STUDY TITLE: Lifestyle Intervention in Preparation for Pregnancy (LIPP)

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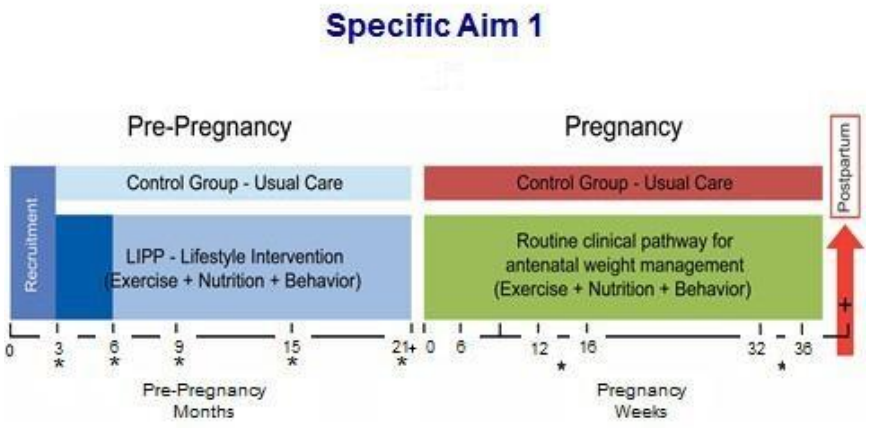
1. Study Schema Figure 7 in LIPP grant



Lifestyle intervention program. *HNRCA or CTRC visit for body composition, OGTT, inflammatory markers; + post-partum: newborn body composition, cord blood, placenta at TMC.

Please note that in accordance with Tufts IRB, the format of this protocol is Boston area specific with addendums for each site detailing local research activities.

2. Introduction

2.1 Background and Rationale

Lifestyle interventions during pregnancy: are they successful? There have been numerous randomized clinical trials (RCT) examining lifestyle interventions for obese women during pregnancy. These RCTs had primary outcomes that included avoiding excessive gestational weight gain (GWG), and decreasing adverse perinatal outcomes, such as gestational diabetes (GDM), preeclampsia and large for gestational age (LGA) neonates (17,18,19,20,21). A recent Danish RCT on lifestyle intervention

consisting of dietary guidance, free fitness club membership, and personal coaching initiated between 10-14 weeks' gestation (18) reported that although there was a decrease in GWG in the intervention group (7.0 vs. 8.6 kg, p=0.01), paradoxically infants in the lifestyle intervention group had higher birth weight (3,742 vs. 3,593 g, p=0.04) compared to controls. Other recent RCTs (UPBEAT and the Norwegian Fit for Delivery Trial) suggest that behavioral lifestyle interventions do not prevent GDM or reduce the incidence of LGA babies despite reducing maternal dietary glycemic load, GWG, and maternal skinfold measures, and increasing physical activity (22,23). At least five meta-analyses published in the past four years, all concluded that lifestyle interventions initiated during pregnancy, while having some success in reducing excessive GWG, had minimal positive effects on adverse pregnancy outcomes including decreasing fetal overgrowth (24, 25, 26, 27, 28). Although some advocate weight loss in obese women during pregnancy, these recommendations are based on extrapolation of epidemiological data (29, 30, 31). Investigators in the UPBEAT trial noted that increasing the intensity and duration of the lifestyle intervention was likely to be impractical for most obese women (22) Further, we reported an increased risk of small for gestational age (SGA) neonates and decreased lean body mass (head circumference and length) in neonates of overweight/obese women with inadequate GWG (32).

Post-delivery weight loss: is it possible and safe, and what is the effect on subsequent pregnancies? Publications related to postpartum weight loss have been primarily retrospective cohort studies reporting that ~10-20% of overweight/obese women had a decrease in weight between pregnancies (33, 34, 35, 36). The interval between pregnancies was 12 months in 30% and 24-36 months in 40-57% of subjects (34, 37). Inter-pregnancy weight loss in overweight/obese women was associated with a decreased risk of GDM or preeclampsia (35, 36, 38, 39) in a subsequent pregnancy. Three studies reported a decreased incidence of LGA in overweight/obese women who had an inter-pregnancy decrease of as little as 1-2 BMI units (34, 37, 38). The inter-pregnancy decrease in weight was not associated with an increased risk of SGA infants (34).

Four RCTs reported that postpartum lifestyle intervention was effective in facilitating weight loss compared to a control group. Lovelady reported weight loss of 0.5 kg/week between 4-14 weeks postpartum in overweight women who were breastfeeding. Weight loss did not affect infant growth (40). Similarly, Colleran reported that a 16- week diet and exercise intervention resulted in a 5.8 kg weight loss in overweight and obese lactating women without adverse effects on infant growth (41). Stendall-Hollis showed that overweight women, most of whom were exclusively breastfeeding, lost 3-4% of body weight and significantly decreased biomarkers of inflammation (42). O'Toole reported a mean 5.6 kg weight loss at 12 weeks; weight loss was primarily fat mass (43). A recent meta-analysis of 11 studies with 769 subjects reported a significant mean -2.57 kg (95% CI -3.6 to -1.5) weight loss in the intervention group. In a subgroup analysis, the most effective interventions were those with objective goals; use of heart rate monitors, pedometers, and exercise combined with intensive dietary intervention, and these interventions produced a -4.1 kg (95% CI -5.2 to -3.5) weight loss (44). Therefore, supervised intensive lifestyle intervention in overweight and obese postpartum women is feasible, efficacious, and safe, in lactating women. The concept that lifestyle intervention before pregnancy is necessary to improve placental function and fetal development is strongly supported by scientific and clinical observations, and is gaining traction as a viable paradigm to improve perinatal metabolic outcomes (45).

Summary: Lifestyle intervention initiated during pregnancy helps reduce excessive GWG; however, it has not been successful in reducing fetal overgrowth. Based on our research we conclude that lifestyle intervention needs to be initiated prior to conception. Just as women with pre-existing diabetes need to normalize glucose before pregnancy to decrease the risk of congenital anomalies, obese women must improve metabolic conditioning before pregnancy to decrease complications of fetal overgrowth during pregnancy (46). We reported an increased expression of lipogenic and inflammatory genes in placenta of obese women in the early first trimester, before any phenotypic change becomes apparent (47). The intervention proposed in this application includes an established exercise, diet and behavioral modification program that have proven, through prospective RCTs, to be successful in improving overall metabolic function by decreasing

body weight/adiposity in obese non-pregnant individuals (48). Collectively these data provide evidence that lifestyle intervention initiated prior to conception will have short- and long-term benefit for the mother and her baby.

Relevant Preliminary Data by PI and Co-Is

Maternal obesity/hyperinsulinemia affects placental growth and gene expression in early pregnancy. Lean and obese women were recruited prior to a planned pregnancy and underwent longitudinal clamps, and intravenous glucose tolerance tests (IVGTT). There was a 3.7-fold increase in insulin response to the IVGTT in obese vs. lean women. The increase in insulin response in early pregnancy correlated with placental growth and fetal adiposity at term ($r=0.64$, $p=0.0001$) (59).

Increased insulin response in obese women results in altered expression of placental lipid metabolism genes. We recruited 48 lean and obese women scheduled for elective first trimester termination. After metabolic evaluations placentas were obtained for molecular analysis. At 9 weeks gestation, insulin resistance, leptin, IL-6 and C-reactive protein (CRP) were increased in obese vs. lean subjects. CRP correlated with maternal BMI and insulin sensitivity. Using global gene profiling analysis of trophoblast cells, we identified 355 genes differentially regulated in obese vs. normal weight women. Pathway analysis revealed a significant decrease in the expression of genes regulating β- oxidation, cholesterol and steroid synthesis.

Since β-oxidation and initial steps of steroid biosynthesis occur in mitochondria, these data suggest impairment of trophoblast mitochondrial function during early pregnancy in insulin resistant obese women. We further postulate that in obese women, hyperinsulinemia and/or defective insulin action alter placental gene expression. We cultured first trimester human trophoblast cells, with and without insulin (100 nM), to investigate insulin effects. In trophoblasts derived from lean women, 2,875 genes were differentially regulated by insulin, whereas only 87 genes were regulated in obese women. These data suggest that the effect of insulin on trophoblast cells is less robust in obese women (60). By improving maternal insulin sensitivity and decreasing β-cell response through lifestyle intervention before conception, the decrease in insulin response will affect placental programming of genes related to lipid metabolism and mitochondrial function. This will result in decreased placental lipid accumulation and nutrient availability to limit neonatal adiposity. Thus, interventions aimed at improving maternal metabolic function should begin before initiation of molecular changes. These data are highly supportive of our hypothesis that when lifestyle intervention is initiated after placental gene expression has been programmed the effects are not reversed.

Potential subjects for recruitment: We have a track record of successfully recruiting and retaining women prior to a planned pregnancy for longitudinal metabolic studies (15,50,51,52,53,54,55,56,57,58). While the number of subjects in the individual protocols were in the range of 20-25, there were multiple protocols over 20 years that were funded by 6 different R01 or Program Project mechanisms, (total $n > 200$). Note; these longitudinal studies involved clamps, muscle and fat biopsies as well as estimates of energy expenditure and body composition. In this protocol we will initiate recruitment in our postpartum unit after the index or subsequent delivery. Using our perinatal database of all obese women (for the purposes of this proposal defined as a BMI 25-35 kg/m2 for the general population, and 23-32.5 kg/m2 for Asian populations as defined by the WHO guidelines, 95), we estimated the time from delivery of their first infant to their next pregnancy. Approximately 1,665 women (32%) became pregnant within 18 months and 2328 (45%) within 24 months of their first delivery. We anticipate the time interval will be considerably less if we recruit women planning to conceive, who do not have a history of infertility and because the number of unplanned pregnancies is decreasing (3). Further, we have a record of excellent success with retention in previous postpartum women (pre-pregnancy relative to the LIPP). We also have follow-up of women and their children at 4, 8 and 12 months postpartum for body composition studies (61). To date, we have recruited over 250

women (>85% of eligible subjects) and their offspring from the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) follow-up study 8-12 years after delivery.

Inter-pregnancy weight loss or gain: In a sub-cohort of our population (n=126), we examined the effect of maternal weight change from the first to second pregnancy. Of the 126 women, 20 (16%) lost weight and 106 (84%) gained weight between pregnancies. In the subsequent pregnancy, women who lost weight delivered infants with a minimal change in mean birth weight, whereas women with an increase in inter-pregnancy weight delivered infants that were on average 107g heavier at birth. These data are consistent with a report (34) from more than 10,000 births in obese women. Inter- pregnancy weight loss was associated with a lower risk of LGA infants (OR: 0.61; 0.52-0.73), whereas weight gain was associated with an increased risk of LGA. We examined body composition of neonates in successive pregnancies where mothers (BMI-25-35 kg/m2) had either gained (n=10) or lost weight (n=5). Women with an inter-pregnancy weight loss had a mean decrease in birth weight of 349 g (183 g fat and 40 g lean mass). In those with an inter-pregnancy weight gain, there was a mean 63 g increase in birth weight, which was comprised of 33 g body fat and 11 g lean mass. There was a net 216 g difference in fat, but only a 51g difference in lean mass between women who lost and gained weight. The effect of weight loss between pregnancies **was primarily due to a change in neonatal fat**. We have also reported that neonatal fat mass is significantly correlated with childhood adiposity (15).

Importance of weight loss for restoration of insulin sensitivity postpartum: We used euglycemic clamps and molecular approaches to identify mechanisms that regulate insulin sensitivity in obese women during late pregnancy and one year postpartum (62). Women who did not lose weight within one year after delivery did not reverse pregnancy-related insulin resistance, and fasting glucose and plasma TNF-α remained elevated. Further, in the absence of weight loss, skeletal muscle TNF-α gene expression and 312Serine-IRS-1 phosphorylation did not recover to pre-pregnancy levels. These data suggest that women who fail to lose weight postpartum are at greater risk of increased insulin resistance in a subsequent pregnancy. The progressive insulin resistance may accelerate the decline in β-cell function and development of type 2 diabetes. In contrast, women who were followed for one year postpartum, but lost significant weight (63), experienced normalization of fasting glucose and insulin, insulin sensitivity, and insulin signaling in skeletal muscle. These data highlight the importance of postpartum weight loss to the metabolic health of the mother.

Lifestyle intervention induces weight loss and improved metabolic function: exercise and caloric restriction in obesity - a randomized clinical trial. We conducted a prospective study in 24 obese (BMI: 34.3 ± 5.2 kg/m2) adults randomized to exercise alone, or exercise combined with moderate caloric restriction (-500 kcal/d (48). Both interventions improved insulin sensitivity ($p<0.001$), and cardiovascular risk factors (systolic/diastolic blood pressure, waist circumference, glucose and triglycerides; $p<0.05$). Total abdominal, subcutaneous, and visceral fat, aerobic capacity, total and LDL cholesterol also improved. These data support the efficacy of our weight loss intervention in reducing body weight and improving insulin sensitivity in obese individuals.

Mechanisms related to pancreatic β-cell function: effects of lifestyle induced weight loss on β-cell function and the GLP-1 response to glucose. We examined the effect of exercise/diet induced weight loss on β-cell function, insulin secretion, and the incretin (GLP-1 and GIP) response to nutrient stimulation as part of NIH funded obesity and insulin resistance studies. In obese adults, a low glycemic diet combined with exercise induced weight-loss and reduced postprandial hyperinsulinemia (48, 64, 65). In contrast, a high glycemic diet impaired pancreatic β-cell and intestinal K-cell function, despite weight loss. Our research nutritionist will counsel participants to eat a Mediterranean-style diet during the lifestyle intervention. Counseling will be

consistent with the subject's metabolic status, food availability, and cultural needs. We have also shown (data in review) that lifestyle-induced weight loss decreases the GLP-1 response to glucose. There are no published data on the effects of lifestyle-induced weight loss on GLP-1 responses to nutrient stimulation in pregnancy. The current application will address this knowledge gap.

Preliminary Data

Placental lipid metabolism pathways are altered in obese women at term. We measured fatty acid metabolism gene profiles in placentas of 40 lean and 40 obese women as part of our NIH-funded placental lipid studies. Carnitine palmitoyltransferase (**CPT**) 1B, the rate-limiting enzyme in the mitochondrial fatty acid oxidation pathway, is lower in term placentas of obese women, while key genes in fatty acid esterification and accumulation (acetylCoA carboxylase (**ACC**), steroyl CoA-desaturase (SCD) 1, and fatty acid binding protein 4 (FABP4) are higher. Consistent with these gene pathways, ^{3}H-palmitate esterification was higher in primary trophoblasts isolated from 11 obese women compared to 5 lean women. These data provide a potential mechanism for our finding that placental lipid content is 17% higher in obese (N=15) vs. lean (N=14) women. In a RCT (PMC), we found that overweight and obese women randomized to omega-3 fatty acid supplementation starting in the early 2nd trimester had lower expression of lipid esterification genes (i.e. DGAT1 by 22%, adipophilin (PLIN2) by 33%) and lower placenta esterified fatty acid content compared to placebo (32±8 vs. 43±12 nmol/mg), but fatty acid oxidation genes and neonatal fat mass were not improved (66). In fact, birthweight increased 25% with omega-3 supplementation. Further, omega-3 did not alter 3H-palmitate oxidation in vitro, but decreased 3H-palmitate esterification ~50% in trophoblasts isolated from obese women (N=11). These data suggest that:
1) Intervention initiated during pregnancy in obese women does not improve placental mitochondrial function or neonatal adiposity, and 2) lowering lipid esterification and storage in placentas of obese women without improving mitochondrial function (i.e. β-oxidation) increases neonatal adiposity, potentially due to greater lipid flux to the fetus. These data are consistent with our premise that lifestyle intervention pre-pregnancy can alter the progression of impaired β-oxidation to lipid accumulation and increased fat deposition, because changes in mitochondrial function may begin early in pregnancy. We expect that pregravid lifestyle intervention will result in improved placental mitochondrial function (i.e. β-oxidation), which will be measurable at term and associated with reduced fatty acid esterification and lipid accumulation, and result in lower neonatal fat mass accretion.

Summary: These preliminary studies: 1) confirm our experience and expertise in conducting RCTs in pregnant women and their offspring; 2) show that the proposed lifestyle intervention approach achieves weight loss in obese adults and is feasible and successful in our hands; 3) provide strong clues as to which mechanisms of action (including placental programming, improved β-cell function, and reduced insulin resistance) are responsible for the improved glucose and lipid homeostasis in obese patients after weight loss interventions. Collectively, these data offer strong supportive evidence for the studies outlined next.

Background and Significance

Obesity during pregnancy: Obesity is a major health concern during pregnancy (4). In the United States 55.8% of the female population aged 20-39 years is overweight or obese, based on body mass index (BMI, kg/m2) criteria (4). Further, 7.5% in this age group have a BMI >40 kg/m2 (5). Equally concerning, 17% of U.S. children and adolescents are obese (6) and mean term birth weight is increasing in developed nations (7, 8). We have reported a significant increase of 116 g in mean term birth weight in Cleveland since 1975 (9). High maternal pregravid BMI, and not gestational weight gain (GWG), was the

strongest correlate for a high birth weight. Although some have suggested that the increase in birth weight may have reached a plateau (10), we have found that this is secondary to factors such as earlier gestational age of delivery (11). Of greater concern is the significant increase in the Ponderal Index (weight/length3) in our neonatal population over the last decade (11).

Pre-pregnancy obesity or excessive gestational weight gain - relationship to fetal overgrowth: In 2009 the Institute of Medicine (IOM) reexamined the 1990 GWG guidelines. Thirty-eight percent of normal weight, 63% of overweight, and 46% of obese women gained weight in excess of IOM guidelines (12). In Cleveland, 59% of overweight and 52% of obese women had excessive GWG. These percentages are higher in African American and Hispanic women. In obese women excessive GWG was a significant risk factor for cesarean delivery and postpartum weight retention, and not related to development of preeclampsia or gestational diabetes (**GDM**). High GWG was associated with an increased risk of preterm birth **but was not related to increased risk of fetal overgrowth** (12). Thus, excessive GWG is a primary risk factor for maternal post- partum weight retention, which increases the risk of maternal pregravid obesity in a subsequent pregnancy.

Maternal obesity in early pregnancy more than doubles the risk of obesity in offspring between the ages of 2 to 4 (13). Boney reported that (LGA) neonates of mothers who were obese, and/or developed GDM, were at increased risk for developing the metabolic syndrome between ages 6 to 11 (14). Maternal obesity *but not GDM* had a 2-fold greater risk for metabolic syndrome in the offspring. In our 8-10-year follow-up studies, maternal pregravid BMI, independent of GDM or GWG, was the strongest predictor of childhood obesity and metabolic dysfunction (15). In a meta-analysis, Philipps reported that although maternal diabetes is associated with increased childhood BMI z-score, this was no longer significant when adjusted for maternal pre-pregnancy BMI (16). Hence, maternal pregravid obesity is not only a risk factor for offspring adiposity at birth, but also for the long-term risk of obesity and metabolic dysfunction in the offspring independent of maternal GDM or excessive GWG.

RELEVANCE AND USEFULNESS OF THE OBJECTIVES

Clinically, the proposed study provides feasible methods to improve the short and possibly long-term metabolic health of obese women and their offspring. Scientifically, this study will provide fundamental insights into the physiological and molecular mechanisms underlying maternal metabolism before pregnancy and effects on maternal/placental physiology.
The actual lifestyle interventions (healthy eating and increased physical activity are not in themselves unique techniques in pregnancy. The uniqueness or innovation is the use of these techniques in order to affect an improvement in fetal adiposity in a subsequent pregnancy. Studies are not unique in pregnancy. The innovative aspects of this proposal are the following:

1) The lifestyle intervention will begin prior to a subsequent pregnancy. We are uniquely positioned to recruit overweight/obese women immediately after index pregnancy. This allows us to conduct a "run-in period" of actual recruitment and randomization by ~3 calendar months (±27 days) postpartum. Our experienced research team is one of the few to successfully recruit large numbers of women prior to a planned pregnancy for metabolic studies.
2) The period between pregnancies will allow sufficient time for the lifestyle intervention to decrease weight and result in improved insulin sensitivity, β-cell function, adiponectin concentrations and decreased inflammation, in contrast to lifestyle intervention initiated during the second trimester of pregnancy.
3) This proposal has the potential to fulfill, by providing level 1 evidence one of the objectives of the 2009 IOM report. Research recommendation S-7 suggests that by providing pre-conceptual services to obese women, such as diet and physical activity,

with the goal of achieving a healthy weight before conceiving, will represent "a radical change to the care provided to obese women of childbearing age" (49).
4) Our proposal incorporates a trans-disciplinary team (nutrition, exercise physiology, molecular biology, obstetrics, and endocrinology). We will test our hypothesis that the intervention translates into cellular and molecular modifications relating to insulin action pathways. Only by employing a well-disciplined longitudinal program, based in the community with sufficient support, will our subjects recognize that lifestyle intervention represents a life course alteration that will have long-term benefits for both mother and child.
Rationale: We conclude, based on our previous research (15, 50, 51, 52, 53, 54, 55, 56, 57, 58), that maximum metabolic benefits relating to fetal body composition and metabolism can only be achieved by improving the pregravid metabolic condition of obese women. Therefore, Specific Aim 1a will examine how maternal lifestyle intervention initiated prior to a planned pregnancy results in improvements in maternal insulin sensitivity, β-cell function, adiponectin concentrations and a decreased inflammatory milieu. Specific Aim 1b provides a physiological translational link between improved maternal metabolism and neonatal adiposity and metabolic condition. Specific Aim 2 will provide evidence that lifestyle intervention improves placental mitochondrial function and lipid oxidation, and decreases lipid esterification/storage thereby providing a mechanism whereby the maternal pre-pregnancy metabolic milieu regulates placental nutrient function and subsequent fetal growth.
Is there an active control group?

☑ **Yes** ☐ **No**

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

- The control group will be provided with current recommendations for healthy eating (myplate.gov) and exercise recommendations (American College of Sports Medicine; ACSM) to achieve a healthy lifestyle. Additionally, the control group will receive healthy pre-pregnancy and pregnancy guidelines from the American College of OB/GYN (ACOG). In brief, the control group will be asked to consume a balanced diet, limit added sugar and saturated fat and increase physical activity for 30 minutes a day for at least 5 days per week.
- The lifestyle data to date during pregnancy has not shown any benefit of lifestyle interventions during pregnancy as compared to the control group. The hypothesis to be tested is that a lifestyle intervention BEFORE pregnancy will have benefit in a subsequent pregnancy. The information provided to the control subjects will in the vast majority of situations be of greater content, specifics and recommendations than are currently provided to overweight and obese women in clinical practice.
- The control group will not be exposed to any risks beyond what is currently recommended by the ACOG for overweight and obese women planning a pregnancy, i.e. eating healthy food, increasing physical activity and return to pre-pregnancy weight.

2.2 Risks to Subjects

Adequacy of Protection Against Risks

Risks include the following:

Subjects will be asked to use an acceptable means of birth control at least until they have completed the 6-month post-partum follow-up visit. The type of birth control they choose will be determined by them and their health care provider. There are no risks in performing the metabolic studies when pregnant; they will be performed at 12-16 and 32-36 weeks of pregnancy. We would not wish to perform them outside the defined study windows to avoid extra visits or obtaining blood that cannot be used as part of the research.

- When blood is drawn from a vein there will be some temporary discomfort and the minimal risk of local bruising, infection or blockage of the vein. We will take suitable precautions including trained staff who are certified to draw blood in volunteer subjects using an indwelling catheter, and minimal size needles to obtain the samples will help to minimize these risks.
- There are no risks to performing the Indirect Calorimetry to estimate resting energy expenditure.
- The possible risks of the oral glucose tolerance test consist of occasional nausea and very rarely vomiting or lightheadedness.
- There are no known risks from using the Bod Pod method to measure body fat composition.
- For those randomized to the Lifestyle Intervention group, risks include possible injury such as muscle and joint sprains during the exercise sessions.
- While exercising or performing the $VO_{2\,max}$ test, subjects might experience fatigue or shortness of breath. They might also feel like their heart is pounding very fast or very hard and experience dizziness, heart rhythm abnormalities, chest pain, or very rarely a heart attack or death. They could stumble and fall off the treadmill. If subjects have any of these experiences, they are instructed to tell the research team. Also, when the mask is on their face, this might feel uncomfortable.
- There are no known additional risks from performing the metabolic tests.
- There is the potential risk of loss of confidentiality of the results of metabolic testing. We make every effort to minimize these risks by keeping paper records in a locked cabinet in a locked office. Any information held in electronic form will be kept on a secure computer in the research area of the hospital. Information containing personal data, for example birthday will be de-identified using a research hospital code number.

Delivery:

- Taking blood from the placenta and the umbilical cord will not harm subjects or their baby.
- Measuring baby's length, weight and fat thickness will not hurt the baby.
- There are no known risks to measuring baby's body composition with the Pea Pod device. It is possible that the baby will become upset while lying in the chamber of the Pea Pod during the 2-minute measurement period; however, the baby at no time will be subjected to any type of discomfort. The baby is placed in a warm, molded plastic "infant seat" type structure. The clear acrylic enclosure allows the infant to always be in the view of the operator.
- There are no risks to mother or infant related to completing these questionnaires. We will instruct subjects that "Some questions on the questionnaires are of a sensitive nature and you may feel uncomfortable answering these questions. You can choose not to answer any questions you are not comfortable completing".

We will maintain contact with the subjects, via phone, email or text messaging and plan to meet with her at any of her postpartum visits to her obstetrical care provider. At that time study staff will review inclusion/exclusion criteria and review factors such as blood pressure and clinical OGTT if the patient had GDM. The study coordinator will also make sure that subjects who required a cesarean delivery will be able to partake in the lifestyle regimen if they are randomized into that group. The PI at each site will make final decision about eligibility. At this time, the potential subjects who agree to participate in the study will be scheduled for a metabolic evaluation visit at the Tufts CTRC and HNRCA, or the Brigham & Women's Hospital (BWH) Research Facility at 221 Longwood prior to randomization.
The consent document for this protocol will be approved by the Institutional Review Board (IRB) at Tufts Medical Center. Written informed consent will be obtained from all subjects participating in the project.

b. Protection Against Risk

The exercise component of the intervention at Tufts will be under the direction of Dr. Roger Fielding, a Co-I on this project. The exercise component of the intervention at BWH will be

under the direction of Dr. Kieran Reid. Methods to ensure safety with the exercise include screening for abnormalities on EKG and supervision by a certified lifestyle coach. At the time of the exercise testing in the HNRCA (VO2 testing), Dr. Fielding or Dr. Catalano will have available Dr. Lisa Ceglia to review any EKGs that he deems questionable. If there is an acute problem the subjects will be brought to the emergency Dept. at Tufts Medical Center. If there is a non-acute finding on the EKG the subject will be referred to her own PCP or if the subject does not have a PCP Dr. Ceglia will provide the name of a cardiologist. Subjects with a questionable cardiac history will either be excluded based on exclusion criteria of if questionable history at the time of explaining the protocol to the subject, we will ask that the subject be cleared by her own physician before being allowed to be included in the LIPP study. Similar precautions will be taken at BWH.

In order to ensure safety with the nutrition intervention, all food provided to subjects as part of this study will be prepared by the HNRCA or BWH Research Facility research nutrition team in the metabolic kitchen of the HNRCA or Research Facility or if the visits are taking place at the CTRC, food provided will be prepared by the nutrition team in the Tufts Medical Center kitchen. The diet will be based upon the Mediterranean diet, which is an eating pattern that focuses on fruits, vegetables, legumes, nuts, lean proteins and fish, while limiting red meats and sweets high in added sugar. Additionally, olive oil is used as the main fat in the diet. The caloric needs of the participants in the lifestyle modification program will be calculated from resting metabolic rate, which will be measured by indirect calorimetry, adjusted initially by a factor of 1.3 to account for the exercise program. Further adjustment in caloric content will be made to attain a weight loss of >5% of pre-pregnancy weight or closest weight to pre-pregnancy based on best clinical data. This will be achieved by a negative caloric balance of 500 Kcal/day to attain a rate of weight loss of 1 lb/week. Participants will be instructed by the LIPP team so dietary intake includes healthy choices for fat (mono and polyunsaturated) and protein (fish, nuts, legumes, and poultry) and substitution of dietary fat and simple sugars by healthy carbohydrates (fruits, vegetables, whole grains). If weight loss is not being achieved, participants will be recommended to use meal replacements (commercially available) to facilitate caloric restriction as was used with great success in the Look AHEAD trial. Once the weight loss target is attained, subjects will enter into a maintenance dietary phase, the aim of which will be to maintain or reduce further weight (up to 15% of baseline weight).

During pregnancy, subjects randomized to the lifestyle intervention program will be advised to maintain their exercise regimen as tolerated per ACSM and ACOG guidelines. During pregnancy, all subjects will have dietary recommendations based upon the ACOG guidelines and delivered by the clinical nutrition program at Tufts Medical Center and Brigham & Women's Hospital in order to gain weight as recommended by the 2009 IOM guidelines. Drs. Catalano and Fielding will oversee the studies in the HNRCA and Dr. Reid and Dr. Sen will oversee the studies in the BWH Research Facility.

The Specific Aims of this proposal require that we only include women who plan to become pregnant. We will include assessment of growth/body composition of their neonates as the primary outcome measure. As such, we anticipate that approximately 50% of the neonates will be male. We believe that because the investigators have broad experience with lifestyle interventions in non-pregnant women, metabolic studies during pregnancies and experience with neonatal measurements, that there will be minimal risk to the women and infants participating in this study. The PIs and CO-Is are all experienced clinicians and NIH funded investigators. The facilities of Brigham & Women's Hospital, the HNRCA and Tufts Medical Center are located next to and within hospitals. The outpatient facilities where the lifestyle

interventions take place will be in the HNRCA, BWH Research Facility or community where the participants work and live.

Medical intervention will be available for all subjects enrolled in this program for medical emergencies related to adverse events. If there is a chronic medical condition resulting from participation in this study, the subject will be responsible per Tufts MC IRB regulations. We have conducted randomized control trials in the past and have experience with using DSMBs. We have had no issue with either data entry or reporting of adverse events.

- Subject identifier number will identify all of the material collected as part of the study. The information will be kept on the REDCap database. Any paper records will be kept in the study coordinator's office in a locked cabinet.
- There are no unknown risks to this study. The metabolic testing has been conducted by the PIs (Catalano at Tufts, Kirwan at the Pennington and Calles at Case Western) at each of the sites for a number of years with no adverse effects. The lifestyle intervention group will be under the supervision of trainers who will have employed healthy eating and exercise as a component of their research for a number of years. The intervention groups will be overseen by Kirwan (Case Western and the Pennington), Fielding (Tufts) and Reid (BWH and MGH).
- The research nutritionist reviews the diets of each subject in the intervention arm and makes adjustments regarding the Mediterranean diet based on the individual's specific food allergy.
- There are no risks to the fetus or embryo for healthy eating and exercise during pregnancy as these are the current recommendations of the ACOG. If a woman becomes severely dehydrated, this could decrease blood flow and oxygen to the fetus. This situation is often accompanied by symptoms as noted above in the mother. Care will be taken to avoid these conditions because of the presence of certified trainers in the intervention group.
- *If applicable, describe risks to people other than the participating subject, e.g., risks to family members, friends, others or risks to the community. NA*
  There may be benefit to the family because of women most commonly doing the greatest share of meal planning for the family.
- *If applicable, describe risks to study investigators or staff performing the study procedures due to research with high risk populations (e.g. prisoners, intravenous drug users, patients with major psychiatric issues, etc.).* NA

2.3 Potential Benefits to Subjects

Potential Benefits of the Proposed Research to Human Subjects and Others

There are potential benefits to subjects and others from this proposed research. The potential benefits to subjects in the lifestyle intervention arm are that they will be able to participate in a supervised diet and exercise program to decrease weight and improve metabolic function in preparation for a future pregnancy. If indeed the intervention proves successful, not only will there be immediate benefit to the subject herself, but there is the real possibility the intervention will have long term benefit for the prevention of obesity and metabolic complications for both the mother and her offspring. Even subjects assigned to the usual care or control arm of the study will have the benefit of being given specific advice as to an optimal diet and exercise regimen before pregnancy based on the metabolic evaluation in the HNRCA or CTRC in TMC and the Research Facility at 221 Longwood in BWH. While we call this usual care, the information the usual care subjects receive from their OB provider on lifestyle interventions for overweight and obese women is not nearly as detailed as the usual care subjects receive in the LIPP study. The metabolic results from testing will be available to the nutritionist and lifestyle coach. In routine clinical care neither this information nor a

detailed meeting with a nutritionist and lifestyle coach are available in the course of a routine postpartum visit.

There are direct benefits to both the intervention and usual care groups as described previously. There will be a benefit to all subjects who are recruited from this study, i.e. women delivering at Tufts MC, Pennington, BWH or any other affiliated institutions participating in the LIPP study.

Importance of the Knowledge Gained

The importance of the knowledge to be gained by this proposal is the ability to determine if Lifestyle Interventions in Preparation for Pregnancy can achieve concrete (real) results in the efforts to stem the epidemic of obesity in women and their offspring. This study will also provide information as to the mechanisms of why lifestyle intervention before rather than only during pregnancy results in improved outcomes. Although the concept of pre-pregnancy lifestyle intervention has been recognized previously as potentially worthwhile, there have been few if any studies employing such a study design, because of the perceived difficulty in recruiting women prior to pregnancy. Based on our history of conducting research studies in women before during and after pregnancy, a study design of recruiting obese women immediately after their index pregnancy and incorporating the community into the lifestyle interventions, we believe that this approach will have applicability in many other populations.

The risks to subjects are minimal. They are the standard of care we should provide to all our obese patients considering future pregnancy. Our ability to provide more than a minimal amount of information is hampered by the lack of resources particularly for women seeking care. If successful, as proof of principle, we believe this protocol can serve as a paradigm for a wider initiation of lifestyle interventions before pregnancy.

We believe that the minimal risks associated with this proposal are reasonable in relation to the anticipated benefits, because to date much of the medical efforts relating to obesity have been focused on treatment; whereas, the goal of this proposal is not only treatment of the mother but prevention of obesity in her offspring. If successful this program will be expanded to include a much larger population; for example, potentially involving a larger multi-center approach or possibly involving the Maternal Fetal Medicine Networks. Last, we will also plan long-term follow-up studies of the mothers and their children to test the hypothesis that there are long-term benefits to lifestyle interventions in preparation for pregnancy.

2.4 Alternatives

- The alternatives to participating in this study are the routine information provided to overweight and obese women by their obstetrical health care provider at the time of their postpartum visit.
- Overweight and obese women MAY be advised to increase physical activity and eat healthy but rarely see a nutritionist or a trainer about the specific lifestyle activity based on their baseline metabolic status.
- Subjects could go to a reputable health club in the area and ask for a personal trainer and nutritionist for either a 1-time session (usual care) or sign up for personal training Intervention group).

3 Objectives

- *Describe the purpose, specific aims, or objectives of the study (i.e. the reason for performing the study in terms of the scientific question to be answered).*
  Studies evaluating lifestyle intervention in obese women during pregnancy have reported limited success in decreasing excessive gestational weight gain, and have failed to achieve

the key outcome of breaking the obesity cycle and reducing neonatal adiposity or birth weight. Although some investigators advocate weight loss during pregnancy in obese women, these recommendations were based on extrapolation of retrospective epidemiological data. Of concern, we reported increased small for gestational age babies and decreased lean body mass in neonates of obese women with weight loss or inadequate gestational weight gain. Based on our research, optimal outcomes from lifestyle interventions are likely to be temporal and therefore must be initiated prior to conception to first improve maternal metabolic function, and subsequently, placental/fetal growth. Several large retrospective cohort studies support our hypothesis. For example, women who lost weight between pregnancies had fewer large for gestational age babies in contrast to women who increased inter-pregnancy weight. In addition, prospective randomized controlled trials have shown that postpartum weight loss is achievable without adverse maternal or neonatal outcomes, these studies include women who breastfed. Based on these observations, we propose a randomized control trial to determine the effect of lifestyle intervention initiated prior to a planned pregnancy on improving neonatal metabolism and adiposity.

Our over-arching hypothesis is that the maternal pre-pregnancy metabolic condition determines the obesogenic in-utero environment, which affects programming of placental mitochondrial function and metabolic pathways, promoting lipid accumulation and neonatal adiposity. Our rationale is based on the need to establish the most effective time to introduce an intervention that will break the obesity cycle in mothers and their children. Understanding how pregravid metabolic conditioning improves maternal physiology and cellular and molecular function in pregnancy will provide the empirical data to support the intervention. We have a highly successful record of - recruiting women who are planning a pregnancy, obtaining compliance in longitudinal studies, and in long-term follow-up of mothers and their offspring. Lifestyle intervention will be initiated prior to conception to decrease maternal body fat, inflammation, insulin resistance, and β-cell dysfunction. Our trans- disciplinary team has the required expertise in lifestyle interventions, management of obesity, and in human physiology that is needed to determine the effects of these interventions on maternal metabolism and fetal- placental growth and function. We will recruit 200 women to pursue the following specific aims:

Specific Aim 1: To investigate the physiological significance of lifestyle intervention in preparation for pregnancy (LIPP) on maternal and neonatal metabolism and adiposity.

Sub-Aim 1a. The working hypothesis is that a lifestyle intervention, initiated after the index pregnancy, will facilitate lower maternal insulin resistance, inflammation, weight, adiposity, incretin response, and energy expenditure compared to usual care. We will perform longitudinal measures of body composition, insulin sensitivity and secretion and blood markers of inflammation. We will link these changes to improvements in neonatal adiposity and insulin sensitivity.

Sub-Aim 1b. The working hypothesis is that women who initiate a lifestyle intervention before the subsequent pregnancy will deliver a baby with lower birth weight and fat accretion; insulin resistance; and cord blood markers of inflammation. We will measure body composition using anthropometrics and air displacement plethysmography (Pea Pod), umbilical cord glucose, insulin, leptin, IL-6, hsCRP, and adiponectin.

Specific Aim 2: To determine the molecular effects whereby lifestyle intervention initiated before pregnancy can improve placental mitochondrial lipid oxidation and accumulation. The working hypothesis is that lifestyle intervention before pregnancy will increase placental mitochondrial lipid oxidation and decrease lipid esterification/storage (Fig. 2). Isotope-

labeled fatty acid oxidation and esterification will be quantified in vitro in placental explants. We will measure markers of mitochondrial lipid metabolism and content using qRT-PCR and ELISA approaches. Placental lipids will be quantified and correlated with neonatal adiposity and maternal metabolism.

Impact of the study: Clinically, the proposal provides feasible methods to improve short and long-term metabolic health of obese women and their offspring. Scientifically, this study will provide fundamental insights into the physiological and molecular mechanisms underlying maternal pregravid metabolism and effects on maternal/placental physiology.

Specify which are the primary or secondary aims or objectives of this study as applicable.
The primary objective is the main question. Secondary objectives are goals that will provide further information for the study
We have not developed secondary aims for the study. These will be considered once the primary study is underway and secondary funding is sought. For example, we are in preliminary discussions with Dr. Economos in the school of nutrition to look at any spillover effect of the LIPP study on other family members. These discussions are at the initial stages and have not yet been fully developed or sought additional research funds.

4 Enrollment and Withdrawal

4.1 Inclusion Criteria

- *Describe the criteria that define who will be **included** in the study as a numbered list.*

Human Subjects Involvement, Characteristics and Design
We will recruit a total of 100 in the Greater Boston area and 100 in the Greater Baton Rouge area (PBRC)) overweight/obese subjects (defined as pregravid BMI 25-35 kg/m^2, and 23-32.5 kg/m^2 for Asian populations as defined by the WHO guidelines, 95) at the time of their index pregnancy, hereafter defined as obese for the purposes of the proposal) determined by review of the electronic medical record (EMR) after delivery of their index child.
Inclusion criteria:
All subjects will meet the following criteria by the time of randomization at 3 calendar months postpartum:
1) Planning another pregnancy within the next 24 months
2) Planning to deliver in the greater Boston or Baton Rouge area during their next pregnancy
3) A previous full-term singleton pregnancy (gestational age > 37 weeks)
4) 18 to 40 years of age at the time of enrollment into the study
5) Vaginal or cesarean delivery
6) Normal glucose tolerance or gestational diabetes (GDM), but without evidence of postpartum diabetes as defined by a 75 g 2-hr oral glucose tolerance test (OGTT) or HbA1c per ADA criteria.
7) Normal blood pressure or mild preeclampsia but postpartum blood pressure not requiring medication
8) Bottle or breast feeding
9) Normal thyroid function (determined by TSH concentration in blood), normal blood cell count and normal kidney and liver functions. Lipid profile with triglyceride 0levels not higher than 400 mg/dl (fasting) and LDL levels not higher than 200 mg/dL (if levels are above 180 mg/dL, potential participant should attain approval via letter from their PCP/OB).
11) No clinical signs or symptoms of cardiovascular disease or any other disease or condition that may contraindicate participation in exercise training (i.e. COPD, 2Severe asthma, orthopedic abnormalities)
13) Using contraception

- *Indicate specifically whether you will include any of the following special populations:*
- Pregnant women yes
- Lower age limit is 18

4.2 Exclusion Criteria

- *Describe the criteria that define who will be **excluded** in the study as a numbered list.*
  All subjects will NOT meet the following criteria:1) Pre- or post-delivery diabetes
  2) Post-delivery hypertension requiring medication
  3) Asthma requiring more than occasional use of a sympathomimetic inhaler, but not chronic inhaled steroids
  4) Inflammatory bowel disease
  5) Need for any assisted reproductive technologies to become pregnant based on best clinical judgement
  6) Medical or obstetrical contraindication to the defined exercise program or diet
  7) Tobacco, excessive alcohol use (greater than 2 drinks/day) or illicit drug use
  8) Eating disorders such as bulimia
  9) Gastric surgery to lose weight including banding or bypass procedures
  10) Any psychological or psychiatric condition that may impair participation in the lifestyle intervention program
  11) Multiple pregnancy
  12) HIV, or hepatitis B or C based on patient's history
  13) Congenital heart disease unless accompanied by a letter from the subject's cardiologist clearing them to exercise
- *Specify whether a study subject may participate in another research study while participating in this research study.*
  Yes as long as there is no conflict with LIPP as the primary research study.

4.3 Withdrawal of Subjects

Subjects who do not become pregnant at the end of the intervention or usual care component (21 months postpartum) are still included in the study. They will not undergo further evaluation but will continue to be followed until either they become pregnant or the study terminates. Contact will be maintained through a brief monthly check-in phone call from study staff. For the intervention arm, this will include a reminder of body weight goal and strategies to sustain weight loss. Testing will resume during pregnancy at the specified intervals of 12-16 and 32 to 36 weeks of pregnancy.

- *Describe anticipated circumstances under which subjects will be withdrawn from the research without their consent.*
  We have had subjects we asked to withdraw from the study because of their not returning for their metabolic assessments in the metabolic research centers, no shows at scheduled training sessions, smoking (which is an exclusion criteria).
  If a LIPP subject becomes pregnant prior to 16 weeks after randomization or becomes pregnant before the 3-month postpartum randomization, i.e. no baseline measurement, they would be asked to withdraw.
- *Describe procedures that will be followed when subjects withdraw or are withdrawn from the research, including the possibility of partial withdrawal from study intervention with continued data collection.*
  We will plan to maintain demographic data for subjects who have withdrawn from the study in order to compare with subjects who have been compliant with the study protocol in order to determine if there was bias in subject compliance.
- *Include any necessary safety precautions to be applied to subjects who withdraw or are withdrawn (tapering drug doses, evaluative x-ray, etc.).* NA

4.4 Recruitment and Retention

4.4.1 Local Recruitment Methods

Describe the following attributes of the recruitment plan for the local Tufts site:

- *When, where, and how potential subjects will be recruited.*
- We plan to approach subjects, assuming they and their newborn are stable, on the postpartum unit of Tufts Medical Center (Tufts MC) after their delivery. The PI and/or

the study coordinator will inform the subjects about the study. Subjects will be identified as being eligible based on information obtained at the time of their delivery. Potential subjects will be interviewed by the PI and/or the study coordinator to determine eligibility and potential interest in the LIPP project. They will be given a brochure describing the study. If potentially interested we will obtain contact information, such as email and phone numbers and contact potential subjects prior to their 4-6 week postpartum visit. Before their postpartum visit with their OB health care provider, study staff will contact them to determine if they might be interested in participating in the LIPP study. If they are, one of the recruiters will meet the subject at their postpartum visit, explain the study in more detail, and provide the potential subject with a copy of the consent form. The subjects will have until 3 calendar months (+/- 3 full weeks) to decide to join the study and be scheduled for metabolic testing. We created an IRB-approved informational website that is linked to on the MIRI lab page at Tufts MC and distributes ads via social media and print linking to the informational website. If a potential participant would like to ask questions related to the study to a current or former participant, LIPP study staff will initiate this communication (via email, text or phone call) with the approval of both individuals.

- *Source of subjects (for example, patient population, local community, etc.).*
  Postpartum patients at TMC.
- *Methods that will be used to identify potential subjects.*
  Review of EMR charts. Patients who are found to meet exclusion criteria during screening, such as history of HIV, or hepatitis B or C, will not be recruited and their PHI will not be recorded.
- *Materials that will be used to recruit subjects.*
  Primarily brochures and speaking with potential subjects, as well as sharing the study website page with those who are interested. The brochure will be given to all potential subjects until we have reached our recruitment goals.
- *When subjects respond to recruitment material, describe the information that will be provided to them about the study and the information that will be collected from subjects (e.g. name, telephone number, etc.) or submit a telephone/email script that will be used.*
  We do not use a script. Subjects will be given a copy of the brochure and if interested will be given a copy of the consent form to be reviewed with the study coordinator.
    - *If data will be retained for subjects that are determined to be ineligible, specify how privacy and confidentiality of these potential subjects will be maintained. Data will be retained until the primary manuscript is written noting differences in the study population that have determined to be ineligible for whatever reason based on inclusion/exclusion criteria.*
    - *Also, submit the screening log to the IRB for review.*
      The screening log template has been submitted with the application.
    - *We will retain non PHI information number of subjects etc. who are determined not to be eligible.*
    - Please refer to the DSMB reports we have submitted.
- *If recruitment material is being mailed or otherwise distributed, describe where/how, the distribution list will be obtained.*
  The information will be given to potential subjects at the time of the postpartum visit. The same brochure will be sent via email to potential subjects prior to their postpartum visit to remind them of the specifics of the LIPP study.
- *Specify how and why the listed recruitment methods will be effective in attracting the targeted subject population.*
  The recruitment methods have been successful in recruiting the first 20 subjects in Cleveland at MetroHealth Medical Center.

4.4.2 Study-Wide Recruitment Methods

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

☑ **Yes** ☐ **No**

If **Yes**, describe the following:

- *Methods of recruitment not under the control of the local Tufts site.*

There will be no further recruitment at the Pennington site in Baton Rouge; we will continue to follow subjects until they deliver at the PBRC (please see section 11.1 for site specific information). There will also be no further recruitment at Massachusetts General Hospital (MGH) but recruitment will occur at Brigham and Women's Hospital (BWH) in Boston in coordination with TMC. Potential participants in the Lifestyle Intervention in Preparation for Pregnancy (LIPP) study will be recruited from eligible women who have delivered infants at BWH. Women will be approached on the Labor and Delivery and Postpartum units of BWH following delivery of their infant following a screening of medical records for exclusions including entering restrictive charts ('breaking the glass'). Women who express interest will be given an IRB approved flyer and contact information will be collected. Contact information will be transferred to Tufts study staff via a Mass General Brigham maintained secure Dropbox. Tufts study staff will reach out by email, phone, or mail to women who agree to be contacted and will offer study participation. As an alternative to in-person recruitment, women who are missed in-person will receive a letter via the Mass General Brigham patient portal with an opt-out option. If women do not opt-out, the LIPP study staff will contact them in 3-4 weeks via email or phone number by way of the listed contact information in their medical records. All subsequent study procedures will be conducted at TMC and BWH for those women who elect to participate. For women who deliver at MGH or BWH, delivery protocol will be followed accordingly for each site. At the time of delivery, LIPP study staff from all Boston-area sites (MGH, BWH and TMC) will collect study sample data, maternal and infant data from medical charts, and conduct maternal questionnaires.

- *When, where, and how potential subjects will be recruited.*
  Brigham and Women's Hospital (BWH) in Boston, MA.
- *Methods that will be used to identify potential subjects.*
  Similar to what we will use at Tufts. Data will be provided if requested and have IRB approval.
- *Materials that will be used to recruit subjects.*
  Submit a copy of each recruitment-related document with the application. For advertisements, attach the final copy of the print advertisement. When advertisements are audiotaped or videotaped for broadcast, attach the final audio/video. You may submit a script of the proposed audio or video advertisement for IRB review before taping the final version in case revisions are requested. Once recommended changes are made, the final version is to be submitted to the IRB for review and final approval.

4.4.3 Payment

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

☑ **Yes** ☐ **No**

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

- *Any proposed payment or incentive for subjects (e.g., a specific description of the incentive, its value both in US and local currency [if international], local household income information, provided to whom). State if the payment/incentive value could pose undue influence on the subject's decision to participate. Subjects in the intervention group*
- *Payment amount.*

PAYMENT

Subjects randomized to the LIPP group, will receive up to approximately $2500 of value as follows:

- $40 for the first study metabolic visit, $50 for the second study visit, and each study visit will then increase by $10. The total compensation of the seven study visits is $490 for participation in this study.
- The study will provide 750 ml bottles of extra virgin olive oil (EVOO) throughout the study.
- If a subject withdraws from the study, they will be paid for the portion of the study that they have completed.
- After delivery and the measurements of the baby have been completed, subjects will receive a $50 gift card for completing the study.
- Subjects will be compensated for the cost of parking while at the research visit.
- Taxi or public transportation services to and from the study visit can be arranged at no expense to subjects, if needed. Costs for childcare may be reimbursed during LIPP visits or training if needed.
- A FitBit system to track step count, exercise time, and body weight throughout the study will be provided.
- Subjects will receive free access to the exercise facilities used in the study or we will provide a membership to an exercise facility until subjects become pregnant or for a period of up to 24 months.

Subjects randomized to the usual care (control) group, will also receive up to approximately $2250:

- $250.00 per each of the 7 metabolic visits, with a $500.00 study completion bonus at the end of the study. The maximum number of visits is seven (5 postpartum and 2 during pregnancy) for a total payment of up to $2,250.
- Subjects will be compensated for the cost of parking while at the research visit.
- Taxi or public transportation services to and from the study visit can be arranged at no expense if needed. Costs for childcare may be reimbursed during LIPP visits or training if needed in accordance with the local site average amount per hour for the duration of the visit (see below- LIPP Childcare).
- *How payment will be made (e.g., cash, check, Greenphire ClinCard).*
  Payment will be made using a pre-paid debit card, i.e. Greenphire ClinCard. Subjects who are legally unable to accept payment from Tufts Medical Center (e.g. subjects on a student or work visa) will receive store or credit card gift cards as tokens of appreciation.
- *To whom payment will be made (subject, parent [which one], LAR).*
  Payment will be made to the study participant.
- *When payment will occur.*
  Payment will occur after each study visit and after delivery and measurement of the baby.
- *The payment schedule to be used, including the payment schedule and amount for subjects who withdraw or are withdrawn from the study.*
  Please see above.

4.4.4 Reimbursement

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

☑ **Yes** ☐ **No**

If **Yes**, describe the reimbursement plan and procedures, including*:*

- *What qualifies for reimbursement and whether pre-approval is needed.*
  All subjects will be reimbursed for parking or transportation for study visits. Subjects in the intervention arm of the study will be reimbursed for a membership in an exercise facility, transportation to the exercise facility and childcare if needed.
- *How subjects will be required to document expenses for reimbursement (e.g., provide receipts).*
  Expenses will be paid either through providing receipts or through arrangements with the exercise facilities directly to the LIPP grant through Research administration.
- *How and how often subjects will request reimbursement.*
  Subjects can request after each study visit. Subjects in the intervention arm can request reimbursement on a monthly basis for transportation to exercise facilities, travel to the exercise facility or childcare if needed.

- *How reimbursement will be made (e.g., cash, check, Greenphire ClinCard).*
  Greenphire ClinCard
- *The reimbursement schedule (when will subjects be reimbursed)*
  Please see above.

Consider developing a handout to provide to subjects detailing the above information. NA

LIPP Childcare

In order to support the LIPP research and create ease for participating mothers, the LIPP Study will offer support to mothers with childcare costs for the duration of a visit. This will be done when requested by participants. Participants will be required to show a form of evidence of cost (i.e., receipt, Venmo screenshot, etc.) for the study records. Upon approval by coordinator and/or principal investigator, participants will be reimbursed through their ClinCard. In accordance with the 2020 regional average of babysitting childcare in the Boston area, the study will reimburse up to $19/hour during the allotted visit time. Below are the expected visit durations and extent of coverage possible:

Visit 1-5: 6 hours → childcare covered up to 7 hours x $19 = $133.00

5 Study Design

5.1 Study Timelines

- *Describe the duration of an individual subject's participation in the study.*
  The time that the subject will be in the study is variable. The minimum time is 13 months (4 months in study and 9 months of pregnancy). The maximum time is 28+ months. The pre-pregnancy component of the study is variable. We list 18 months in study/21 months post-partum but if the subject becomes pregnant after 21 months post-partum, she will be followed in the pregnancy component. Hence, we list 28+ months.
- *Describe the duration anticipated to enroll all study subjects at the Tufts study site.*
  Subjects will have their initial testing and randomization 3 calendar months (+/- 3 full weeks) after their delivery. Enrollment begins when the Informed Consent is signed.
- *Describe the estimated date for investigators to complete this study (complete primary analyses).*
  The initial grant ended on 11/30/2022 and we are on an approved no-cost extension until 11/30/2023.
- *Describe the study procedures that will be accomplished at each study visit.*
  At the HNRCA, CTRC or BWH Research Facility, the following metabolic testing/procedures will be performed:

  Metabolic evaluations will be performed at baseline (3 months ± 3 full weeks postpartum). Follow-up evaluations will be performed after 6 (± 3 full weeks) and 9 months (± 3 full weeks), 15 (± 3 full weeks) and 21 months (± 3 full weeks) prior to pregnancy (Figure at the top of the protocol form) up to a maximum of 18 months. Once a subject becomes pregnant and has dating and viability confirmed by ultrasound, metabolic evaluations will continue at 12-16 and 32-36 weeks of gestation. However, if an individual participant has scheduling issues or there are extenuating circumstances with scheduling visits, we may schedule outside of the above windows.

  Maternal Body Composition: Anthropometry measurements will include height, weight, and hip and waist circumferences. Total body fat will be measured by whole body plethysmography (Bod Pod; Cosmed, Rome, Italy), (70). We will use a hydration constant of 76% for fat free mass during late pregnancy (71). If a BodPod is not available we will use skin calipers to take skinfold measurements of the triceps and mid-axillary skinfold to estimate body fat.

  Resting Energy Expenditure: Resting Metabolic Rate (RMR) will be determined after an overnight fast using the Cosmed OMNIA metabolic cart (Cosmed, Rome Italy) with a

canopy system. Participants will relax in a quiet low-light metabolic room for 30 min prior to obtaining a 30-min measure of exhaled breath. Oxidative glucose metabolism will be estimated and urine samples will be obtained before and after the measure in order to calculate non-protein RQ (NPRQ). These data will be used in conjunction with Specific Aim 2 and will be correlated with placental mitochondrial function during pregnancy.

Exercise Capacity: An incremental graded treadmill test will be performed at baseline (3), 6, 9, 15, & 21 months postpartum time points in both groups. In addition, for the intervention group additional VO_2 max testing will be performed at approximately 4 & 5 months postpartum. Oxygen consumption, heart rate, and ratings of perceived exertion will be performed as previously described (48, 64, 65, 72).

Insulin Sensitivity and β-Cell Function: A 75 g oral glucose tolerance test (OGTT) will be used to assess postprandial glycemia, and insulin sensitivity and secretion. After an overnight fast, blood samples will be drawn at -10, 0, 30, 60, 90, 120, & 180 minute time points. C-peptide data will be analyzed using a combined model approach to provide pre-hepatic insulin secretion rates, insulin sensitivity, disposition index, and relate insulin and C-peptide kinetics (73). Plasma glucose will be measured using the glucose oxidase method (YSI; Yellow Springs, OH). Insulin will be assayed by RIA (Millipore, Billerica, MA). A fasting blood sample will be collected in lieu of the OGTT during pregnancy visits when there is a lack of funding. The OGTT will be collected during pregnancy again if additional funding is secured.

Enteroinsular Axis Responses: Plasma samples (with appropriate additives) will be obtained to measure incretin hormones (glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP), and satiety-related gut peptides (cholecystokinin (CCK), and peptide YY (PYY). Measurements will be made under static (fasting) and dynamic (glucose-stimulated) conditions. Blood samples for these incretin measurements will not be collected when there is a lack of funding, but may be collected again if additional funding is secured.

Metabolic and Inflammatory Biomarkers: Fasting blood samples will be obtained to measure CBC, TSH, HbA1c, lipid panel, and total free fatty acids (FFA). Adipocytokines (adiponectin, leptin interleukin-6, interleukin-8, TNF-α and hsCRP) will be measured using ELISA (R&D Systems, Minneapolis, MN). All samples from each subject will be stored at -80ºC and run in the same assay at completion to decrease variability.

Questionnaires: IRB approved questionnaires will be administered at each visit that capture changes in lifestyle, mood, breastfeeding status, diet and exercise. These data will provide a generic measure of physical and mental health through assessment of physical functioning, bodily pain, limitations due to physical, personal, or emotional problems and well-being, energy/fatigue, and general health perceptions. During pregnancy the questionnaires will be administered at 12-16 and 32-36 weeks gestational age, either at visits or over the phone. Diet information may be collected via an electronic interface, the ASA24 (see “Electronic Diet Data Collection” below).

For women who deliver at MGH or BWH, delivery protocol will be followed accordingly for each site. At the time of delivery, LIPP study staff from all Boston-area sites (MGH, BWH and TMC) will collect study sample data, maternal and infant data from medical charts, and conduct maternal questionnaires.

Addendum: In cases where there are adverse conditions in which samples cannot be obtained for lab results or in instances where there is equipment failure, measures may be repeated for accurate time point data with the participant's consent.

Neonatal measures

Measuring Fat Mass in the Infant: We have extensive experience in estimating body composition in neonates (77,78,79,80,81,82,83,84,85,86,87,88), and were one of the first centers to procure a Pea Pod (pediatric air densitometry), which is housed in the neonatal intensive care nursery at TMC.

Insulin Resistance at Birth: At birth we will obtain umbilical cord blood for insulin, C-peptide and glucose to estimate insulin resistance using HOMA (89). Full lipid profile, CRP, and the adipokines IL-6 and leptin (an excellent marker of neonatal fat mass) will be measured in cord blood as described above.

Usual Care Group

- Subjects assigned to this group, will receive normal standard of care that includes advice and recommendations to improve their health and will be monitored by the study staff. This will be provided via electronic communication or at the time of the exercise capacity test at the HNRCA or BWH Research Facility.
- Subjects receive information on post-pregnancy diet/weight loss from our research staff.

Lifestyle Intervention in Preparation for Pregnancy Group (LIPP)

- Subjects randomized to this group will participate in a supervised diet and exercise program. They will work with personal trainers and Lifestyle Coaches to assure safety and compliance to the intervention.
- **Dietary Intervention (Weight Loss)**: During the first 12-weeks of lifestyle intervention, we will lower the amount of food subjects eat daily with an emphasis on eating healthy foods, and replacing those unhealthy foods with healthy fats, like the fats found in extra virgin olive oil (i.e., a Mediterranean dietary pattern). To help with this change, subjects will be provided with 750 ml bottles of extra virgin olive oil as needed throughout the study. Every 2 weeks, subjects will record their food intake for 24 hours using a phone app. Nutrition counseling will also be performed utilizing an app. The app is called MyFitnessPal. It is free and will be provided with instructions on how to download it (from the App Store). It consumes approximately 160 megabytes, which is a fraction of a percent of a typical 15 GB phone and will sync with the FitBit. For subjects who have difficulty in tracking meals, a variety of behavioral techniques (goal setting, self-monitoring, and general nutrition education) as administered by the lifestyle interventionist will be used to increase compliance.
- **Supervised Exercise Intervention**: During the first 12-weeks of the program, subjects will exercise 3-5 days each week for up to 60 minutes each day. The exercise will consist of walking/jogging on a treadmill, cycle and/or elliptical. Sessions will be conducted at the HNRCA, BWH Research Facility or an exercise/fitness facility in their area. Subjects will not be required to have a pregnancy test for each of their supervised exercise sessions. However, if they think they may be pregnant they will be told to inform their trainer. Subjects will be instructed to keep their heart rate within a prescribed limit (80-85% of maximum) and will be provided with a heart rate monitor and watch to make it easy to check their heart rate. They will also be provided with a FitBit system (including an Aria electronic scale) to track step count and metabolic functions (e.g. heart rate, body weight). They will be allowed to keep the FitBit at the conclusion of the study. Subjects will be instructed to wear the FitBit throughout the day and a heart rate monitor when

exercising and to notify the investigators if there is any change in their medications (prescription, over-the-counter, herbals) during the period of this study. All exercise sessions will be recorded in an exercise training log and supervised by a certified exercise physiologist/trainer. There are cases where this supervision may occur remotely if necessary.

- **Dietary Intervention (Weight Maintenance):** During the first phase of weight maintenance (2A) subjects will attend approximately 2 supervised group sessions per week. Each session will last approximately 60 minutes. These sessions will include structured exercise (e.g., Zumba, jazzercise, stroller walking) and we will review subjects' diet records.
- After 3 months and the desired weight loss, subjects will move to the next Phase (2B). During this phase, subjects will be required to attend approximately 1 supervised session per week. If they do not maintain their weight loss, they will return to the previous phase for more supervised weight management. Alternatively, if they maintain their weight loss after 3 months, they will progress to the third Phase (2C) of the program until they become pregnant.
- The third phase (2C) consists of no supervised exercise sessions. However, subjects and their Lifestyle Coach will talk on the phone weekly to review their progress, including FitBit exercise data, weight, and diet. Each telephone call is expected to last approximately 5 minutes.
- **Electronic Diet Data Collection:** Dietary assessment is a standard-of-practice for clinical trials. The ASA24, or Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool, is a web-based tool that enables multiple, automatically coded, self-administered 24-hour dietary recalls. The ASA24 guides participants through steps to record all foods and beverages they have consumed over 24-72 hours. The ASA24 was developed by the National Cancer Institute at the National Institutes of Health and has been validated for accuracy.
- Participants will be assigned an ASA24 ID, Username and Password from each study site.
- The 24-hour recall usually takes 20–30 minutes, a similar length of time if an in-person 24-hour recall was conducted.
- The format and design of the ASA24 are modeled on the interviewer-administered Automated Multiple Pass Method 24-hour recall developed by the US Department of Agriculture (USDA). This recall requires multi-level food probes to accurately assess food types and amounts.
- The ASA24 software (most recent version ASA24-2020) is programmed with a food database which includes all foods available from USDA's Food and Nutrient Database for Dietary Studies (FNDDS) database. In addition, the software includes pictures of foods in multiple portion sizes to help respondents estimate portion size.
- Data files include nutrients, foods, pyramid food groups, and Healthy Eating Index (HEI) variables. The software can quickly compute nutrient and food group estimates for each recall day. The software also has the capacity to accommodate languages other than English.

✷ **Respondent confidentiality is maintained within ASA24**

- ASA2 does not collect any identifying data about Respondents, either from Researchers or directly from Respondents.
- Respondent data are protected by industry standard security controls. All data entered into ASA2 at the Respondent's computer are encrypted by the Internet browser (e.g., Internet Explorer, Firefox) before they are transmitted to ASA24 servers using Secure

Socket Layer protocol, or SSL. SSL allows for the authentication of the sending and receiving computers.

- Only a particular study's investigator (study PIs, intervention staff) and the ASA24 operations team can access response data using usernames and strong passwords.
- IP address information is accessed for the purpose of routing information between the server and the Respondent's computer. Often, the IP address is that of the user's Internet Service Provider (ISP). IP addresses are not stored or tracked by ASA24.

Virtual Sessions

- Counseling sessions for either the control or intervention groups may be conducted via a virtual format (Zoom, etc).
- These sessions may be one-on-one or group sessions discussing the control/intervention counseling information described above.
- Participants will be provided a secure link and, if it is a group session, advised on the group nature of the sessions. Participants are asked NOT to share the personalized, secure link to anyone else to protect the privacy of each participant.

For both groups, once healthy pregnancy is confirmed, we will notify subjects' obstetrical healthcare provider regarding their participation in the study. Subjects will be asked to follow a diet for healthy weight gain during pregnancy and to maintain moderate physical activity based upon their level of physical activity before pregnancy based on ACOG guidelines and their primary obstetrical care provider. All subjects will be provided with the ACOG patient information guides on healthy eating and exercise during pregnancy. They will have a repeat of all the studies excluding VO2 max testing at TMC, the HNRCA or BWH Research Facility as described previously at 12-16 weeks and 32-36 weeks of pregnancy.

Below is a detailed schedule for all planned evaluations and screenings associated with the study as described above, if additional funding is secured:

	3 months post-partum	Randomization	4 months post-partum	5 months post-partum	6 months post-partum	9months post-partum	15 months post-partum	21 months post-partum	Pregnancy	12 - 16 weeks	32 - 36 weeks	Delivery	
CRU Visit - LIPP & Usual care groups													
Baseline Laboratory studies	X												
Resting Metabolic Rate	X				X	X	X	X		X	X		
OGTT	X				X	X	X	X		X	X		
Body composition	X				X	X	X	X		X	X		
Neonatal measurements													X
Placental measurements													X
LIPP Group													

Exercise Tolerance	X		X	X	X	X	X	X					
Nutritional assessment	X		X	X	X	X	X	X		X	X		
3-5 Days/Week Supervised exercise intervention	X		X	X	X								
Weight maintenance phases 2A, 2B or 2C						X	X	X		X	X		
Usual care group													
Exercise Tolerance	X				X	X	X	X					
Nutritional assessment	X				X	X	X	X		X	X		

Below is the schedule for all planned evaluations and screenings, if there is a lack of funding:

	3 months post-partum	Randomization	4 months post-partum	5 months post-partum	6 months post-partum	9months post-partum	15 months post-partum	21 months post-partum	Pregnancy	12 - 16 weeks	32 - 36 weeks	Delivery	
CRU Visit - LIPP & Usual care groups													
Baseline Laboratory studies	X												
Resting Metabolic Rate	X				X	X	X	X		X	X		
OGTT	X				X	X	X	X					
Fasting Blood Draw										X	X		
Body composition	X				X	X	X	X		X	X		
Neonatal measurements													X
Placental measurements													X
LIPP Group													
Exercise Tolerance	X		X	X	X	X	X	X					
Nutritional assessment	X		X	X	X	X	X	X		X	X		
3-5 Days/Week Supervised exercise intervention	X		X	X	X								
Weight maintenance phases 2A, 2B or 2C						X	X	X		X	X		
Usual care group													
Exercise Tolerance	X				X	X	X	X					

5.2 Procedures

- Is there a placebo control arm?

  ☑ **Yes** ☐ **No**

  If **Yes**, describe the following:
  - *The scientific, methodological, and medical reasons to use a placebo.*
    There is no placebo per se. We are using the term usual care.
  - *The care that will be given to subjects who receive placebo. If this is local standard of care, specify that.*
    Local standard of care plus more detailed information about healthy eating and exercise from study staff at the time of the visits or via electronic communication.
  - *All potential risks to the placebo group and how the risks will be minimized.* NA
- *Provide a description of all research procedures being performed as follows:*
  - *How individuals will be screened for eligibility. Specify screening that will take place prior to informed consent and screening that will take place after informed consent.*
    Screening before consent will be performed by a review of the delivery logs on Labor and Delivery on a daily basis using the screening log form. Contact information with be obtained and the subjects who express interest before their postpartum visit will be met by study staff to review in detail the study objectives, protocol and procedures.
  - *Procedures being performed to monitor subjects for safety or to minimize risks.*
    Study subjects in the intervention arm will have frequent contact with lifestyle trainers and nutritionists initially approximately 3 times/week, then approximately 2 times, then approximately one time per week in person. Assuming the subject is maintaining her weight contact will then be continued with frequent phone calls and via email, text or downloading of data from Fit Bit.
  - *All drugs and devices used in the research, their regulatory approval status, and the purpose of their use.* NA
  - *The source records that will be used to collect data about subjects. (Attach all surveys, scripts, and data collection forms.)*
    Please see attached documents.
- *Describe the following concerning pregnancy testing and birth control:*
  - *Whether pregnancy testing will be conducted on women of reproductive potential? If testing will not be conducted, provide the reason.*
    Pregnancy testing will be determined on all pregnant women at the time of metabolic visits. Additionally, a POC test will be performed by LIPP staff when a VO_{2max} test is done outside of the metabolic visit. We will not do pregnancy testing once the subjects enter the pregnancy component of the study.
  - *What birth control methods **women** of reproductive potential will be instructed to use. If women will not be instructed about acceptable methods of birth control, clarify why.*
    Subjects are instructed to use birth control for at least 7 months post-delivery. The choice of birth control will be noted but the choice will be up to the subject and her medical provider.
  - *What birth control methods **men** of reproductive potential will be instructed to use. If men will not be instructed about acceptable methods of birth control, clarify why.* NA
- *Specify which procedures, tests, visits, etc. described above are part of usual standard of care at Tufts and which are performed solely for research purposes. All the studies obtained as part of the protocol are for research purposes.*
  Subjects will be given a copy of their results to share with their health care provider.
- *Clarify which tests are routinely performed for clinical care, but are providing data for the research, and which tests are only performed for research purposes.*

None of the testing performed as part of the protocol is for clinical purposes. Subjects are free to share the information, for example, the results of the OGTT, with their health care provider.

5.3 Evaluations

Will you perform any laboratory tests for this study?

☑ **Yes** ☐ **No**

If **Yes**, describe the following:

- *List all laboratory tests to be done as part of the study (e.g., hematology, clinical chemistry, urinalysis, pregnancy testing).*
  CBC without differential, urine pregnancy test, HbA1c, Lipid profile, Basic metabolic panel, Liver panel, TSH, urine urea nitrogen. The OGTT will be performed to measure insulin sensitivity and beta cell response.
- *Differentiate screening laboratory test(s) from those taken after enrollment.*
  - *List special assays or procedures required to determine study eligibility or assess the effect of the intervention (e.g., immunology assays, pharmacokinetic studies, images, flow cytometry assays, microarray, DNA sequencing). For research laboratory assays, include specific assays, estimated volume and type of specimen needed for each test. For procedures, provide special instructions or precautions. The above are the screening laboratory tests. If the subject has diabetes, hypertension, hyperthyroidism etc. she will be ruled out based on exclusion criteria. The special assays listed above are*
    NA
- *Include specific test components and estimated volume and type of specimens needed for each test.*
  The initial visit will obtain a total of ~91cc (baseline eligibility labs plus OGTT). Subsequent visits will obtain ~83cc only for the OGTT component of the study. When there is lack of funding, subsequent pre-pregnancy visits will only obtain ~49cc of blood for the OGTT and pregnancy metabolic visits will obtain ~25cc for a fasting blood sample. Blood collection procedures may revert to those previously-approved if additional funding is secured.
- *Specify laboratory methods to provide for appropriate longitudinal and cross-sectional comparison (e.g., use of consistent laboratory method throughout study, use of single, central laboratory for multi-site studies).*
  The glucose from the OGTTs will be obtained real-time onsite or in the MIRI lab. The estimated intra-assay coefficient of variation is ~ 2%. The rest of the laboratory studies will be batched so that each subject's complete analysis is obtained at one time. The intra-assay coefficient of variation varies depending on the test but is generally less than 5% and the inter-assay coefficient of variation is generally less than 10%.
- *If more than one laboratory will be used to perform study tests, specify which evaluations will be done by each laboratory.*
  The Tufts Medical Center and Brigham & Women's Hospital labs will perform the routine laboratory studies at the 3-month postpartum metabolic visit. A urine pregnancy test as a POC test will be performed before each metabolic visit, except the pregnancy visits; additionally, a urine pregnancy POC test will be performed by LIPP staff when a VO_{2max} test is done outside of the metabolic visit. The glucose and cytokine measures will be performed in the Maternal Infant Research Institute (MIRI) and BWH Research Facility, and the insulin and enteroinsular axis response will performed at the Pennington Biomedical Research Institute.
- *Specify if the laboratory tests that will be performed are in compliance with Clinical Laboratory Improvement Amendments (CLIA) of 1988. If not, explain why.*
  All laboratory tests performed in the TMC and BWH hospital laboratories will be performed in compliance with Clinical Laboratory Improvement Amendments (CLIA) of 1988. The studies performed in the MIRI and PBRC will be performed for research purposes only.
- *If samples will be stored for the purpose of this study, describe the preparation, handling, and storage of specimens, including specific time requirements for processing, required temperatures,*

aliquots of specimens, where they will be stored, how they will be labeled, and what will happen to the samples after the study is over (e.g. will be destroyed).
Samples from CTRC or the HNRCA will be placed on ice and then brought to the MIRI during the 3 hour OGTT or fasting blood draw, or processed on site. Samples will be aliquoted in separate vials in the MIRI to avoid freeze thaw cycles. Samples will be bar coded using a unique identifier code for subject name and study visit. Samples not run real time, will be stored in -80°Cfreezers in the MIRI. Samples not used at the completion of the study will be destroyed. The cord blood samples at the time of delivery will be brought to the MIRI for processing (glucose), and then aliquots stored at -80°C for analysis of insulin, cytokines and lipids. Samples from the BWH Research Facility will be handled in a similar manner but in the BWH research lab.
The placentas obtained at the time of delivery will be fixed for histological analysis, submerged in RNA Later, or snap frozen in liquid nitrogen and stored at -80°C for molecular analysis. For a subset of the subjects, we will obtain fresh placental tissue for lipid metabolism activity assays.
After participants have completed the study, insulin, c-peptide, and incretin samples will be sent to the PBRC site for analysis. Similarly, inflammatory markers, other biomarkers as noted and placental samples, will be shipped to TMC or BWH for analysis. An MTA will be obtained from the respective sites prior to shipping.

5.4 Collection and Storage of Human Biological Specimens (Tissue Banking)

Will biological specimens be stored for **future, unspecified**, research?
Yes, please see Form 6 and attached letter.

x ☐ **Yes**

6 Ethics and Protection of Human Subjects

6.1 Informed Consent Process

Will subjects be required to provide informed consent?

☑ **Yes** ☐ **No**

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

- *Where the consent process will take place (e.g. a private clinic room.)*

Informed consent will take place at the postpartum visit or at the time of the 3-month postpartum metabolic visit before testing.

- *Anticipated amount of time a potential subject will have to make a decision about participation in the study.*

Three calendar months.

- *Processes to ensure ongoing consent throughout the study.*

Frequent visits and contacts and the ability to take the consent form home before needing to sign consent at or prior to the baseline visit. In addition to the re-consent process, participants will be asked to provide verbal consent at the beginning of their early pregnancy visit (12-16 weeks gestation). Verbal consent for neonatal measures will also be collected after participants deliver.

- *State if you will follow "SOP: Informed Consent Process for Research (HRP-090)".*

We will follow the SOP: Informed Consent Process for Research (HRP-090)".

- *Non-English Speaking Subjects* NA
  - *Describe the consent procedures that will be used to enroll non-English speakers (for example, the use of IRB approved Short Forms per the IRB's Short Form policy). If IRB approved Short Forms will not be used describe who will conduct the consent interview, use of interpreters, use of IRB approved translated documents, etc.*
  - *If non-English speakers are being excluded, provide the ethical and scientific justification, including whether this would be equitable. For example, if non-English speakers are eligible for the study and could potentially benefit from participation, it would not be equitable to exclude them.*

The study is very detailed and involves 1-2 years of participation in the program. If we are able to hire a research coordinator who is proficient in a language other than English we will consider recruiting non-English speakers. We will consider the use of interpreters for non-English speakers after the study is up and running at TMC/HNRCA and BWH.

Refer to the IRB Short Form Policy on the IRB website for information on enrolling non-English speakers.

- *Process to Document Consent in Writing*
  - *State if you will follow "SOP: Written Documentation of Consent (HRP-091)". If not, describe how consent will be documented in writing.*
    We will follow the SOP: Written Documentation of Consent (HRP-091)
  - *If you will obtain consent, but will not document consent in writing, attach a consent script and confirm the following (you may use Tufts IRB ICF templates to create the consent document or script):* NA

6.2 Waiver or Alteration of Consent Process

This applies for studies where informed consent will not be obtained, required information will not be disclosed, or the research involves deception. NA

- Is a waiver or alteration of the consent process being requested for this study?
  ☐ **Yes** ☑ **No**
- *Is a waiver of the consent process being requested for parents for research involving children?*
  ☐ **Yes** ☑ **No**
- Is a waiver of the consent process for planned emergency research being requested?
  ☐ **Yes** ☑ **No**

6.3 International Research

- *Refer to the IRB's International Checklist and International Guidance to ensure that all relevant information described in those documents is included in this protocol.* NA

6.4 Confidentiality

- *Describe the procedures in place to maintain confidentiality as follows:*
  - *Where and how data or specimens will be stored.*
  - The hard copy of the information on the study record and the ICF will be stored in a locked cabinet in a locked room. The electronic version of the data will be stored in REDCap. Information that will be downloaded for analysis will be de-identified first. A copy of the ICF will be given to each subject and a copy retained in the subject's medical record. BMC, MGH & BWH will operate in a similar manner with all collected participant information being stored in a locked cabinet in a locked room. Transfer of interested parties contact information will occur via secure email or a secure Mass General Brigham maintained Dropbox.
    - *Specifically, state where the study records, including both electronic and/or paper study documents including signed ICFs/assent forms, will be retained (stored) during the study (state the location for original document plus any copies that are made, e.g., if a copy of the ICF will be retained in the subject's medical record).*
  - *How long the data or specimens will be stored.*
    Until the completion of the study and data analysis is complete.
  - *Who will have access to the data or specimens?*
    Only study-wide LIPP, BWH Research Facility, CTRC, HNRCA or MIRI laboratory staff

- *Who is responsible for receipt or transmission of the data or specimens locally?*
  LIPP study staff who are members of the MIRI (P Catalano, P O'Tierney-Ginn and study coordinator Li Yin Cheok) and BWH Research Facility. The minimum amount of information required for scheduling will be shared with laboratory or university staff that are involved in study visits. Data and specimens will be in a coded data set using a unique LIPP study code number. Physiological data will be sent via the BWH Research Facility, HNRCA or CTRC staff.
- *How data and specimens will be transported.*
  BWH Research Facility, HNRCA or MIRI LIPP study staff will transport specimens. Data and specimens will be de-identified using a unique LIPP study code number.
- *Whether data will be coded.*
  Yes we will use bar code identification
  - *If so, specify if there is a key to the code that matches the subjects' study identification number with their name and who will have access to it.*
    Any key to bar code information will be kept in the LIPP research study coordinator's office
- *Whether videotapes and/or photographs of subjects will be capable of identifying the study subject. If so, indicate who will have access to (be able to view) these items and how long the videotapes or photographs will be retained for the study and what the plan is for their destruction.* NA
- *If identifiable screening data will be retained for the study, clarify why, and who will have access to it. Submit the screening log.*
  Screening log has been submitted. Access will be through the LIPP research study coordinator and/or PI.
  It will be necessary to include HIPAA data on the screening log so that when approaching the potential subject postpartum one can refer to specific inclusion and exclusion criteria. Further, the screening log will be used as a contact source for the patient at the time of the postpartum visit and in order to contact via email or text message.
  We have been very careful about ensuring privacy of study subjects whether screened or enrolled in our studies. The paper versions of the screening log will be maintained in a locked room in a locked file cabinet. The electronic version will be kept on a Tufts Medical Center server in a secure data file. Once the data is entered onto the REDCap database maintained by LIPP study staff at PBRC, the information will be de-identified and use only a secure unique study number.
- *Clarify whether confidential genetic information will be collected from subjects. NA*
  Data and specimens should be stored in a secure location only accessible by the research team.
  The PI must ensure that study documents are stored in a manner that protects the privacy of subjects and the confidentiality of study data.

- *Indicate whether a Certificate of Confidentiality will be obtained. A Certificate of Confidentiality should be obtained for research involving collection of information that, if disclosed, could have adverse consequences for subjects or damage their financial standing, employability, insurability, or reputation. For more information, refer to NIH's Certificates of Confidentiality Kiosk.* NA

6.5 Provisions to Protect the Privacy Interests of Subjects

- *Describe the steps that will be taken to protect subjects' privacy interests. "Privacy interest" refers to a person's desire to place limits on with whom they interact or to whom they provide personal information.*
  Personal information will only be available to LIPP, BWH Research Facility, MIRI, CTRC and HNRCA study staff. All other information will be de-identified for example for statistical analysis or DSMB review.

- *Describe the steps that will be taken to make the subjects feel at ease with the research situation in terms of the questions being asked and the procedures being performed. "At ease" does not refer to physical discomfort, but the sense of intrusiveness a subject may or may not experience in response to questions, examinations, and procedures.*
  The consent form will describe the study procedures in detail. The study protocol will be explained to the subjects as well. At the time of the visit, the LIPP study staff (PI when available and study coordinator) in addition to the CTRC, HNRCA or BWH Research Facility staff will be present to answer any ongoing questions. For those subjects randomized to the intervention group, HNRCA or BWH Research Facility staff will be present during the visit as well as the training sessions. Hence, there will be continuity of staff with all subjects in the LIPP study.
- *Under what circumstances will the research team access sources of information about the subjects.*
  The only time the LIPP research team will access sources of information about potential subjects is when the postpartum delivery records are screened for subject eligibility and the outcome pregnancy and delivery.

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

- *Describe the plan to periodically evaluate the data regarding both harms and benefits to assess subject safety as follows: We will continue to have a DSMB to evaluate subject benefit and harm.*
  - Study data, including safety data, untoward events, and efficacy data, will be reviewed on a regular basis.
    Please refer to the DSMB charter.
  - *Who will review the data?*
    The DSMB this will include representatives from Case Western Reserve in Cleveland, TMC in Boston and PBRC in Baton Rouge. This has been made available to the IRB
  - *How the safety information will be obtained and documented (e.g., case report forms, by telephone calls with participants, printouts of laboratory results, etc.).*
    Per the format laid out in the DSMB which was in place in Cleveland.
  - *The frequency of data collection, including when safety data collection starts.*
    Safety collection starts when the first subject is enrolled into the study, in particular for the intervention group starting training.
  - *The frequency or periodicity of review of cumulative data.*
    This will depend on how there is progression of recruitment. In Cleveland we had a DSMB approximately twice in the first year of the grant. We will have a minimal of 1 DSMB meeting per year.
  - *The statistical tests for analyzing the safety data to determine whether harm is occurring.*
    The LIPP study statistician who will be located at Pennington Biomedical in Baton Rouge will determine this.
  - *Any conditions that trigger an immediate suspension of the research or other action for the research.*
    Any severe adverse reaction will trigger a review and determination if this was related to the research protocol.
  - *The plan might include establishing a data monitoring committee which addresses all the above.*
- *Describe the entity responsible for monitoring the data, and their respective roles (e.g., the investigators, the research sponsor, a coordinating or statistical center, an independent medical monitor, a Data and Safety Monitoring Board (DSMB) /Data Monitoring Committee (DMC), and/or some other entity, and the timeframe for reporting events to this entity.*
  - *Submit a copy of the DSMB/DMC Charter if the study is monitored by a DSMB/DMC.*
    We have submitted the DSMB Charter for the LIPP study in Cleveland as well as the two DSMB reports (both open and closed) to the Tufts Medical Center IRB.

6.7 Compensation for Research-Related Injury

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

☑ **Yes** ☐ **No**

If **Yes**, describe the available compensation in the event of research related injury including*:*

- *What this compensation will be (e.g. free medical care, payment for treatment, compensation for lost wages, dependent care, etc.).*
  There is the potential for risk to the subjects in the intervention group, sprains and fatigue after exercise. These have not been of any severe degree to affect study participation based on the first year of experience in Cleveland. There will be no compensation for any injury or loss of employment.
- *Who will provide this compensation (e.g. sponsor, institution, etc.)?* NA
- *What will count as a qualified harm (e.g., physical, psychological, economic, social, or other injury)?* Qualified harm will be determined by a serious adverse event related to the study protocol as determined by the DSMB
- *Whether only injuries "related" to study, participation will be covered. If yes, clarify how it will be determined what injuries are considered related, and who will decide this.* NA
- *Will the study distinguish between injury (short-term, resolvable) and impairment (often longer-term, potentially manageable, but not resolvable) and if so, why.* NA
- *The process subjects must follow to obtain compensation. If no funds are set aside for research-related injury and subjects and/or their insurance will have to pay for the treatment of such injuries, include a statement such as: "No funds have been set aside for research-related injury. You or your insurance carrier will be required to pay for medical care associated with your research-related injury."*
  This has been similar to the language in all of our previous consent forms.
  "No funds have been set aside for research-related injury. You or your insurance carrier will be required to pay for medical care associated with your research-related injury."

6.8 Economic Burden to Subjects

Does the research involve any costs to subjects?

☐ **Yes** ☑ **No**

6.10 Vulnerable Populations

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

☑ **Yes** ☐ **No**

If **Yes**, describe the following:

- *Any preclinical studies, including studies on pregnant animals, and clinical studies, including studies on non-pregnant women, that have been conducted that provide data for assessing potential risks to pregnant women and fetuses.*
  The preliminary data that was obtained in Cleveland was data that included the number of women who might be eligible based on inclusion and exclusion criteria. The other preliminary data was the review of the average time to the second pregnancy. None of these information provided data related to risk to subjects relative to intervention.
- *Whether the risk to the fetus is caused solely by interventions or procedures that hold out the prospect of direct benefit for the woman or the fetus or if there is no prospect of benefit to the fetus, the risk to the fetus is NOT greater than Minimal Risk, and the purpose of the research is the development of important biomedical knowledge which cannot be obtained by any other means.*

There is no risk to the fetus from participation in the study. The ACOG recommends that pregnant women partake in physical activity during pregnancy for 30 min at least 5 days a week. Our study protocol recommends that all subjects follow ACOG guidelines for diet and physical activity and gain weight according to IOM recommendations from 2009.

- *The biomedical knowledge that is expected to result from this research for this population.*
  Importance of the Knowledge Gained
  The importance of the knowledge to be gained by this proposal is the ability to determine if Lifestyle Interventions in Preparation for Pregnancy can achieve concrete (real) results in the efforts to stem the epidemic of obesity in women and their offspring. This study will also provide information as to the mechanisms of why lifestyle intervention before rather than only during pregnancy results in improved outcomes. Although the concept of pre-pregnancy lifestyle intervention has been recognized previously as potentially worthwhile, there have been few if any studies employing such a study design, because of the perceived difficulty in recruiting women prior to pregnancy. Based on our history of conducting research studies in women before during and after pregnancy, a study design of recruiting obese women immediately after pregnancy and incorporating the community into the lifestyle interventions, we believe that this approach will have applicability in many other populations.
  The risks to subjects are minimal. They are the standard of care we should provide to all our obese patients considering future pregnancy. Our ability to provide more than a minimal amount of information is hampered by the lack of resources particularly for women seeking care without considerable personal resources. If successful, as proof of principle, we believe this protocol can serve as a paradigm for a wider initiation of lifestyle interventions before pregnancy.
- *How any risk of this research is the least possible for achieving the objectives of the research?*
  Risk will be minimized by having trained lifestyle intervention staff supervise physical activity and nutritionist the healthy eating for the Intervention group. The usual care group will receive benefit from having specific goals provided by our research team but not specific follow-up per the study protocol.
- *How mothers providing consent are informed of the reasonably foreseeable impact of the research on the fetus or neonate.*
  Mothers are informed by the PI of the study and potential benefits of healthy eating and exercise before pregnancy. They will also be informed of the foreseeable impact of the research on their fetus/neonate.
- *Specify that no inducements, monetary or otherwise, will be offered to terminate a pregnancy and that in the case of a fetus, the fetus is not the subject of a planned abortion.* NA
- *Specify that individuals engaged in the research will have no part in any decisions as to the timing, method, or procedures used to terminate a pregnancy or in determining the viability of a neonate.* NA

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

☐ **Yes** ☑ **No**

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

Neonates will be included in the study as an outcome measure.

a. ☒ *Check to confirm that you will follow "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)" to determine whether a prospective subject has or has not attained the legal age for consent to treatments or procedures involved in the research under the applicable law of the jurisdiction in which the research will be conducted. (e.g., individuals under the age of 18 years). If this SOP will not be followed, describe how this (attainment of legal age for consent or not) will be determined:*

b. *How permission to participate in the study will be obtained from the parents or legal guardians:* See Informed Consent section above. Permission will be obtained from parents or legal guardians as described in that section.

c. *The assent process of children as follows:*
   i. *Any waiting period available between informing the prospective subject and obtaining the assent:* N/A
   ii. *Any process to ensure ongoing assent:* N/A
   iii. *Research team members involved in the assent process:* N/A
   iv. *How long children will have to consider study participation:* N/A
   v. *Steps that will be taken to minimize the possibility of coercion or undue influence:* N/A
   vi. *Steps that will be taken to ensure the subjects' understanding:* N/A
   vii. *If assent will not be obtained from children, specify why:* All children enrolled will be infants without the capacity to provide informed assent.
   viii. *If children reach 18 years of age while in the study describe the following:*
      1. *The plan to obtain written informed consent from the subject at age 18 years:* N/A
      2. *Who will be responsible for managing the plan:* N/A
      3. *Where the consent discussion will take place:* N/A
      4. *What will happen if the subject cannot be located to provide consent at age 18 years:* N/A

Will minors under the age of 18 who are:
   i) married, widowed, divorced; or
   ii) the parent of a child; or
   iii) a member of any of the armed forces; or
   iv) pregnant or believes herself to be pregnant; or
   v) living separate and apart from his/her parent or legal guardian, and is managing his/her own financial affairs

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

☐ **Yes** ☑ **No**

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

☐ **Yes** ☑ **No**

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

☐ **Yes** ☑ **No**

Will prisoners be enrolled?

☐ **Yes** ☑ **No**

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

☑ **Yes** ☐ **No**

If **Yes**, describe the following:

- *Justification for specifically recruiting and enrolling students and/or employees into the study.*
  Women who are overweight or obese delivering babies at TMC will be given the opportunity to enroll like any other subject who meets inclusion criteria.
- *How potential coercion will be eliminated.*
  By strictly following inclusion and exclusion guidelines.
- *Recruitment methods to be applied specifically to students or employees. If the same recruitment methods previously described in the protocol will be used, then state that.*
  N/A, The same criteria used for recruitment will apply to all potential subjects. No specific recruitment aimed at employees

- *Additional safeguards included to protect the rights and welfare of students and employees.*
  No other than has already been described.
- *Protections to ensure that a subject's participation or early withdrawal from the study will not affect his/her status as a student or employee. Per the statements in the approved subject consent form*
- *Submit a letter from the appropriate institutional official (e.g., Department Chair, Vice-President) who oversees the students and/or employees attesting to the fact that the employee or student's participation in the research is acceptable and that coercion has been minimized.*

7 Adverse Event Monitoring

7.1 Definitions

- *Define adverse events (AEs), serious adverse events (SAEs), and unanticipated problems for your study.*
  Please refer to the DSMB definitions

During the course of the LIPP study, all adverse events (AEs) and serious adverse events (SAEs) will be monitored, documented, and if causal reported to BWH, TMC and the PBRC and the Data Safety Monitoring Board (DSMB). The Chair of the DSMB will be located at the PBRC. Every attempt will be made to follow a subject who has had negative reaction to any study intervention or procedure.

General Reporting Guidelines

Adverse Events (AEs)

An Adverse Event (AE) is any unfavorable medical occurrence, which may include abnormal signs (for example, abnormal physical exam or laboratory finding), symptoms, or disease, temporally associated with, but not necessarily considered related to, the subject's participation in the LIPP research study. These events are usually of a non-serious nature and are not reportable in accordance with the Institutional Review Board guidelines of the respective hospitals/ Universities for "Reportable Events," as they do not pose risk to study subjects.

Adverse events that take place during the course of the LIPP study will be classified under the Common Terminology Criteria for Adverse Events (CTCAE), a descriptive terminology used for Adverse Event (AE) reporting. AEs will be categorized as expected or unexpected in terms of their nature, severity, or frequency as outlined in the consent form. AEs will be graded using the CTCAE system and relatedness assessed by the PI and presented to the DSMB at DSMB meetings.

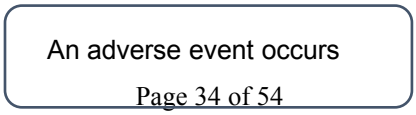
Serious Adverse Events (SAEs)

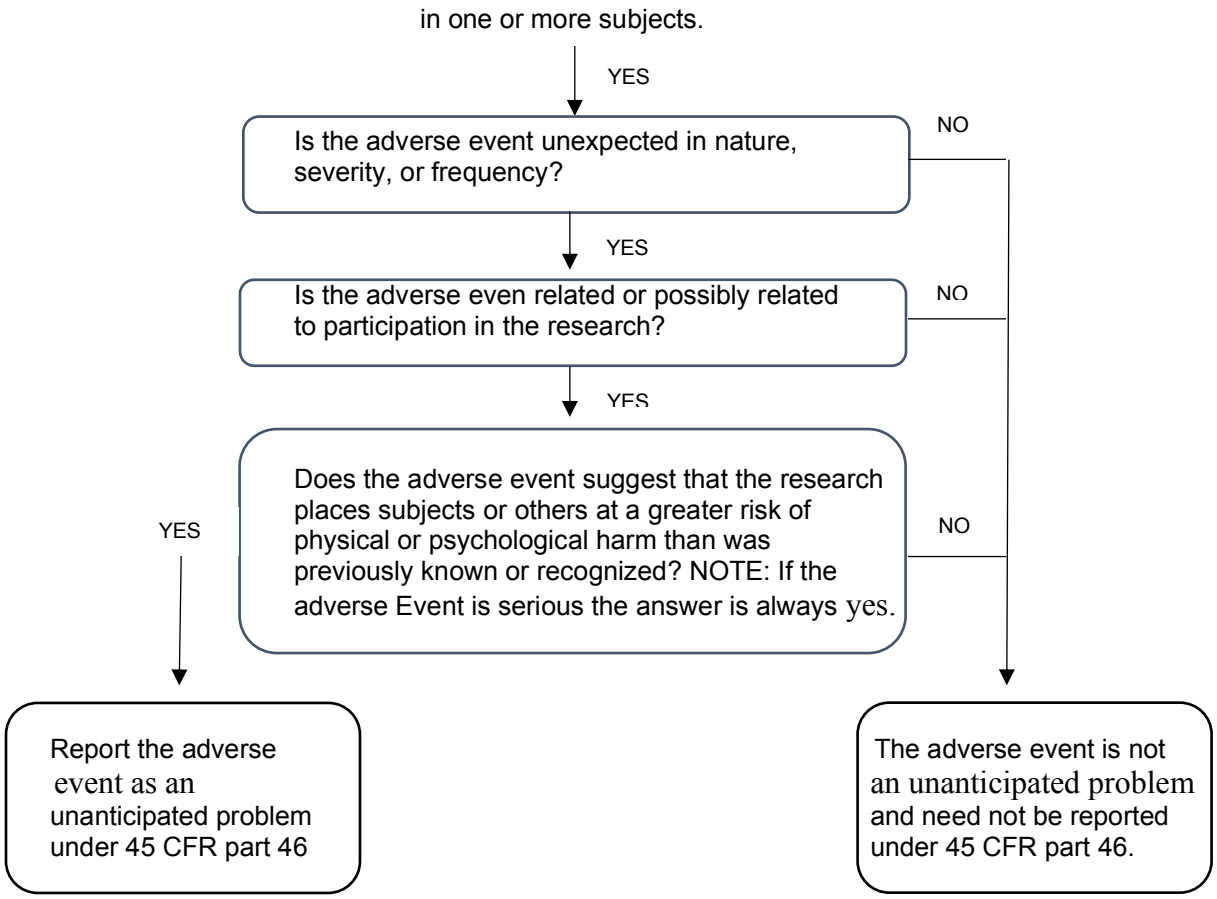
Serious Adverse Events (SAEs) or "Unanticipated Problems Involving Risk to Subjects or Others," are characterized as symptoms which are harmful to the subject and result in significant outcomes which are reportable to the respective IRBs and the study's DSMB.

Examples of serious adverse events are:

- Death
- Life threatening medical events that place a subject at immediate risk of death from the event as it occurred
- Results in impatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity as a result of the study intervention
- Events 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.

The flow chart below provides an algorithm for determining whether an adverse event meets the definition of an "unanticipated problem involving risk to subjects or others."





Adverse Events which follow the above figure, that meet the criteria of unexpected, fatal or life-threatening, and related or possibly related to the research intervention must be reported to the IRB and the DSMB within 24 hours of learning of the event. If necessary, a subsequent follow-up report with the details of the event will be submitted as well. All other reportable adverse events will be submitted to the IRB within 10 working days of the investigator learning of the event. Reportable events will be submitted through the respective IRB's system.

AE and SAE Assessment Guidelines Specific to the LIPP Study

Key Criteria: to determine if the event is unexpected, related to participation in the research study and involves risk to human subjects or others.

If an adverse event meets the criterion of unexpected, related or possibly related, and poses risk of harm to other subjects, the study coordinator will notify one of the Multiple Principal Investigators, Dr. Patrick Catalano or Dr. John Kirwan, within 24- hours who will then inform the DSMB Chair and the Tufts Health Sciences IRB; the reviewing IRB of record.

To evaluate AEs and SAEs, a licensed medical professional will apply the following assessment criteria:

Event Expectedness

Unexpected Medical Events:

These are the medical events that are not described in the current IRB-approved protocol or informed consent, taking into account the characteristics of the participant population being studied (e.g. pregnant population).

Expected medical events:*

- Symptoms and risk related to normal pregnancy progression such as nausea, vomiting, fatigue, urinary frequency, increased or decreased appetite, transient dizziness, mild back or breast pain or discomfort, mild mood changes, miscarriage, preterm delivery, still birth, low birth weight, problems with placenta and birth defects,

- Symptoms related to fasting for at least 8-hours and related hypoglycemia: headache, nausea, and lightheadedness
- Symptoms related to hypoglycemia such as fainting, fast heart rate, sweating, shakiness, difficulty paying attention, sudden moodiness or irritability, unconscious
- Symptoms related to venous blood draw such as bruising, bleeding, swelling, and pain, fainting, lightheadedness and infection at the injection site.
- Symptoms related to consuming the glucose solution for the diabetes testing such as brief nausea, vomiting, tiredness, lightheadedness and upset stomach.

* While the above medical events may be expected, they will be collected for outcome purposes.

Event Severity using Common Terminology Criteria for Adverse Events (CTCAE)

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on these general guidelines:

- Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 Moderate; minimal, local or noninvasive intervention indicated limiting age-appropriate instrumental activities of daily living.
- Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care.
- Grade 4 Life-threatening consequences; urgent intervention indicated.
- Grade 5 Death related to AE.

Event Relatedness

Related: The incident, experience, or outcome is more likely than not caused by study procedures.

- A reasonable time course would be within the 1-4 hours the subject is receiving oral glucose tolerance testing.
- Symptoms abate once the study product is discontinued and patient has received a snack or drink. Symptoms are not explained by other factors such as concomitant medications or current illness

Possibly Related: There is reasonable possibility that the incident, experience or outcome may have been caused by study procedures.

- A reasonable time course would be within 24-48 hours the subject either fasting for the study or receiving the oral glucose tolerance testing.

- Symptoms may be explained by other factors such as concomitant medications or current illness

Adverse Event

$VO_{2\ max}$	Falling off treadmill
$VO_{2\ max}$	Exhaustion
$VO_{2\ max}$	Feeling like heart is pounding very fast or very hard

$VO_{2\ max}$	Dizziness
$VO_{2\ max}$	Heart rhythm abnormalities
$VO_{2\ max}$	Chest pain
$VO_{2\ max}$	When the mask is on the face, this might feel uncomfortable
Structured exercise	Feeling like heart is pounding very fast or very hard
Structured exercise	Dizziness
Structured exercise	Heart rhythm abnormalities
Structured exercise	Chest pain
Laboratory studies	Bruising at IV site
OGTT	Nausea from glucose load
OGTT	Bruising from IV site
Resting Metabolic Rate	Claustrophobia
Bod Pod	Claustrophobia
Neonatal Anthropometrics	Bruising from skinfold calipers
Maternal anthropometrics	Bruising from skinfold calipers

Serious Adverse Event

$VO_{2\ max}$	Heart attack
$VO_{2\ max}$	Death
Structured exercise	Heart attack
Structured exercise	Death

7.2 Reporting Procedures

- *Describe the protocol-specific reporting procedures, including who will be responsible for each step (e.g., PI, Data Coordinating Center, Medical Monitor), which forms should be completed, timeframes for reporting, how reports will be distributed, and what follow-up is required.*
- *Include specific details of reporting procedures for:*
  - *Deaths, life-threatening events, pregnancies PI*
  - *Other SAEs PI or study coordinator*
  - *Other AEs study coordinator*
  - *Other UPs study coordinator*

  All the details are noted in the DSMB charter. The reporting mechanisms are noted above, since different adverse events are reported based on the degree of severity
- *Ensure that the reporting procedures meet the reporting requirements of the FDA, NIH, OHRP, sponsor, study leadership and any other regulatory body that applies to the study, as applicable.*
  NIH will receive a yearly non-competitive renewal notice of the progress of the study and any serious adverse events related to the study protocol.

7.3 Reportable New Information

- *Indicate also, that reportable new information will be reported to the IRB per the Tufts Health Sciences IRB's Reportable New Information policy. If your reporting plan to the IRB differs from the IRB's policies, please describe it in detail.* NA
  Reportable new information will be reported to the IRB per the Tufts Health Sciences IRB's Reportable New Information Policy

8 Statistical Considerations

8.1 Study Endpoints

- *Describe the primary and secondary study endpoints*.
  The primary outcome measure of this proposal is lower neonatal adiposity at birth in the LIPP group relative to: 1) the usual care/control group. As secondary outcomes we anticipate that prior to a subsequent pregnancy, LIPP will produce significant improvement (absolute and percent changes) in maternal weight and body composition, and more importantly improved insulin sensitivity, β-cell function, incretin response to glucose, lipid and inflammatory biomarkers, compared to the Control group. We also expect reduced insulin resistance, umbilical cord lipids and inflammatory profiles in babies born to LIPP subjects compared to Controls.
- *Describe any primary or secondary safety endpoints.*
  The risk of having a small for gestational age baby or preterm delivery will be followed closely in the intervention group, as this is a theoretical risk.

8.2 Statistical Analysis

- *Describe the statistical analyses that will be performed for this study*
  Statistical Analysis
  The primary analyses of Specific Aim 1a will be intent-to-treat comparisons of the LIPP and Control groups in regard to changes in maternal insulin sensitivity, BMI, and fat mass. The comparisons will be performed first with two-sample t-tests at α=0.05. Should any imbalance of confounding factors in the groups be recognized, linear regression models that include significant differences (e.g. GDM) would be used to perform covariate adjustments. Based on our 1-year postpartum follow-up studies (62,63), we will have 90% power to detect an absolute or covariate-adjusted improvement in insulin sensitivity of 30% and an 80% power to detect an improvement as small as 25% in the LIPP vs. Control group. Corresponding 95% confidence intervals (95% CI) for the absolute or covariate-adjusted differences or percent improvements in insulin sensitivity between groups will be reported. We estimate the standard deviation (SD) of change in BMI from randomization until subsequent pregnancy to be 5.1 kg/m2. We will have 90% power to detect an absolute or covariate-adjusted difference in BMI of 2.6 kg/m2 and 80% power to detect a difference of 2.26 kg/m2 and 90% power to detect an absolute or covariate-adjusted difference in fat mass of 5.9 kg and 80% power to detect a difference of 5.1 kg between groups prior to the second pregnancy.
  The primary analysis for Specific Aim 1b is an intent-to-treat comparison of LIPP versus Control neonates with respect to fat mass at birth. The comparison will be performed using a two-sample t-test at α=0.05. Linear regression will be performed, which will include weight (body composition measures) of the subject's index child as a covariate. Should any imbalance of confounding factors (for example gestational age) in the groups be recognized, linear regression models will be used to perform a covariate adjustment. Based on our preliminary data, we estimate the SD of neonatal fat mass between the LIPP and Control groups to be no more than 225 g. With at least 50 women in each group, (assuming a 50% dropout), the t-test or linear regression will have 90% power to detect an absolute or covariate-adjusted difference of 146 g fat mass between groups. We have 80% power to detect an absolute or covariate-adjusted difference as small as 126 g fat mass between groups. Corresponding 95% CI for the absolute or covariate-adjusted difference in neonatal fat mass between groups will be reported. For secondary analyses, we will be using the same

statistical approach. Based on our preliminary data, we estimate the SD of birth weight to be 700 g; with 50 neonates in each group we will have 90% power to detect an absolute or covariate-adjusted difference of 455 g in birth weight and 80% power to detect a 393g difference between groups. Additional secondary analyses will include umbilical cord cytokines. Comparisons will be performed using a two-sample t-test at a significance level of α=0.05; however, Mann-Whitney U tests or log transformations will be employed if data are not normally distributed. Linear regression models, including confounding factors, will be used to perform covariate adjustments. Based on our published data (80), we estimate the standard deviations of umbilical cord IL-6 and CRP to be 3.4 pg/ml and 7,900 ng/ml, respectively. With 50 women in each group, we will have 90% power to detect an improvement in IL-6 and CRP levels of 50% and 42%, and 80% power to detect an improvement of 42% and 36%, respectively.

The primary goal of Aim 2 is to determine the effect of lifestyle intervention prior to pregnancy on placental mitochondrial fatty acid oxidation at term. We hypothesize that placental β-oxidation will be higher in the LIPP group. We will conduct an intent-to-treat analysis using two sample t-test or non-parametric Wilcoxon rank-sum test to assess differences between groups. Regression analysis will be used to assess the association of placental β-oxidation and enzyme activity with maternal inflammatory cytokine levels and insulin resistance in early pregnancy, along with neonatal fat mass with adjustment for gestational age and gender. Descriptive statistics, such as mean, median and range will be calculated for all variables. Power and sample size analysis based on our preliminary mitochondrial β-oxidation data in obese women (38±14 nmol/mg/hr) showed that a sample size of N=18/group achieves 80% power to detect a difference of 25% between groups using a two-sample t-test with a significance level of 0.05.

- *Provide a power analysis.*
  These are included in the statistical analyses.

8.3 Number of Subjects

- *If this is a multicenter study, specify the number of subjects to be enrolled in total across all sites.*
  220.
- *Specify the number of subjects to be enrolled at the Tufts site. Subjects who sign an ICF are considered "enrolled". For studies that have a separate screening ICF, this number is the number of subjects who sign a screening ICF.* 100
  - *Provide the rationale for enrolling this number of subjects at the Tufts site.*
    This is approximately 50% of the study recruitment goals of 200. In addition to the already recruited participants in Cleveland and Baton Rouge, the Boston sites anticipate to recruit the remaining subjects to meet the goals.
  - *Estimate the number of subjects expected to be enrolled at the Tufts site (i.e. sign the screening or study ICF) as well as the number needed to complete the study at the Tufts site.*
    We anticipate recruiting 100 subjects in the Greater Boston area and expect a 40 to 50% dropout for various reasons.
  - *If a large number of withdrawals and/or dropouts are expected, explain why.*
    It is not unusual for a lifestyle intervention study lasting over a number of years to have a 40% dropout rate. This has been addressed in the statistical analysis.

8.4 Data Management

- *Describe the data analysis plan, including descriptions of the data.*
  This is described in the statistical analysis
- *Provide a power analysis.*

This is included in the statistical analysis.

- *Describe the steps that will be taken to secure the data (e.g., training, authorization of access, password protection, encryption, physical controls, certificates of confidentiality, and separation of identifiers and data) during storage, use, and transmission.*
  All research staff for the LIPP grant has undergone CITI training and have research experience. The PI of the project has 30 years of clinical research training and has not had any research violations of authorization of access, password protection, encryption, physical controls, certificates of confidentiality, and separation of identifiers and data during storage, use, and transmission. We anticipate that the study coordinator of the LIPP grant will have as a minimum a Bachelor's degree and clinical research experience.
- *Describe any procedures that will be used for quality control of collected data.*
  The Study coordinator, the PI and Statistical staff will ensure that the data has been collected and cleaned appropriately. Any data that is judged to be an outlier will be reassessed going back to the original source. In the past we have reanalyzed approximately 10% of data to make sure that results are consistent with first time analyses.
- *Describe how data and specimens will be handled study-wide:*
  - *What information will be included in that data or associated with the specimens.*
    The non-PHI study subject identifier as well as which visit the data was collected will be noted and entered into REDCap.
  - *Where and how data or specimens will be stored.*
    Data will be stored on REDCap. Hard copies will be maintained in a locked cabinet in a locked office of the study coordinator.
  - *How long the data or specimens will be stored.*
    Until the study is completed. Hard copies will be kept as long as the data are being analyzed. Non-PHI data will be stored on an encrypted hard drive.
  - *Specify who will have access to the data or specimens.*
    The PI, study coordinator and staff in the HNRCA and BWH Research Facility will have access to the data. The statistical staff and REDCap team will have access to the non-PHI data.
  - *Specify who is responsible for receipt or transmission of the data or specimens.*
    The study coordinator and PI.
  - *Specify how data and specimens will be transported.*
    By research staff from the LIPP, BWH Research Facility, MIRI and HNRCA.
  - *Specify the plan for study data retention and storage (accounting for research team turnover).*
    The PI will maintain the data along with Dr. Kirwan the MPI at the Pennington.

8.5 Randomization

Will subjects be randomized?

☑ **Yes** ☐ **No**

If **Yes,** describe the following:

- *The randomization procedures, including the ratio of subjects randomized to each study arm.*
  Yes through a computer generated randomization stratified for overweight vs. obese and breastfeeding status.
- *The blinding procedures if the study will be blinded.*
  N/A, only the statistician analyzing data will be blinded.

9 Drugs or Devices

Will the research involve drugs?

☐ **Yes** ☑ **No**

Will the research involve devices?

☐ **Yes** ☑ **No**

10 Study Administration

10.1 Setting

- *Describe the sites or locations where your research team will conduct the research.*
  At Tufts Medical Center, HNRCA, MIRI, TMC CTRC, TMC Labor and Delivery and TMC newborn nursery. At Brigham & Women's Hospital, the BWH Research Facility, BWH Labor and Delivery and BWH Newborn Nursery.
- *If the research will take place at an international site, refer to the International Guidance and International Checklist.* NA
- *Describe the following:*
- Where research procedures will be performed.
  At Tufts Medical Center, HNRC, MIRI, TMC Labor and Delivery and TMC newborn nursery. At Brigham & Women's Hospital, the BWH Research Facility, BWH Labor and Delivery and BWH Newborn Nursery.
    - *The composition and involvement of any community advisory board.*
      We will consider a community advisory board in Boston. We had such a board as part of the LIPP project in Cleveland.
    - *If a hospital stay will be required solely for research and if so, the expected duration of the hospital stays.* NA
    - *If inpatients will be enrolled, clarify if study participation will require an increase in the length of hospital stay, and if so, for how long.* NA
    - *For research conducted outside of Tufts and its affiliates:* Please refer to section *Multi-site Research*
        - *Describe the site-specific regulations/customs affecting the research*
        - *Describe the site-specific local scientific review and ethical review structure and procedures.*
        - *Describe how the study team is/will become knowledgeable of the local study site's culture, customs, regulations, and society.*

10.2 Registration

- *Describe the steps the research team will take to ensure that a subject is appropriately enrolled or registered in the study prior to receiving any study intervention (e.g. describe and submit any protocol eligibility checklist that will be used, specify who on the research team will confirm eligibility and that consent was documented, etc.).*
  The PI will be responsible to make sure that the subject has a thorough understanding of the research and procedure, the goals of the study and how they may be randomized into either study arm, i.e. usual care or intervention. The study coordinator will be a Master's or Bachelor's level person with research experience. They will obtain the written consent. The PI or Co-Investigator will review the study with the subject and will be available either in person or via telephone to answer any questions a potential subject may have before the subject has any evaluation

10.3 Resources Available

- *Specify the role and tasks delegated to each research team member.*

Pennington Biomedical Research Center – Research team members will perform analysis of data, and interactions with participants during recruitment, evaluation in the clinical research unit, for nutrition, and for lifestyle changes. Please see addendum for site specific procedures.
Massachusetts General Hospital & Brigham and Women's Hospital-- Potential participants will be recruited from eligible women who have delivered infants at MGH and BWH. TMC study staff will reach out by email, phone, or mail to women who agree to be contacted at a later date and will offer study participation. Consent and all subsequent study procedures will be conducted at Tufts Medical Center or

Brigham & Women's Hospital for those women who elect to participate. Cord blood and placental collection will be done according to standard of care at the respective hospitals. BWH or MGH LIPP research staff will collect designated tissue specimens from the research participant's hospital. Samples will be collected according to protocol, aliquoted and processed as needed and placed on ice or liquid nitrogen. The designated samples will either be transported to TMC/MIRI for further processing and storage by LIPP staff until analysis or processed and stored at BWH until analysis. Neonatal anthropometrics will be performed at the institution by LIPP staff and be used as an alternative measure for body fat if a PeaPod is not available. Neonatal anthropometrics may be conducted at the houses of LIPP participants with their consent if a participant delivers at a facility other than an approved LIPP study site and are unable to travel to BWH, MGH or TMC after delivery. Both anthropometrics and PeaPod will be performed otherwise. Additionally, for those participants who deliver at BWH or MGH, LIPP research staff will conduct study sample collection according to the site protocol. In addition to infant measures and study sample collection, LIPP study staff will conduct maternal questionnaires prior to delivery and post-delivery. Before delivery, the Infant Feeding Intention Scale will be conducted; after delivery, questionnaires will include the Breast-Feeding Self-efficacy after Delivery, Infant Feeding Practices Scale and the Holmes-Rahe Life Stress Inventory.

- *At Tufts Medical Center;*
  Patrick Catalano (PI) will oversee the recruitment, metabolic studies of the mother and her baby and data analysis.
  Dr. Michael House (CO-I) will oversee any medical findings that may come up during the course of the study as an MD on staff at TMC.
  Study coordinator (Li Yin Cheok) – recruitment and follow up of subjects for appointment and coordination of lifestyle trainers and nutritionist at TMC, HNRCA and PBRC.
- *HNRCA*
  Roger Fielding (CO-I) will oversee metabolic testing of non-pregnant subjects and supervise lifestyle trainers and nutrition staff.
- Lifestyle fitness trainer will work with subjects randomized to intervention arm and work with Drs. Fielding and Catalano to ensure fidelity to intervention protocol.
- *At Brigham & Women's Hospital;*
  Dr. Sarbattama Sen (PI) will oversee the recruitment, metabolic studies of the mother and her baby and data analysis.
  Dr. Kieran Reid will oversee metabolic testing of non-pregnant subjects, work with subjects randomized to intervention arm and work with Dr. Catalano to ensure fidelity to intervention protocol.
- Study coordinator will be responsible for recruitment and follow up of subjects for appointment at BWH and MGH.
- *Describe the qualifications (e.g., training, experience) of the PI and research team to perform their roles. Provide enough information for the IRB to determine the PI and research team are qualified to conduct the proposed research.* Please refer to the Biographical sketches of Drs. Catalano, Kirwan, O'Tierney-Ginn, Fielding and Sen.
- *Specify the coverage plan to address any issues (including subject safety issues) that occur while the PI is away and/or unavailable.*
  The research team member designated to serve, as the acting PI in the PI's absence should have similar training and expertise as the PI.
  Drs. Catalano and Fielding will cross cover for the LIPP subjects at Tufts, MGH and the HNRCA.
  Drs. Catalano and Sen will cross over for the LIPP subjects at BWH. The study coordinator and Lifestyle coach will cross cover for each other.
- *Describe other resources available to conduct the research, for example:*

- *Feasibility of recruiting the required number of suitable subjects within the proposed recruitment period. For example, how many potential subjects do you have access to? What percentage of those potential subjects do you anticipate recruiting?*
  We will have access to subjects delivering at TMC, approximately 1300/year. To increase the likelihood of meeting our recruitment goal, we will also be recruiting at BWH, who see a higher volume of deliveries; approximately 9000/yr and 3800/yr, respectively. If recruitment is not adequate, we will explore recruiting at the other hospitals in Boston for example, BI hospital.
  We are in the process of collecting demographic data from the Tufts delivery charts to determine eligibility of patients delivering at TMC.
- *Confirm that the amount of time you will spend on the research is adequate to conduct and complete the research.*
  The PI has no clinical duties and although budgeted at 20% effort will easily spend 40 to 50% effort on the LIPP project.
  Facilities HNRCA, MIRI, TMC, BWH Research Facility
- *Availability of medical or psychological resources that subjects might need TMC*
- *Process to ensure the research team members have adequate oversight and are adequately trained regarding the protocol, study procedures, and their roles and responsibilities.*
  Study staff have met once a week in Cleveland and that will continue at Tufts via telephone and Zoom conference with the PBRC and Brigham & Women's Hospital.

10.4 IRB Review

- *Specify that an appropriate IRB registered with the OHRP, will review and approve this study.*
- *Specify that any amendments to the protocol or informed consent documents will be reviewed and approved by the IRB prior to use, unless required to eliminate an apparent immediate hazard to subjects.*
  We have developed a DSMB to oversee the LIPP study.
  We confirm that any amendments to the protocol or informed consent documents will be reviewed and approved by the IRB prior to use, unless required to eliminate an apparent immediate hazard to subjects.

10.5 Multi-Site Research

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

☑ **Yes** ☐ **No**

If **Yes**:

- *Describe the processes to ensure pertinent and timely communication among sites, including:*
  - *When and how sites will be provided with the current version of the protocol, informed consent form, and other study documents.*
    When the IRB has approved the protocol and ICF this will be provided to LIPP study staff at the PBRC and BWH. The HNRCA site is functionally part of Tufts and any IRB approval for the HNRCA will go through the primary site at the MIRI.
  - *That all required approvals will be obtained at each site (including approval by the site's IRB of record).*
    This will be done before recruitment at the PBRC. At the Pennington, BWH and Tufts sites, we will recruit and follow the same protocol at each site.
  - *How all modifications will be communicated to sites, and will be reviewed and approved (including approval by the site's IRB of record) before the modification is implemented.*
    We continue to have biweekly phone conference calls with staff at TMC, BWH and the Pennington. This is currently ongoing and will continue once we have IRB approval from TMC.

- *That all participating sites will safeguard data as required by local information security policies.*
  The PIs (Catalano and Kirwan) have worked collaboratively not only on the LIPP project but also for a number of years. They have multiple projects and manuscripts where they have been co-investigators. Both are experienced and have excellent records regarding patient safety and data integrity.
- *Assurance that all local site investigators will conduct the study appropriately.*
  The PIs (Catalano and Kirwan) have worked collaboratively not only on the LIPP project but also for a number of years. They have multiple projects and manuscripts where they have been co-investigators. Both are experienced and have excellent records regarding patient safety and data integrity. The training for study staff at the Pennington has been completed as they have relocated to Baton Rouge with Dr. Kirwan. At BWH, Dr. Sen will ensure that all procedures will be conducted according to the study protocol. At Tufts and MGH, the study coordinator will work with the PI and Co-I regarding the study recruitment and protocol. We have a weekly telephone conference to review procedures etc.
- *That all non-compliance with the study protocol or other reportable new information will be reported in accordance with local policy.*
  The PI confirms that all non-compliance with the study protocol or other reportable new information will be reported in accordance with local policy.

- *Describe the method for communicating the following information to participating sites:*
  - Problems – Weekly telephone conference calls.
  - Interim results at quarterly meetings.
  - Study closure at the time that data is analyzed and secondary analyses identified.
  - Amendments as the situations demand amendments will be made.
    Any changes to the protocol will be made at both sites and discussed at the weekly telephone conference.
  - Research related communications at weekly LIPP conference calls.
- *Specify which site is the Data Coordinating Center.*
  The Pennington
- *Specify if Tufts' data will be shared outside of Tufts (e.g., with other investigators, sponsor, etc.) and how data will be shared.*
  Data will be shared with the Pennington on an ongoing basis. At the completion and publication of the study, data will be made available to other investigators per NIH guidelines.
- *Describe any collaborations not described above, such as:*
  - *Tufts investigators with multiple affiliations that would engage other institutions in research (e.g., Tufts making direct payment to another institution for the research, the research is being conducted on behalf of another institution).*
    The PI has a bioscientific appointment (no salary) at MetroHealth Medical Center in Cleveland. The LIPP grant has transferred to Tufts and Tufts will be the coordinating center for the LIPP grant, Dr. Catalano has percent effort on other NIH grants at Tufts as well as at other institutions. His percent effort is covered through research administration
  - Each participating site will complete a Form 5 (continuing review form). The Tufts PI will collect and submit these to the Tufts Health Sciences IRB for the 2 external relying sites.
  - Each participating site will complete and submit the self-audit tools to the Tufts PI, a summary of which will be submitted to the Tufts Health Sciences IRB at the time of continuing review (summary of any issues identified during the self-audit). This summary can be described in the cover letter accompanying the continuing review application. The original self-audit checklists will be available to the Tufts Health Sciences IRB upon request:
    - File Review Checklist
    - Participant File Checklist

10.6 Community-Based Participatory Research

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

Will this study involve community-based participatory research?

Not at this time, possibly at a later date

☐ **Yes** ☑ **No**

10.7 Sharing Results with Subjects

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

☑ **Yes** ☐ **No**

If **Yes**, describe the following:

- *Rationale for sharing these results.*
  Routine clinical data can be shared with PCPs to potentially avoid additional testing and expense.
- *How results will be shared.*
  Please refer to the patient results form.
- *For individual subject results, specify if subjects have the option to opt-in or opt-out of receiving these results or allowing these results to be shared with others.*
  The subjects have the option to opt out of receiving their study results and can determine if they will share them with others.
- *Any referral policies (i.e. for confirmation of any individual subject results).*
  If a subject is screened out of the study, for example meeting exclusion criteria, the subject will be offered to contact the appropriate health care provider. In Cleveland, one subject was determined to be ineligible because of hypertension, which required treatment, and the subject was referred to her PCP for medical treatment.
- *Whether testing of research specimens is being conducted in, a laboratory certified (CLIA-approved) to conduct diagnostic testing. If patient-specific research results are reported from the laboratory and those results will or could be used for the diagnosis, prevention, or treatment of any disease or impairment, or the assessment of the health of human beings, the laboratory must be CLIA certified.*
  All laboratory studies provided to patients are performed at CLIA approved laboratories at TMC.
  - *If the research tests are experimental or of unknown or unproven clinical significance and the results will be provided to the source individual or physician or placed in the source individual's medical record, provide the rationale for this.*
    NA

11 Addendums

11.1 Site Specific Changes: Pennington Biomedical Research Center

Section 2.2 Risk to Subjects

- *Adequacy of Protection Against Risks*
  - The site PI or designee (Medical Investigator, Dr. Daniel Hsia, MD) will make the final decision regarding eligibility.

Section 4.4.1 Local Recruitment Methods

- *Source of subjects (for example, patient population, local community, etc.).*

  - Postpartum patients at local Baton Rouge area hospitals that perform deliveries, women attending local women's health events, women responding to web- and flyer-based recruitment advertisements.
- *Methods that will be used to identify potential subjects.*
  - Trialfacts: A web-based recruitment screening process (https://trialfacts.com/).
    - Trialfacts creates all the advertising and recruitment material used in the recruitment process, including study information pages, landing pages, pre-screening questionnaires, and online advertisements. Pennington Biomedical Research Center creates an additional landing page and screening questionnaire for those that pass the Trialfacts pre-screening process.
  - Physician assisted recruitment at Baton Rouge General, whereby providers at Baton Rouge General discuss the study and handout flyers to potential participants at their discretion.
  - Posted flyers throughout the Greater Baton Rouge area in approved locations.
  - Community events
- *Materials that will be used to recruit subjects.*
  - Handouts, flyers, physician referrals and online screening material will be used to recruit subjects.
- *When subjects respond to recruitment material, describe the information that will be provided to them about the study and the information that will be collected from subjects (e.g. name, telephone number, etc.) or submit a telephone/email script that will be used.*
  - Subjects will be contacted via phone by research staff and/or directed to Pennington's Web Screener Initiator (Web screener link: https://my.pbrc.edu/Clinic/Screener/Steps/Step1).
  - *If data will be retained for subjects that are determined to be ineligible, specify how privacy and confidentiality of these potential subjects will be maintained. Data will be retained until the primary manuscript is written noting differences in the study population that have determined to be ineligible for whatever reason based on inclusion/exclusion criteria.*
    - Pennington Biomedical has a Notice of Privacy Practices for Protected Health Information (provided below).
- *If recruitment material is being mailed or otherwise distributed, describe where/how, the distribution list will be obtained.*
  - An email listserv with IRB approved information will be sent approximately every quarter to people who have subscribed to the PBRC email list.

- *Specify how and why the listed recruitment methods will be effective in attracting the targeted subject population.*
  - Pennington Biomedical does not provide women's health and reproductive services and as such, necessitates community involvement and hospital partnerships for recruitment. This has been successful in enrolling 12 research participants to date.

Section 5.1 Study Design

- *Resting Energy Expenditure*
  - Resting Metabolic Rate (RMR) will be determined after an overnight fast using a Deltatrac II indirect calorimeter (Datex-Ohmeda, General Electrics, Finland) with a canopy system.
- *Neonatal Measures*
  - A PEAPOD is housed at Pennington Biomedical Research Center.
  - Pennington Biomedical staff will perform requisite tissue collection at local Baton Rouge hospitals dependent on where the participant delivers (Legal agreements are provided for Baton Rouge General and Women's Hospital, while the Ochsner legal agreement is pending). After hospital discharge, participants will travel to Pennington Biomedical where a PEAPOD measure and neonatal anthropometrics will be performed. If travel is difficult, neonatal anthropometrics may be obtained at respective hospitals.

- Additionally, a medical release form will be obtained from the participant for the purpose of obtaining the antenatal chart.
- Finally, neonatal anthropometrics, caliper measurements and circumference measures may be conducted at the houses of PBRC-enrolled study participants if they are unable to travel to the PBRC site after delivery.

- *Lifestyle Intervention Group*
  - Supervised Exercise Intervention
    - Exercise sessions will be conducted at Pennington Biomedical Fitness Center by the Intervention Resources Department at Pennington Biomedical. (See additional descriptions below.)

Section 5.3 Evaluations

- All laboratory tests will be performed by the Pennington Biomedical Clinic, Kirwan Lab Team and/or analyzed by the Clinical Chemistry Core at Pennington Biomedical. (See additional descriptions below.)
- *Sample Handling & Storage*
  - Sample processing and storage will occur locally at PBRC, following the description in the parent protocol.
- *Cord blood collection and placenta collection at delivery*
  - This process is described in detail in our Standard Operating Procedures (SOP 2304 Placenta Collection and Processing). In brief, LIPP research staff (Caitlin Hebert) will collect tissue specimens from the research participant's delivering hospital. Samples will be collected according to protocol and placed on ice or liquid nitrogen. The samples will then be immediately transported by Caitlin Hebert to PBRC for further processing and storage by LIPP staff (Kirwan Lab personnel) until analysis. The clinical chemistry core will perform glucose and lipid profiles on cord blood samples. When the research participant is admitted to their delivering hospital's delivery unit, they will hand the PBRC Placental Biospecimens Delivery Unit Information sheet to the attending physician.

Section 6.1 Informed Consent Process

- The PBRC site provides additional informed consent to the infants born to enrolled mothers. This informed consent is performed with the LIPP participant at the PBRC site during the final study visit (infant measurements) or may be conducted offsite if participants are unable to travel to PBRC after delivery.

Interventional Resources
Expertise including Facilities, Equipment, & Resource Descriptions

Fitness Center

Pennington Biomedical includes a 2300-square-foot Exercise Training Facility. The facility offers state-of the-art equipment, professional intervention technicians, and optimal training data-capturing capabilities. Staff have the ability to collect and enter data in real-time via a standing desk with dual monitors to operate the heart rate software, databases, and drive files as needed. The cardiovascular fitness training room contains 12 treadmills, 6 stationary bikes, and 2 elliptical while the strength training room has an extensive set of machines and free weights. There are 3 private rooms for intervention related counseling in a one-on-one or small group setting. For measurements, there's a private exam room complete with scale, stadiometer, and computer. In addition, there's a meeting room space capable of outfitting group exercise classes, group presentations, study orientations or introductory sessions, or childcare facility for study participants. The Fitness Center is supported by a trained staff composed of full-time exercise interventionists and students working on exercise-related degrees.

The facility is participant friendly and includes televisions, reading materials and enthusiastic staff who are well trained in customer service. Further, there are locker rooms equipped with lockers and showers available exclusively

for the use of study participants. The facility has a defibrillator, electronic scales, and multiple heart rate monitors systems (Polar and Zephr Bioharness). There is an outdoor walking track as well. The scenic walking track is 12 feet wide, paved, lighted and well-maintained. While the track is over half a mile in length there are 3 intermediate circle turn-outs that can be used to create shorter walking courses. All exercise intervention areas at Pennington are located in buildings far from the clinical assessment area with virtually no opportunity for assessment staff to accidentally observe exercising participants.

We have developed a real-time web-based system (EDIN2.0) that allows exercise interventionists to efficiently maintain accurate records of each exercise session. The progression of each participant is tracked both within individual sessions and during the entire study. Exercise can occur on a treadmill, elliptical, or cycle ergometer with the speed/grade/resistance modulated in order to keep participants at their target heart rate (±3 beats) derived from the maximal exercise testing. EDIN also allows for data capture of acute energy expenditure submaximal tests and manual entries such as outdoor exercise or resistance training.

Resource Descriptions

Exercise Interventionists & Research Specialists

Interventional Resources has 7-10 full time interventionists or specialists, and approximately 8-10 students (varied by paid, interns, and volunteer status). The Interventionists and Specialists are primarily responsible for participant contact activities which include but are not limited to conducting orientations, run-ins, collecting assessment data, physical activity or other devices, randomization assignment, and setting up and conducting the physical activity and other interventions. They are responsible for ongoing participant contact, maintaining participant records, tracking compliance and completing data entry requirements.

11.2 Opt-In Ancillary Studies

11.2.1 Breast Milk Collection

Aim: To determine the impact of the lifestyle intervention on breastfeeding outcomes and breast milk composition of the index pregnancy.

Hypothesis: Our overall hypothesis is that women randomized to the lifestyle intervention group will have higher rates of breastfeeding and less pro-inflammatory breast milk composition with a lower omega-6/omega-3 fatty acid ratio.

Methods:

a. Breastfeeding outcomes: Our **exposure** will be randomization group and our **outcomes** will be amount of breastfeeding versus formula feeding, duration of any breastfeeding and duration of exclusive breastfeeding. Study staff will administer validated breastfeeding outcome questionnaires every other week via text message or email to quantify duration of exclusive and any breastfeeding. Statistical analysis: We will use independent samples t-test to analyze the association between participation in the lifestyle intervention program on breastfeeding rates during each timepoint of questionnaire administration. Next, we will use multivariate logistic regression to further examine the adjusted association of lifestyle intervention with rates of breastfeeding, adjusting for key covariates.

b. Breastmilk composition: Our **exposure** will be participation in the lifestyle intervention program. Our **outcomes** will be breast milk cytokines, PUFAs, leptin and insulin. Study staff will instruct mothers to collect breast milk samples and provide a 20 mL sample for this study. Participants will provide breast milk samples before initiating the intervention and then at routine study visits scheduled at 3- and 6-months following intervention initiation. If mothers indicate that they plan to stop breastfeeding based on questionnaires, we will attempt to collect a breast milk sample prior to cessation of breastfeeding. PUFAs will be measured using established methods of ultra-high performance liquid chromatography-heated electrospray ionization tandem mass spectrometry UHPLC-HESI/MS-MS. Insulin will be measured using ELISA or radioimmunoassay. For statistical analysis, we will use independent samples t-test to analyze the association between participation in the lifestyle

intervention program and the mean concentrations of these breast milk markers. We will then use multivariate logistic regression to further examine this association, adjusting for key covariates.

c. Procedures: At each study visit, if a participant is still breastfeeding and consent has been signed for this ancillary sub-study, study staff will collect one 10-20mL sample of breast milk. Instructions will be provided at the previous study visit so that if desired, a participant can pump their sample at home in the morning before arrival. If the sample is provided at the study visit, participants will be asked to pump a full breast using their own pump or a disposable manual breast pump that we provide. If pumped at the visit, study staff will thoroughly mix/shake the full sample and pour 10-20ml into the provided sterile container; if at home, the participant will be given instructions on how to prepare the sample. The remainder of the sample will be returned to the subject. This sample will be processed in the laboratory and archived at -80^{o}C at Brigham and Women's Hospital. The samples will be carried by the BWH LIPP study staff to BWH where they will be stored in freezers in a secured room in a secured building by subject ID number and date of collection to be used for future analysis.

The impact of the proposed supplemental work lies in identifying modifiable factors that may impact breastfeeding outcomes, offspring growth and adiposity. Results from this study could provide further insight into the role of lactational programming in intergenerational obesity.

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