumulation, in turn, is associated with increased cellular diacylglycerol (DAG) and ceramide levels, activation of novel protein kinase C (nPKC) isoforms and phosphorylation of the insulin receptor (at Thr1160), which inhibits its kinase activity (9,36-42). These findings provide a potential mechanistic framework for understanding how intracellular lipid accumulation may cause IR and lead to the development of T2D. It is unknown whether similar changes in intracellular TGs, DAGs and/or phosphorylation of the insulin receptor contribute to changes in insulin sensitivity during reproductive cycles.

While accumulating evidence demonstrates that breastfeeding reduces the risk of developing T2D, the mechanisms mediating this risk reduction are unknown. We propose that the reduction in risk of T2D is a consequence of changes in fuel and energy metabolism associated with the metabolic demands of milk production. Decreases in plasma glucose and insulin levels during lactation likely contribute to lipid mobilization from maternal stores, allowing NEFAs to be redirected from peripheral tissues to the lactating mammary gland in order to support milk TG secretion (43,44). Thus, our working hypothesis is that lactation mobilizes lipids from maternal tissues to be used for milk secretion and, in turn, this improves insulin sensitivity in women who lactate. We hypothesize that those who do not lactate have persistent pregnancy-related intracellular lipid storage and IR, increasing their long-term risk of T2D.

4. **Research Plan:** Summarize the study design and research procedures using non-technical language that can be readily understood by someone outside the discipline. **Be sure to distinguish between standard of care vs. research procedures when applicable, and include any flowcharts of visits specifying their individual times and lengths**. Describe the setting in which the research will take place.

Aim 1: Two groups of 16 women with a history of GDM will be recruited for participation. Group 1 will consist of women who exclusively or mostly breast-fed for at least 4-6 months (< 6 ounces of formula/24 hours at 6-9 weeks of delivery)[1] and who delivered within the past 18 months; Group 2 will consist of women who exclusively or mostly formula-fed (no breastfeeding or < 3 weeks of breastfeeding)[1] and who delivered within the past 18 months.



Post-partum OGTT visit (standard of care): 6-8 weeks after delivery, patients from the Yale Medicine's Maternal-Fetal Medicine Clinic with a history of GDM during pregnancy undergo an OGTT as part of standard of care. At this visit, they may be approached by research staff about participating in this study. If they are interested in participating, they will sign the consent form at this visit and they will then be

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asked to complete questionnaires assessing their infant feeding practices, including, but not limited to, overall experience (sources of support, barriers/difficulties faced), frequency/number of feeds, and type of feeds (breastfeeding, expressed breastmilk, formula). Women who are lactating will be given the option to provide us with 2-4 mL of expressed breastmilk, which will be kept frozen and its metabolite content will be studied, including milk lipids, protein, lactose and nucleic acids. The breastmilk will be stored for the duration of the study and will not be shared.

Approximately 8 weeks later, these women will be contacted by research staff and asked about their infant feeding practices. If their feeding practices match the description of Group 1 or Group 2, they will be invited to return for Visit 1, Visit 2 and Visit 3 (described below).

Screening visit (Visit 1): For women who did not attend the post-partum OGTT visit or who are not patients at the MFM Clinic, they will be consented at this visit. All participants will be screened at the YNHH Research Unit (HRU) or Church Street Research Unit (CSRU with a medical history and exam. Blood will be collected for HbA1C, Cr, ALT, AST, TSH, hematocrit and fasting lipid panel, and a urine pregnancy test will be administered. Oral glucose tolerance tests (OGTT) will be performed after an overnight fast. A nurse will insert an intravenous (IV) catheter and subjects will ingest 7.5 oz of glucola, which contains 75 g of glucose in flavored water. Blood samples will be taken at certain time points before and after glucola ingestion for the measurement of plasma glucose and insulin concentrations. Approximately 60 mL of blood will be drawn at this visit.

Continuous glucose monitor (CGM) placement (Visit 2): For up to 10 days prior to Visit 3, eligible participants will be asked to wear a CGM (Dexcom G6 Pro). At the visit for CGM placement (either at CSRU or HRU): 1) a trained study staff member will explain to the participant how to use the CGM, and 2) a trained study staff member will insert the CGM's plastic wire-like tip under the participant's skin with the use of the CGM applicator. This visit will take approximately 15 minutes.

The CGM consists of a sensor that measures glucose levels from the interstitial tissue. A transmitter that captures and stores data from sensor. The sensor is sterile and comes in an unopened package. Each sensor and transmitter are single use only and disposable. The Dexcom G6 Pro does not require any calibration. This means that no finger sticks are required for its use. Participants will need to return the sensor within 30 days of sensor placement.

The Dexcom G6 Pro data is blinded to study participants. Study staff will explain to the participants that intense exercise may cause the sensor to loosen due to sweat or movement of the sensor. If the CGM sensor/transmitter falls off while the participant is wearing it, we will ask them to return to the HRU or CSRU so that a new sensor/transmitter can be re-inserted. Subjects will be provided with our contact information in the event that they have any questions, concerns or issues related to the CGM.

After completing the 10-day CGM session, the sensor will be removed by a trained study staff member. To remove the sensor, the adhesive patch will be gently peeled off from the skin. Removal of the sensor is a painless procedure. The sensor/transmitter will be discarded after use. CGM removal will occur during Visit 3.

Hyperinsulinemic-euglycemic clamp (Visit 3): After an overnight fast, eligible subjects will return to the YNHH HRU. Two IV catheters will be inserted: one into a distal arm or hand vein that will be placed in a heated box to allow for the sampling of arterialized venous blood; the second into an antecubital vein for administration of a primed (200 mg/m2) and continuous infusion of [6,6-2H] Glucose and [2H5] Glycerol

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(non-radioactive stable isotopes) as well as for collecting blood samples for insulin and glucose measurements. After an equilibration period lasting 2 hours., the 2-step euglycemic-hyperinsulinemic clamp study will be performed. A primed-continuous infusion of regular insulin will be given at 8 mU/(m2 × min) during the first 2 hours, and increased to 40 mU/(m2 × min) for the subsequent 2 hours. Plasma glucose levels will be measured every 5 min, and a variable glucose infusion will be initiated to maintain levels at ~90 mg/dl. Plasma samples will be drawn at baseline and throughout the infusion period for measurement of isotope enrichment, insulin, NEFA, glycerol turnover, and additional hormones which may be altered with lactation. Approximately 160 mL of blood will be drawn at this visit.

Also at this visit, we will perform indirect calorimetry a total of 3 times (once before and twice during the insulin clamp). A transparent plastic hood will be placed over the participant's head for 30 minutes each time. A hose connects the hood to a calorimeter, which measures oxygen consumption and carbon dioxide production and calculates resting energy expenditure.

After the insulin clamp is completed, body composition will be assessed using whole body DXA. The DXA scan will be performed in the HRU to measure fat distribution, body fat, lean body mass and bone density.

Questionnaires: Women will be asked to complete questionnaires assessing their infant feeding practices, including, but not limited to, overall experience (sources of support, barriers/difficulties faced), frequency/number of feeds prior to introduction of solid foods, and type of feeds (breastfeeding, expressed breastmilk, formula). These questionnaires can be completed on a computer or smart phone at the woman's convenience. The questionnaires will be adapted from the CDC (https://www.cdc.gov/breastfeeding/data/ifps/questionnaires.htm) and the "Breastfeeding Self-Efficacy Scale".

Aim 2: Retrospective chart review

1. Estimated number of records to be reviewed: 500/year
2. Criteria for inclusion/exclusion: Women with a history of GDM who had an OGTT 6-8 weeks post-partum as part of standard of care at Yale Medicine's Maternal-Fetal Medicine Clinic in the past 3 years and throughout the duration of this study. (May 1,2016 until April 30, 2024)
3. Information collected and recorded from medical records:
    a. Data to be recorded from the chart:
        i. MRN
        ii. Sociodemographic information (age, race/ethnicity, preferred language, marital status, insurance, tobacco use)
        iii. Pre-pregnancy height/weight/BMI
        iv. GDM history (date of diagnosis, management)
        v. Date of delivery and delivery method
        vi. Weight/BMI on day of delivery
        vii. Gravida and parity
        viii. Date of OGTT during pregnancy and post-partum
        ix. OGTT lab results (during pregnancy and post-partum)
        x. Weight/BMI on day of OGTT (during pregnancy and post-partum)
        xi. Infant feeding practices
        xii. Infant birth weight/percentile and weight/percentile at 2 months of age

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b. Type of medium that will be used to record the information and the plans for maintaining confidentiality and security of the data: CSV file. The file will be stored on a secured server on an encrypted laptop and/or computer that is stored in a locked office.
c. Who will have access to the data, and how access to the data storage (whether paper-based or electronic) will be monitored: All data will be available to study personnel and stored on a secured server on an encrypted laptop and/or computer that is stored in a locked office.

4. How will the data and/or identifiers be destroyed when no longer needed for research purposes? If it will not please explain why data must be retained, for how long and how it will be kept secured: All data comes from the EPIC EMR and therefore cannot be destroyed and will be retained indefinitely. All de-identified data will be stored on a secured server on an encrypted laptop and/or computer that is stored in a locked office.

5. Genetic Testing N/A ☒

6. **Subject Population:** Provide a detailed description of the types of human subjects who will be recruited into this study.
Aim 1:
   - Group 1: 16 women (ages 18-45, HbA1C < 6.5%) with a history of GDM who exclusively or mostly breast-fed (< 6 ounces of formula/24 hours at 6-9 weeks of delivery)[1] for at least 4-6 months and who delivered within the past 18 months
   - Group 2: 16 women (ages 18-45, HbA1C < 6.5%) with a history of GDM who exclusively or mostly formula-fed (no breastfeeding or < 3 weeks of breastfeeding)[1] and who delivered within the past 18 months

7. **Subject classification:** Check off all classifications of subjects that will be specifically recruited for enrollment in the research project. Will subjects who may require additional safeguards or other considerations be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

☐Children	☐ Healthy	☐Fetal material, placenta, or dead fetus
☐Non-English Speaking	☐ Prisoners	☐Economically disadvantaged persons
☐Decisionally Impaired	☐ Employees	☐Pregnant women and/or fetuses
☐Yale Students	☐ Females of childbearing potential	

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects?
Yes ☐ No ☒

8. **Inclusion/Exclusion Criteria:** What are the criteria used to determine subject inclusion or exclusion?
Aim 1:
Inclusion Criteria:
   - Women between the ages of 18-45 with a history of GDM (as defined as the American Diabetes Association criteria) who delivered a singleton, live birth at ≥ 35 weeks gestation within the past 18 months
   - HbA1C < 6.5% at time of screening
   - Delivery within the past 18 months
   - Group 1: exclusively or mostly breast-fed (< 6 ounces of formula/24 hours at 6-9 weeks of delivery)[1] for at least 4-6 months

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Exclusion criteria:

- Currently lactating or lactation within the past 1 month at the time of the screening visit
- Diagnosis of diabetes (T1D or T2D) prior to pregnancy
- Current use of any glucose-lowering agents
- Pregnancy related medical problems including preeclampsia
- Major congenital fetal anomalies
- Creatinine > 1.5mg/dL, Hematocrit < 35%, ALT and AST > 2.5X upper limit of normal
- Known psychiatric disorders, alcohol abuse, HIV, hepatitis, renal disease, hepatic disease, untreated heart disease, untreated thyroid disease, active systemic infection or malignancy
- Illicit drug use (by the participant's self-report)
- History of post-partum depression
- Use of weight loss supplements or dieting 6 months prior to study
- Corticosteroid or opiate use within 6 months of study

9. How will **eligibility** be determined, and by whom?
   Eligibility will be determined by a qualified physician or nurse practitioner associated with the research protocol and will be based upon the above inclusion/exclusion criteria.

10. **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.
    Physical examination, venipuncture/IV placement, lab data collection and analysis: Subjects will receive a comprehensive medical history assessment, physical examination and lab testing to ensure good physical health status and eligibility. These are routine medical procedures and should add no risks other than those normally associated with these procedures. Study doctors and/or nurse practitioners will assess the screening laboratory blood work and if any abnormal findings occur, they will provide appropriate medical advice. Potential participants who are excluded from the study due to medical reasons, or those who are in immediate need for medical or psychiatric attention, will be referred to the appropriate facilities at Yale New Haven Hospital. Subjects will be exposed to the risk of venipuncture/IV placement that is routinely done in the HRU and CSRU. Risks associated include: local bruising, hematoma, or infection. If this occurs, appropriate treatment will be provided immediately. On extremely rare occasions, a blood clot or infection might occur, and if this occurs, subjects will be treated at the HRU or CSRU, which is fully staffed with nursing specialists and medical personnel. These risks are mitigated by the use of research nurses who have extensive experience in venipuncture and IV catheterization. Transient vasovagal symptoms, e.g. nausea, sweating, and lightheadedness may also occur during IV catheter placement.

    Oral glucose tolerance test (OGTT): The OGTT is a commonly used outpatient test for diagnosis of type 2 diabetes and gestational diabetes. It is not associated with any specific adverse effects. Occasionally, some patients may experience mild nausea or GI discomfort following drinking the 75-gram glucose drink; however, this typically resolves after eating food.

    Hyperinsulinemic-euglycemic clamp: The infusion of insulin and glucose poses a small risk that plasma glucose will fall below the predetermined level (90-100 mg/dl) resulting in little or no symptoms of hypoglycemia: sleepiness, hunger, anxiety, diaphoresis and tremor exaggerated symptoms. These symptoms are quickly reversed with IV dextrose infusion. The risks of drawing blood and inserting an IV line are detailed above. The studies will be performed under the supervision of a qualified physician or nurse practitioner. All solutions that are administered via IV will be sterile, pyrogen free, and prepared in a sterile environment. During glucose infusion, D20 (20% glucose) will be used to decrease the risk of thrombophlebitis that is associated

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with hypertonic glucose solutions. To avoid hypoglycemia during the hyperinsulinemic clamp, plasma glucose will be checked every five minutes using a bedside glucose monitor. Based on these measurements, the plasma glucose will be adjusted via the D20 infusion.

Phlebotomy: The total amount of blood drawn for this study is 220 mL (including screening, OGTT and hyperinsulinemic-euglycemic clamp). People who are in good health are not usually affected by this kind of blood loss. However, to be safe, subjects will be warned against donating blood for at least 6 weeks before and after completing this study. Additionally, subjects are screened for anemia at the first visit and women with a hematocrit less than 35% will be excluded.

6,6D2-glucose and D5-glycerol: The infusion of 6,6D2-glucose and D5-glycerol involves no radioactivity, and the glucose and glycerol are metabolized just as the unlabeled metabolite. Clinical research studies using these tracers have been used at Yale for decades with no adverse effects. The isotopes are purchased from Cambridge Isotopes, Inc. in powder form which has been certified to be sterile and free of pyrogens. The tracers are made into a solution by the Yale New Haven Hospital Investigational Drug Service (IDS). The solutions are tested to be free of pyrogens and to be sterile before being administered. All certifications are kept with the IDS.

Continuous glucose monitoring (CGM): The CGM poses no major risks to the subjects. Participants may feel a mild discomfort (pin prick sensation) during the sensor insertion. Some individuals may experience bruising of the skin at the insertion site of the CGM sensor, which resolves by itself in a few days. Redness and discomfort (inflammation) can occur at the sensor insertion site. Some individuals may be sensitive to the adhesive that keeps the sensor attached to the skin. If the participant notices significant skin irritation around or under the sensor, they will be instructed to contact us and it can be removed. Rarely sensors may fracture and a small piece may remain under the skin which will need to be removed by a study physician. This may cause mild discomfort, bruising, or temporary bleeding. The participants will also be informed that the sensor must be removed before an MRI, a CT scan, or diathermy treatment.

DXA scanning: DXA scanning for body composition is performed routinely by our group. The DXA measurement poses no major risk to the subject. The amount of radiation the subject will be exposed to is small and is one tenth of the amount of radiation for a chest X-ray.

Indirect calorimetry: Indirect calorimetry is routinely performed by our research team and poses no major risks to the users. Some people may feel claustrophobic and if this happens, we will remove the hood and stop the testing.

Questionnaires: The questionnaires are generally benign in nature. The major inconvenience is the time taken to complete them and a possible breach of confidentiality.

11. Minimizing Risks: Describe the manner in which the above-mentioned risks will be minimized.
IV Insertion and Blood Drawing: Risks of bruising, clotting, and infection will be minimized by having venipuncture performed by trained, experienced personnel under sterile conditions. To avoid injury due to fainting, the antecubital vein catheter will be inserted when the subjects are recumbent. All infused solutions will be prepared in sterile form by the YNHH Investigational Drug Services (IDS) pharmacy and tested for pyrogenicity and sterility before use. During studies 20% dextrose will be used to reduce the risk of thrombophlebitis associated with use of excessively hypertonic glucose solutions (50% dextrose). Women with a hematocrit less than 35% will be excluded. Patients will be advised to refrain from blood donation for 6 weeks after study completion.

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Hyperinsulinemic-euglycemic clamp: The infusion of insulin and glucose poses a small risk that plasma glucose will fall below the predetermined level (90-100 mg/dl) resulting in hypoglycemia, which can be quickly reversed with IV dextrose infusion. To avoid hypoglycemia during the clamp, plasma glucose will be checked every five minutes using a bedside glucose monitor. Based on these measurements, the plasma glucose will be adjusted via the dextrose infusion. The risks of drawing blood and inserting an IV line are detailed above. The studies will be performed under the supervision of a qualified physician or nurse practitioner. All solutions that are administered via IV will be sterile, pyrogen free, and prepared in a sterile environment. During glucose infusion, dextrose 20 (20% glucose) will be used to decrease the risk of thrombophlebitis that is associated with hypertonic glucose solutions (50% glucose).

6,6D2-glucose and D5-glycerol: The infusion of 6,6D2-glucose and D5-glycerol involves no radioactivity, and the glucose and glycerol are metabolized just as the unlabeled metabolites. Clinical research studies using these tracers have been used at Yale for decades with no adverse effects. The isotopes are purchased from (Cambridge Isotopes, Inc.) in powder form which has been certified to be sterile and free of pyrogens. They are made into a solution by the YNHH IDS. The solutions are tested to be free of pyrogens and to be sterile before being administered to the participants. All certifications are kept with the IDS.

Continuous glucose monitoring (CGM): CGM catheter will be placed under sterile conditions by experienced staff members. In case of signs of irritation, inflammation or bruising at the sensor insertion site, the sensor will need to be removed immediately and standard care will be applied. The participants will be given one of the study physician or nurse practitioner's cell phone number to contact for any questions, concerns or problems.

Questionnaires: The major inconvenience of the questionnaires is the time taken to complete them and a possible breach of confidentiality. Study participation is voluntary, and subjects are informed that they are free to withdraw at any time without penalty. All data will be kept confidential except in cases of imminent danger to the participants. Such limits to confidentiality will be clearly explained to participants verbally and in the written consent forms. Good clinical and research practice procedures and HIPAA regulations will be followed. No subjects are identified by name in any of the published literature and only by code in major data storage areas.

12. **Data and Safety Monitoring Plan:** Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.)
    a. What is the investigator's assessment of the overall risk level for subjects participating in this study? We feel that the risks associated with the study proposed should be deemed as moderate, for the following reasons:
        1) We do not view the risks associated with the hyperinsulinemic-euglycemic clamp and glycerol infusion technique as minimal.
        2) Given the established safety and validity of these clamp techniques, we do not view the proposed studies as high risk.
    b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study? N/A
    c. Include an appropriate Data and Safety Monitoring Plan. Examples of DSMPs are available here http://your.yale.edu/policies-procedures/forms/420-fr-01-data-and-safety-monitoring-plans-templates for
        i. Minimal risk

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ii. Greater than minimal

d. For multi-site studies for which the Yale PI serves as the lead investigator: N/A

Greater Than Minimal RiskDSMP

1. Personnel responsible for the safety review and its frequency:

The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency, which must be conducted at a minimum of every 6 months (including when reapproval of the protocol is sought). During the review process, the principal investigator (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment. Either the principal investigator or the IRB have the authority to stop or suspend the study or require modifications.

2. The risks associated with the current study are deemed greater than minimal for the following reasons:

1. We do not view the risks associated with phlebotomy, hyperinsulinemic-euglycemic clamp and glycerol infusion as minimal risks.

Although we have assessed the proposed study as one of greater than minimal risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3. Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator according to the following categories:

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).
b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).
d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
e.) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

4. Plan for Grading Adverse Events:

The following scale will be used in grading the severity of adverse events noted during the study:

1. Mild adverse event
2. Moderate adverse event
3. Severe

5. Plan for Determining Seriousness of Adverse Events:

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Serious Adverse Events:

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In addition to grading the adverse event, the PI will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it results in any of the following outcomes:

1. Death;
2. A life-threatening experience in-patient hospitalization or prolongation of existing hospitalization;
3. A persistent or significant disability or incapacity;
4. A congenital anomaly or birth defect; OR
5. Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the IRB is necessary.

6. Plan for reporting UPIRSOs (including Adverse Events) to the IRB

The principal investigator will report the following types of events to the IRB:

Any incident, experience or outcome that meets ALL 3 of the following criteria:

1. Is unexpected (in terms of nature, specificity, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved protocol and informed consent document and (b) the characteristics of the subject population being studied; AND
2. Is related or possibly related to participation in the research (*possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); AND
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, legal, or social harm) than was previously known or recognized.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) may be medical or non-medical in nature, and include – but are not limited to – *serious, unexpected, and related adverse events* and *unanticipated adverse device effects*. ***Please note*** that adverse events are reportable to the IRB as UPIRSOs only if they meet all 3 criteria listed above.

These UPIRSOs/SAEs will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website. All related events involving risk but not meeting the *prompt* reporting requirements described in IRB Policy 710 should be reported to the IRB in summary form at the time of continuing review. If appropriate, such summary may be a simple brief statement that events have occurred at the expected frequency and level of severity as previously documented**.** In lieu of a summary of external events, a current DSMB report can be submitted for research studies that are subject to oversight by a DSMB (or other monitoring entity that is monitoring the study on behalf of an industry sponsor).

13. Statistical Considerations: Describe the statistical analyses that support the study design.
Group sample sizes of 15 and 15 achieve 80% power to detect a between-group difference of 0.61 in a design with 7 repeated measurements (taken at time 0, 5, 10, 30, 60, 90, 120 min) having a autoregressive AR(1)

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covariance structure when the standard deviation is 1, the correlation between observations on the same subject is 0.6, and the alpha level is 0.05. To account for up to 5-10% missing data due to drop out or poor quality data, we will need to enroll a total of 32 subjects (16 per group) for this study. Data Analysis: Primary endpoints: Whole body insulin sensitivity, endogenous glucose production, whole body lipid turnover rates. Secondary endpoints: Plasma NEFA, TGs, insulin levels, metrics of body composition. Statistical Analysis: Before statistical testing, all continuous variables will be tested for normality of distribution. Summary statistics will be computed. For between-group comparisons of patient characteristics, two sample t-test or Mann-Whitney U test will be used for continuous variables, and Fisher's exact test for categorical variables. Comparisons of repeatedly measured glucose and lipid turnover rates will be made using the mixed model analysis. Fixed factors include group and time and their interactions. A random effect for subject plus an autoregressive covariance matrix will be used to accommodate within-subject correlation of repeated assessments. Linear contrasts comparing differences between groups or different time points within a group will be estimated.

SECTION II: RESEARCH INVOLVING DRUGS, BIOLOGICS, RADIOTRACERS, PLACEBOS

If this section (or one of its parts, A or B) is not applicable, check off N/A and delete the rest of the section.

A. RADIOTRACERS ☒N/A

B. DRUGS/BIOLOGICS [2H5]-Glycerol and [6,6-2H]- Glucose to be used during the clamp procedure. A primed-continuous infusion of regular insulin will be given at 8 mU/(m2 × min) during the first 2 hours, and increased to 40 mU/(m2 × min) for the subsequent 2 hours.

C. Use of Placebo: ☒Not applicable to this research project

1. Continuation of Drug Therapy After Study Closure ☒Not applicable to this project

1. Identify whether FDA approval has been granted and for what indication(s). – N/A

2. All protocols which utilize a drug, biologic or radiotracer not approved by, but regulated by, the FDA, or a radiotracer regulated by the RDRC, must provide the following information: N/A

   a. What is the Investigational New Drug (IND) number assigned by the FDA?
   b. Who holds the IND?
   c. All protocols which utilize a radiotracer not approved by, but regulated by the FDA must provide the IND number:

   Alternatively, use of the investigational radiotracer may be under RDRC/RSC oversight: (check if appropriate)

   _____
   Alternatively, an exemption from IND filing requirements may be sought for a clinical investigation of a drug product that is lawfully marketed in the United States.

   Exempt Category 1
   The clinical investigation of a drug product that is lawfully marketed in the United States can be exempt from IND regulations if all of the following are yes:

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i. The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for

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the drug. ☒Yes ☐ No

ii. The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and the intention of the investigation is NOT to support a significant change in the advertising for the product. ☒ Yes ☐ No

iii. The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. ☒ Yes ☐ No

iv. The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56). ☒Yes ☐ No

v. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs. ☒ Yes ☐ No

2. Background Information: Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this drug is being administered to humans, include relevant data on animal models.

[2H5]-Glycerol and [6,6-2H]- Glucose to be used during the clamp procedure. A primed-continuous infusion of regular insulin will also be given at 8 mU/(m2 × min) during the first 2 hours, and increased to 40 mU/(m2 × min) for the subsequent 2 hours.

Hyperinsulinemic-euglycemic clamp: The infusion of insulin and glucose poses a small risk that plasma glucose will fall below the predetermined level (90-100 mg/dl) resulting in hypoglycemia, which can be quickly reversed with IV dextrose infusion. To avoid hypoglycemia during the clamp, plasma glucose will be checked every five minutes using a bedside glucose monitor. Based on these measurements, the plasma glucose will be adjusted via the dextrose infusion. The risks of drawing blood and inserting an IV line are detailed above. The studies will be performed under the supervision of a qualified physician or nurse practitioner. All solutions that are administered via IV will be sterile, pyrogen free, and prepared in a sterile environment. During glucose infusion, dextrose 20 (20% glucose) will be used to decrease the risk of thrombophlebitis that is associated with hypertonic glucose solutions (50% glucose).

6,6D2-glucose and D5-glycerol: The infusion of 6,6D2-glucose and D5-glycerol involves no radioactivity, and the glucose and glycerol are metabolized just as the unlabeled metabolites. Clinical research studies using these tracers have been used at Yale for decades with no adverse effects. The isotopes are purchased from (Cambridge Isotopes, Inc.) in powder form which has been certified to be sterile and free of pyrogens. They are made into a solution by the YNHH IDS. The solutions are tested to be free of pyrogens and to be sterile before being administered to the participants. All certifications are kept with the IDS.

3. Source: a) Identify the source of the drug or biologic to be used. – information noted above
b) Is the drug provided free of charge to subjects? ☒Yes ☐ No
If yes, by whom? The drug will be provided through funding received by the Principal Investigator, Dr. Belfort De Aguiar for the conduction of this study.

4. Storage, Preparation and Use: Describe the method of storage, preparation, stability information, and for

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parenteral products, method of sterilization and method of testing sterility and pyrogenicity.
Check applicable Investigational Drug Service utilized:

☒, YNHH IDS
☐ Yale Cancer Center
☐ CMHC Pharmacy
☐ West Haven VA
☐ PET Center
☐ None
☐ Other:

D.DEVICES ☒N/A

SECTION III: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. **Targeted Enrollment:**
   a. Targeted for enrollment at Yale for this protocol: 32
   b. If this is a multi-site study, give the total number of subjects targeted across all sites: N/A

2. **Indicate recruitment methods below.** Attach copies of any recruitment materials that will be used.

☒ Flyers	☐ Internet/web postings	☐ Radio
☒ Posters	☐ Mass email solicitation	☐ Telephone
☒ Letter	☒ Departmental/Center website	☐ Television
☒ Medical record review*	☒ Departmental/Center research boards	☐ Newspaper
☒ Departmental/Center newsletters	☒ Web-based clinical trial registries	☒ Clinicaltrails.gov
☒ YCCI Recruitment database	☐ Social Media (Twitter/Facebook):	
☐ Other:		

* Requests for medical records should be made through JDAT as described at
http://medicine.yale.edu/ycci/oncore/availableservices/datarequests/datarequests.aspx

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3. Recruitment Procedures:

a. Describe how potential subjects will be identified.

Potential subjects will be identified as indicated below using the methods identified in #2 above:

- Self-Identify
- Provider Referrals
- Study Team Members
- Existing registries, studies, and websites where participants have previously provided informed consent or a request to be contacted for research utilizing their preferred method of contact when known.
- Joint Data Analytics Team (JDAT)
- Potential subjects may be identified by MFM clinical providers/staff and/or research staff during routine clinical care at the Maternal Fetal Medicine Center at 1 Long Wharf Drive, New Haven, CT06511.

How these options will be utilized is further described in section "b" below.

b. Describe how potential subjects are contacted.

- Self-Identify and Provider Referrals: Participants will self-identify using IRB approved recruitment materials that will be posted locally. Recruitment materials will display the contact information of the study team in addition to a description of the study. Potential participants may also be approached by their medical provider. Additionally, participants may be approached by study personnel at community events, or at various locations where potentially eligible patients are seen when facilitated through the participant's treatment team, including at Yale SOM, YNHH, YMG, and VA Connecticut Healthcare System.
    - Potential subjects may be approached in person, at the time of routine clinical care in MFM clinic. If time does not allow (either a research team member is not available to speak with the potential subject or the potential subject does not have time to discuss the study), potential subjects may be called after their clinical visit to discuss the study. In this situation, the potential subject would be provided with a brochure about the study during their clinic visit and will be told that a research team member may be calling them about a study they are potentially eligible for. The person contacting the potential subject will be a research team member who may be known to the subject or may be calling on behalf of the PI.
- Recruitment through Existing Registries and Studies: Participants will be recruited through the YCCI website/registry/Help Us Discover and ClinicalTrials.gov website. Participants may also be identified through their participation in the Yale University Reproductive Sciences Longitudinal Pregnancy study. Study personnel will use MyChart, traditional mail, telephone or email of patients recruited through this method. If the EPIC registration module provides a preferred method of contact, that method will be utilized. If the participant is not registered in EPIC but has expressed interest in a research study by calling, emailing, filling out an online questionnaire, or speaking with study personnel face-to-face, the patient will be contacted by their means of preference, if stated.
- Recruitment through JDAT: The Joint Data Analytics Team (JDAT) will also be used to query eligible patients in EPIC based on the eligibility criteria outlined above (#8). JDAT will identify and send a study communication to potential participants based on these criterion that fall within the following time period: the last 18 months. Potential participants who do not use MyChart will receive a paper mailing including information about the study. JDAT will not identify the potential participants to the researchers, and therefore the researchers will receive no identifiable information from JDAT.
- Messaging to Study Participants:
  The following wording will be used in the letter or MyChart message patients receive via JDAT describing the study and inviting them to participate:
    - "You are receiving this notification because you may qualify and be interested in a study looking at the effect of breastfeeding on blood sugar, insulin and fat in women with a history of

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gestational diabetes. The Yale New Haven Health electronic health record system has searched medical conditions to find people who may be good matches for research studies. No one has looked at your record and no information has been shared with any research doctor or research team member. Just because you received this message does not mean that you are in a research study or that you have to decide to be in this or any study.
You may be interested and eligible to participate in a research study conducted by Yale University investigators to better understand breastfeeding and diabetes.
To opt-out of research, including opting out of receiving future messages about research studies, please email optout@yale.edu or call 1-877-978-8348 and select option #3.
Title of study: The effect of lactation on insulin sensitivity and lipolysis in women
Principal Investigator: Renata Belfort De Aguiar, MD, PhD
Study Coordinator: Mari-Lynet Knight
Phone # 203-737-4777

The message/mailing to potential study participants will also include the following information, regardless of who sends it:

- "You are receiving this notification because you may qualify and be interested in a study looking at the effect of breastfeeding on blood sugar, insulin and fat in women with a history of gestational diabetes. Approximately 32 participants will be enrolled in this study.
  Confidentiality and Privacy: Any personal health, financial data, and other information gathered in the study will remain confidential and will be stored on a password-protected computer, only accessed by study personnel. When the results of the research are published or discussed, no information will be included that would reveal your identity. We understand that information about you obtained in connection with your health is personal, and we are committed to protecting the privacy of that information. If you would like to learn more about participating in this study, please contact the study coordinator at 203-737-4777.
  Possible Benefits: This research may not benefit you directly. However, knowledge gained from the results may help us to better understand breastfeeding and diabetes.
  Participation in this study is completely voluntary. You are free to decline to participate, to end participation at any time for any reason, or to refuse to answer any individual question at any time. Refusing to participate will involve no penalty or loss of benefits to which you are otherwise entitled (such as your health care outside the study, the payment for your health care, and your health care benefits).
  Questions: If you have any further questions about this study, you may contact the investigator, Renata Belfort De Aguiar, MD, PhD, at 203-737-4777. If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688."

- <u>Screening and Informed Consent Discussion:</u> Research team members will contact interested potential participants to assess eligibility and provide the participant with additional information about the study, including the study procedures, purpose and potential complications. The brief screen via email or phone will ask for the following information to confirm study eligibility:
  - Name
  - Age/Date of Birth
  - Place of Birth
  - Phone Number/Email
  - Address
  - Marital Status
  - Race/Ethnicity

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- Height/Weight
- Medical/Surgical History including obstetric history
- Medications
- Drug/Alcohol Use

It is necessary to collect this information prior to the subject traveling to Yale for an in-person screening visit, in order to determine their eligibility and avoid unnecessary screening and traveling on the part of the participant. This information will be protected according to HIPAA policies and will be destroyed if the individual is determined to not be eligible for the study during this correspondence or is otherwise not interested in participating in the study, unless the subject consents for his/her screening information to be stored for future studies that he/she may be eligible for.

During the in-person screening session, the informed consent form and study details are reviewed in detail by one of the project investigators or key study personnel and the subject will be asked to read the informed consent form (approved by the Yale Human Investigation Committee). The subject will be given time to ask questions and only after that will the subject be asked to give informed consent to participate. The informed consent form and study details will again be reviewed with the subject on each study day prior to beginning the study.

The Principal Investigator in this study or key study personnel will be responsible for obtaining consent.

c. Who is recruiting potential subjects?
All research team members as well as the individuals listed above (JDAT, referring providers, etc.).

4. Assessment of Current Health Provider Relationship for HIPAA Consideration:
Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?
☐Yes, all subjects
☒Yes, some of the subjects
☐No

If yes, describe the nature of this relationship. An investigator may be the treating clinician for subjects recruited from the YNHH Endocrine Clinic/YNHH Diabetes Center or Yale Medicine's Maternal-Fetal Medicine Clinic.

5. Request for waiver of HIPAA authorization: (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only. Note: if you are collecting PHI as part of a phone or email screen, you must request a HIPAA waiver for recruitment purposes.)
Choose one:
☐ For entire study
☒ For recruitment/screening purposes only
☐ For inclusion of non-English speaking subject if short form is being used and there is no translated HIPAA research authorization form available on the University's HIPAA website at hipaa.yale.edu.

i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data: It is necessary to collect this information prior to the subject traveling to Yale for an in-person screening visit, in order to determine their eligibility and avoid unnecessary screening and traveling on the part of the participant. This information will be protected according to HIPAA policies and will be destroyed if the individual is determined to not be eligible for the study during this correspondence or is otherwise not interested in participating in the study, unless the subject

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consents for his/her screening information to be stored for future studies that he/she may be eligible for. In addition, we will need the participant's full name, address, date of birth, place of birth, phone numbers, marital status, height and weight in order to schedule a screening visit at the HRU or CSRU.

ii. If requesting a waiver of **signed** authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data: *Write here*

The investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAA-Covered entity must be accounted for in the "accounting for disclosures log", by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

6. **Process of Consent/Assent:** Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.
   During the in-person screening session at the YNHH Hospital Research Unit (HRU) or Church Street Research Unit (CSRU), the informed consent form and study details are reviewed in detail by one of the study investigators or key study personnel. The subject will be asked to read the informed consent form (approved by the Yale Human Investigation Committee). The subject will retain a copy of this consent to review. The subject will be given time to ask questions and only after that will the subject be asked to sign to give informed consent to participate. The informed consent form and study details will again be reviewed with the subject on each study day prior to beginning the study. Any subject who appears incapable of providing informed consent (e.g., due to apparent cognitive impairment) will be excluded.

7. **Evaluation of Subject(s) Capacity to Provide Informed Consent/Assent:** Indicate how the personnel obtaining consent will assess the potential subject's ability and capacity to consent to the research being proposed.
   We do not plan to recruit subjects with limited decision-making capacity. Potential subjects will undergo a face-to-face interview. At that time, a study clinician will meet the subject, review the informed consent form, explain the purpose of the study and risks associated with participation, and will be available for questions. To ensure that the study subject understands the study, the subject will be asked questions about the study procedures and the risks associated with participation. If any concern arises that the study subject did not fully understand the study, the study clinician may decide that the subject is not suitable for participation. If the subject is still interested after all questions have been answered, a study physician will ask the subject to sign the informed consent form.

8. **Non-English Speaking Subjects:** Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. If enrollment of these subjects is anticipated, translated copies of all consent materials must be submitted for approval prior to use.
   We plan to enroll Spanish-speaking subjects and will provide informed consent forms in Spanish to these subjects. Some members of the research team speak Spanish and are comfortable conducting research visits in Spanish. For all other research team members who do not speak Spanish, a certified translator at the clinic will be utilized.

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As a limited alternative to the above requirement, will you use the short form* for consenting process if you unexpectedly enounter a non-English speaking individual interested in study participation and the translation of the long form is not possible prior to intended enrollment? YES ☐ NO ☒

Note* If more than 2 study participants are enrolled using a short form translated into the same language, then the full consent form should be translated into that language for use the next time a subject speaking that language is to be enrolled.

Several translated short form templates are available on the HRPP website (yale.edu/hrpp) and translated HIPAA Research Authorization Forms are available on the HIPAA website (hipaa.yale.edu). If the translation of the short form is not available on our website, then the translated short form needs to be submitted to the IRB office for approval via modification prior to enrolling the subject. ***Please review the guidance and presentation on use of the short form available on the HRPP website.***

If using a short form without a translated HIPAA Research Authorization Form, please request a HIPAA waiver in the section above.

9. Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study. If you will request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.

☐Not Requesting any consent waivers

☒Requesting a waiver of signed consent:

☒ Recruitment/Screening only *(if for recruitment, the questions in the box below will apply to recruitment activities only)*

☐ Entire Study (Note that an information sheet may be required.)

For a waiver of signed consent, address the following:

- Would the signed consent form be the only record linking the subject and the research? YES ☒ NO ☐
- Does a breach of confidentiality constitute the principal risk to subjects? YES ☒ NO ☐

OR

- Does the research pose greater than minimal risk? YES ☐ NO☐
- Does the research include any activities that would require signed consent in a non-research context? YES ☐ NO ☐

☐ Requesting a waiver of consent:

☐ Recruitment/Screening only *(if for recruitment, the questions in the box below will apply to recruitment activities only)*

☐ Entire Study

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For a full waiver of consent, please address all of the following:

- Does the research pose greater than minimal risk to subjects?

  ☐ Yes ***If you answered yes, stop. A waiver cannot be granted.***

  ☐ No
- Will the waiver adversely affect subjects' rights and welfare? YES ☐ NO☐
- Why would the research be impracticable to conduct without the waiver? *Write here*
- Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date? *Write here*

SECTION IV: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

1. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research?
   - Medical record number
   - Name
   - Address
   - Phone number/email
   - Age/date of birth
   - Race/ethnicity
   - Medical (including obstetric) and surgical history, allergies, medications taken
   - Blood test results

2. How will the research data be collected, recorded and stored?
   Research data will be collected directly from the subject as well as via the electronic medical record. Subjects will be assigned a study number. The principal investigator will create a computer worksheet where the name of the subject's medical information is linked to the coded information. This will be housed on a password protected computer on a secured network. Only the investigators will have access to the computer records, which include the subject's identity, in order to evaluate the information generated by the study. No further public disclosure of this information will be made. One copy of the consent form will be kept in a secured and locked cabinet in the PI's office (which is also locked). The subject's name will be kept separate from the results.

3. How will the digital data be stored? ☐CD ☐DVD ☐Flash Drive ☐Portable Hard Drive ☒Secured Server ☐Laptop Computer ☐Desktop Computer ☐Other

4. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study?
   All identifiable subject information that is collected during recruitment, screening, and participation will appear only on the initial paper forms, which will be kept under lock and key in the academic office of one of the study investigators. All digital files that could link a code number to an individual study subject will be password protected and stored on a shared drive in a password protected secure server, on a laptop or computer which will be kept locked in the PI's office.

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All portable devices must contain encryption software, per University Policy 5100. If there is a technical reason a device cannot be encrypted please submit an exception request to the Information Security, Policy and Compliance Office by clicking on url http://its.yale.edu/egrc or email it.compliance@yale.edu

5. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured.
   Procedures to ensure confidentiality follow the regulations and policies of the Yale University School of Medicine. The security mechanisms specified above will continue to be in place to protect study data.

6. If appropriate, has a Certificate of Confidentiality been obtained? N/A

SECTION V: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)
This research may not benefit the subject directly. However, knowledge gained from the results may help us to better understand lactation and diabetes.

SECTION VI: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. Alternatives: What other alternatives are available to the study subjects outside of the research?
   The alternative is to decline to participate in the study.

2. Payments for Participation (Economic Considerations): Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.
   $50 for the screening visit
   $100 for the hyperinsulinemic-euglycemic clamp visit
   $20 for completing the questionnaires
   $15 for using the CGM
   $15 for providing expressed breastmilk (optional)
   Parking vouchers for the study visits
   Reimbursement for child care during the study visits (up to $15/hr)

3. Costs for Participation (Economic Considerations): Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects.
   All study interventions and procedures will be provided at no cost to study participants.

4. In Case of Injury: This section is required for any research involving more than minimal risk, and for minimal risk research that presents the potential for physical harm (e.g., research involving blood draws).

   a. Will medical treatment be available if research-related injury occurs? Yes
   b. Where and from whom may treatment be obtained? YNHH
   c. Are there any limits to the treatment being provided? No
   d. Who will pay for this treatment? Insurance/patient

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e. How will the medical treatment be accessed by subjects? Medical treatment will be provided by study staff with referral to appropriate care at YNHH as needed

IMPORTANT REMINDERS

Will this study have a billable service? Yes ☒ No☐

A billable service is defined as any service rendered to a study subject that, if he/she was not on a study, would normally generate a bill from either Yale-New Haven Hospital or Yale Medical Group to the patient or the patient's insurer. The service may or may not be performed by the research staff on your study, but may be provided by professionals within either Yale-New Haven Hospital or Yale Medical Group (examples include x-rays, MRIs, CT scans, specimens sent to central labs, or specimens sent to pathology). Notes: 1. There is no distinction made whether the service is paid for by the subject or their insurance (Standard of Care) or by the study's funding mechanism (Research Sponsored). 2. This generally includes new services or orders placed in EPIC for research subjects.

If answered, "yes", this study will need to be set up in OnCore, Yale's clinical research management system, for Epic to appropriately route research related charges. Please contact oncore.support@yale.edu

Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? Yes ☒ No ☐

If Yes, please answer questions a through c and note instructions below.

a. Does your YNHH privilege delineation currently include the specific procedure that you will perform? Yes ☒ No ☐

b. Will you be using any new equipment or equipment that you have not used in the past for this procedure? Yes ☐ No ☒

c. Will a novel approach using existing equipment be applied? Yes ☐ No ☒

References:

1. Feng L, Xu Q, Hu Z, Pan H. Lactation and progression to type 2 diabetes in patients with gestational diabetes mellitus: A systematic review and meta-analysis of cohort studies. J Diabetes Investig 2018; 9:1360-1369
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3. Gunderson EP, Crites Y, Chiang V, Walton D, Azevedo RA, Fox G, Elmasian C, Young S, Salvador N, Lum M, Hedderson MM, Quesenberry CP, Lo JC, Ferrara A, Sternfeld B. Influence of breastfeeding during the postpartum oral glucose tolerance test on plasma glucose and insulin. Obstetrics and gynecology 2012; 120:136-143
4. Gunderson EP, Hedderson MM, Chiang V, Crites Y, Walton D, Azevedo RA, Fox G, Elmasian C, Young S, Salvador N, Lum M, Quesenberry CP, Lo JC, Sternfeld B, Ferrara A, Selby JV. Lactation intensity and postpartum maternal glucose tolerance and insulin resistance in women with recent GDM: the SWIFT cohort. Diabetes care 2012; 35:50-56
5. Gunderson EP, Lewis CE, Lin Y, Sorel M, Gross M, Sidney S, Jacobs DR, Jr., Shikany JM, Quesenberry CP, Jr. Lactation Duration and Progression to Diabetes in Women Across the Childbearing Years: The 30-Year CARDIA Study. JAMA Intern Med 2018; 178:328-337

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