Determinants of gestational weight gain in obese pregnant women

"MomEE"

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PROTOCOL

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1. SUMMARY

In the U.S. the number of obese women entering pregnancy has more than doubled in the past 30 years. Pregravid obesity alone however is not the only cause for concern since two-thirds of obese women gain weight in excess of the 2009 IOM gestational weight gain recommendations and attempts to manage gestational weight gain to date have failed. To improve weight management of obese pregnant women, there is a critical need to deliver specific, evidence-based recommendations on energy intake and energy expenditure (physical activity); the two primary determinants of weight gain in non-pregnant individuals. We will evaluate energy intake and energy expenditure during pregnancy (13 to 37 weeks) and through 12 months postpartum in at least 60 obese women. We will test the hypothesis that obese pregnant women with weight gain above the IOM guidelines, 'High Gainers', will have increased energy intake but no evidence for changes in energy expenditure after adjustment for the weight gained when compared to women with appropriate gestational weight gain, 'Normal Gainers'. The primary outcome variables are 1) energy intake during pregnancy and 12 months postpartum measured with the energy balance method and with a mathematical model of maternal energy intake and 2) the free-living energy expenditure and sleeping metabolic rate (absolute and adjusted for maternal body composition and fetal size) during pregnancy. Secondary outcomes include measurement of changes in physical activity and substrate oxidation, determinants of energy intake including fasting concentrations of leptin, total ghrelin, peptide YY, cholecystokinin and determinants of energy expenditure including urinary epinephrine and norepinephrine, and fasting concentrations of T3, T4 and TSH.

2. BACKGROUND AND SIGNIFICANCE

2.1. Definition and prevalence

Currently in the United States, 31.9% of reproductive age women (20-39 years) are estimated to be obese [1] and 8% severely obese [2]. Obesity at conception as well as excess gestational weight gain leads to adverse health outcomes for the mother [3-6] and more alarming, the infant [6-9] that are sustained for decades later.

In an attempt to counteract these trends, the Institute of Medicine (IOM) performed a detailed review of observational data in pregnant women and infants, and defined, within each BMI classification, boundaries for appropriate weight gain during pregnancy [10]. According to the IOM, gestational weight gain in obese women should be limited to 5-9 kg [10]. To be compliant with the gestational weight gain guidelines, the IOM suggests pregnant women receive dietary advice and physical activity counseling throughout pregnancy. Despite these recommendations, recent data in 227,149 obese pregnant women indicate that more than 55% of obese women in the U.S. exceed the IOM guidelines for gestational weight gain [11]. Furthermore, to date, lifestyle interventions specifically designed to attenuate weight gain in obese pregnant women have failed [12-17]. In order to improve weight management in obese pregnant women, there is a critical need to understand the determinants of gestational weight gain. In support of this contention, the IOM recognized that, "there remains a lack of information to relate dietary intake or physical activity to gestational weight gain even though they are primary determinants of weight gain in non-pregnant individuals" [10].

There is little knowledge of the determinants of gestational weight gain in pregnant women and no studies have been conducted specifically in pregnant women who are obese. Successful weight management programs in non-pregnant individuals rely on individualized prescriptions of energy intake and physical activity [18-21]. Understanding how changes in energy intake, energy expenditure and physical activity during pregnancy contribute to gestational weight gain is critical for designing effective interventions for pregnant women. To advance the management of gestational weight gain in obese pregnant women, studies are thereby needed to: 1) understand the simultaneous changes in energy intake, energy expenditure and physical activity throughout pregnancy and, 2) evaluate these measures in relation to gestational weight gain and the 2009 IOM guidelines for weight gain during pregnancy.

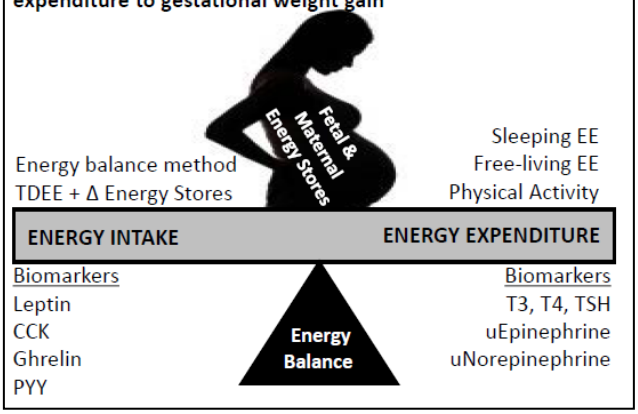
Objective assessments of energy intake can be derived from the energy balance method by summing total daily energy expenditure and changes in body energy stores. We have shown that the energy balance method can be

used to estimate energy intake during weight loss with good accuracy and precision [22, 23] and have preliminary data validating this method during weight gain. Objective assessments of energy intake have not been undertaken in obese pregnant women and almost no data exist. Using data from a landmark study that collected simultaneous measurements of body composition (by the 4-compartment model) and energy expenditure by the doubly labeled water technique [24], we computed energy intake in the 3rd trimester by the energy balance method. In overweight women with gestational weight gain above the 2009 IOM guidelines ('High Gainers'), energy intake in the third trimester was 3503±666 kcal/d compared to 2617±400 kcal/d for women with appropriate gestational weight gain ('Normal Gainers'). As expected, energy expenditure in these women increased in proportion to gestational weight gain. When the change in energy expenditure was adjusted for change in maternal body weight and body composition (mother plus fetus), energy expenditure was higher than expected but not different between 'High Gainers' and 'Normal Gainers'. These preliminary data indicate that a large energy intake and not an impaired increase in the energy expenditure underlie excess gestational weight gain in 'High Gainers'.

2.2. Specific aims and hypotheses

The aim of this study is to test the hypothesis that obese pregnant women with weight gain above the IOM guidelines, 'High Gainers', will have increased energy intake but no evidence for changes in energy expenditure after adjustment for the weight gained when compared to women with appropriate gestational weight gain, 'Normal Gainers' (Figure 1).

Figure 1. Contributions of energy intake and energy expenditure to gestational weight gain



Aim 1: Measure energy intake in obese women during pregnancy. The primary outcome is energy intake over the last 2 trimesters of pregnancy measured by: 1) the energy balance method (using doubly labeled water and change in maternal energy stores by whole-body MRI and fetus size by 3-D ultrasound) and, 2) our energy balance model of gestational weight gain and energy intake.

Hypothesis 1: Energy intake will be significantly larger in 'High Gainers' versus 'Normal Gainers'.

Aim 2: Measure total energy expenditure in obese women during pregnancy. We will combine the most accurate and precise methods to assess sedentary (sleeping metabolic rate in a room calorimeter) and free-living (doubly labeled water) energy expenditure, and physical activity.

Hypothesis 2: Absolute energy expenditure (unadjusted) during pregnancy will increase proportionally to increase in metabolic mass (mother plus fetus) whereas energy expenditure adjusted for maternal body energy stores and fetus size will not differ between 'High Gainers' and 'Normal Gainers'.

Aim 3: Measure energy intake and energy expenditure in obese women 12 months postpartum and their relationships to "postpartum weight loss".

Hypothesis 3: The percentage of weight normalization (postpartum weight loss as a percentage of gestational weight gain) will be proportional to the decreased energy intake from late pregnancy to 12 months postpartum.

3. RESEARCH DESIGN

This is a single arm, prospective study. In this study, we will enroll up to 75 obese, pregnant women to complete at least 60. This study will be performed at Pennington Biomedical Research Center in Baton Rouge, Louisiana. Study outcomes will be assessed at three time points during pregnancy; namely, gestational weeks 13-16 (early), 24-27 (mid), and 35-37 (late); and at two time points postpartum; namely, postpartum weeks 22-25 and 48-56 (postpartum testing). Infants born to the mothers enrolled in the MomEE study will complete 1 study visit before 10 days of life to measure anthropometrics, body composition measurements, and questionnaires.

3.1. Primary outcomes

The primary outcome variables are 1) energy intake during pregnancy and 12 months postpartum measured with the energy balance method and with a mathematical model of maternal energy intake, and 2) the free-living energy expenditure and sleeping metabolic rate (absolute and adjusted for maternal body composition and fetal size) during pregnancy.

3.2. Secondary outcomes

Secondary outcomes include measurement of changes in physical activity and substrate oxidation, determinants of energy intake including fasting concentrations of leptin, total ghrelin, peptide YY, cholecystokinin and determinants of energy expenditure including urinary epinephrine and norepinephrine, and fasting concentrations of T3, T4 and TSH.

4. STUDY POPULATION

4.1. Participants

Up to 75 obese, pregnant women will be recruited for the study. After delivery, the infants born to enrolled mothers will be enrolled to complete 1 study visit before 10 days of life.

4.2. Eligibility Criteria

- Female with singleton, viable pregnancy (confirmed by ultrasound measured ≥ 6,0 gestation)
- BMI ≥30 kg/m^2 (first study measured weight and height)*
- 18-40 years old
- Medically cleared for participation by primary care obstetrician
- Medically cleared for participation by Medical Investigator
- Willingness to allow the study access to information in your medical record
- Willingness to be notified of incidental findings from study procedures

4.3. Exclusion Criteria

Subjects meeting any of the following criteria are **not** eligible to participate:

Clinical

- Hypertension (i.e. SBP >160 mmHg & DBP >110 mmHg)*
- Diagnosis of diabetes prior to pregnancy
- Hb A1c ≥6.5 %*
- Implanted metal objects that render MRI unsafe
- HIV or AIDS (self-reported)
- Severe anemia (hemoglobin <8g/dL and/or hematocrit <24%)**

Psychological

- History or current psychotic disorder or diagnosis of a current major depressive episode or bipolar disorder
- Past history of anorexia or bulimia by medical history or patient report (binge eating disorder is not an exclusion) or current eating disorder
- Actively suicidal defined as a value ≥2 on the BDI-II question 9*

Medications

- Current use of one or more of the following medications: metformin, systemic steroids, antipsychotic agents (e.g., Abilify, Haldol, Risperdal, Seroquel, Zyprexa), anti-seizure medications or mood stabilizers that would be expected to have a significant impact on body weight (e.g., Depakote, Lamictal, Lithium, Neurontin, Tegretol, Topamax, Keppra), medications for ADHD including amphetamines and methylphenidate
- Continued use of weight loss medication including OTC and dietary supplements for weight loss (e.g., Adipex, Suprenza, Tenuate, Xenical, Alli, conjugated linoleic acid, Hoodia, Green tea extract, Guar gum, HydroxyCut, Sensa, Corti-slim, Chromium, chitosan, Bitter orange)

Other Exclusion Criteria

- Recent history of or currently smoking, drinking alcohol or abusing drugs (prescription or recreational)
- Plans to move out of the study area within the next 2 years or plans to be out of the study area for more than 4 weeks in the next 12 months
- Planned termination of pregnancy
- Unwillingness to avoid pregnancy for 12 months following delivery
- Claustrophobia
- Prior or planned (within 1 year of expected delivery) bariatric surgery
- Participant's unwillingness or inability to commit to a 1 year follow-up
- Recurrent pregnancy during the postpartum period of the study

*Measured during screening
**Abstracted from medical record

5. RECRUITMENT

Obese, pregnant women will be presented with the opportunity to participate in this study during their primary care obstetrical appointments by participating referring Ob/Gyns. Community advertisements, outreach and promotional materials may also direct the participant to contact study staff to participate. Study staff will explain the study and the study procedures to interested mothers and may provide them with a copy of the consent form or show them a short summary video for further explanation if mothers are interested.

6. SCREENING

Participants will present to Pennington Biomedical to complete a screening visit to assess their eligibility. Participants will provide informed consent prior to the initiation of study procedures. Height, weight, vital signs and HbA1c (by finger stick) will be measured. Medical, obstetrical, medication and psychological history will be reviewed for exclusionary criteria and questionnaires will be administered. A brief discussion (referred to as a Lifestyle Interview) will be used to assess the participant's understanding of the study and willingness to remain in the study in light of potential obstacles to participation, e.g. work schedule, family responsibilities, travel schedule, driving distance to Pennington Biomedical, etc. The participant's medical record will be abstracted to evaluate her gestational age, pregnancy viability and hemoglobin/hematocrit values. The participant's primary care obstetrician will be requested to provide medical clearance for her participation. If inclusion/exclusion criteria are satisfied, the participant is eligible to participate.

7. ASSESSMENT SCHEDULE AND PROCEDURES

Clinical outcomes (**Table 1**) will be assessed in early (13-16 GA), mid (24-27 GA) and late (35-37 GA) pregnancy and approximately 6 months (22-25 weeks) and 12 months (48-56 weeks) postpartum. Prenatal vitamins will be provided to participants that are deemed eligible and chose to participate. Additionally, adherence to the 2009 IOM gestational weight gain guidelines will be encouraged for all study participants. A brief counseling session will be conducted and IOM materials and a scale will be provided to participants after they are deemed eligible. Participants will be asked to weigh daily throughout the study on the scale that will be provided.

Clinic assessments at each time point can be carried out in a single study visit (excluding equipment/ biospecimen drop offs). Subjects may alternatively elect to have the procedures performed over 2 visits. Subjects will be asked to refrain from alcohol, caffeine and exercise approximately 36 hours prior to testing.

On the morning of testing, subjects will be asked to come to Pennington Biomedical fasting for body weight measurement, urine collection, and accelerometer application. If subjects have given permission, an additional urine sample will be collected and stored for future use. Subjects will report to the clinical research unit in the evening where body weight will be measured and a second urine sample will be collected. Subjects will receive an individualized dose (based on morning body weight) of doubly labeled water and will then enter the metabolic chamber for an assessment of sedentary energy expenditure. A standard dinner will be served at least 1 hour after being dosed with doubly labeled water. Questionnaires will be provided for the participant to

complete while in the chamber. Lights will be turned out at about 10:30 PM. Subjects will be asked to collect all urine overnight. Subjects will be woken in the morning and asked to void. Next, subjects will remain awake, and resting metabolic rate (RMR) will be measured for approximately 30 minutes. Subjects will exit the chamber and their body weight and vital signs will be measured. Accelerometers may be removed and downloaded. A fasting blood draw will be collected and if the subjects have given permission, additional blood will be collected and stored for future use. Subjects will have neck circumference and body composition by BOD POD, and skinfolds measured. Then, subjects will be offered breakfast and RFPM training will be performed. Next, subjects may have a whole body MRI scan to measure total adipose tissue mass and skeletal muscle mass and a 3-D ultrasound to measure the size of her fetus or they may choose to do this at an additional visit if desired. Actigraph will be placed back on the subject's wrist and will be returned with the timed urine samples for the doubly labeled water study collected 6 and 7 days later.

The study visit as described above will be performed at the early pregnancy, late pregnancy and 12 months postpartum (excluding the ultrasound) time points. Additionally at 12 months postpartum, an oral glucose tolerance test will be conducted. At the mid pregnancy time point, weight, vital signs, body composition measurements (MRI, skinfold thickness and BOD POD), neck circumference, 3D ultrasound, blood collection, and blood/urine archive (if consented) will be collected or performed. An abbreviated visit at 6 months postpartum will be conducted to collect weight, vital signs, blood, neck circumference, body composition by BOD POD and skinfold thickness, and blood/urine archive (if consented). Questionnaires will be administered at each visit. Participants will receive handouts with some of their study results. Prenatal vitamins will be provided to participants during pregnancy and compliance assessed at subsequent visits. Participants will be provided a scale to take home to continuously weigh themselves throughout the study. Data from prenatal, delivery, and neonatal records will also be obtained from the participants' respective physicians.

Table 1.	Screening Visit	Early Pregnancy	Mid Pregnancy	Late Pregnancy	Delivery	6 months after delivery	12 months after delivery
(weeks)	Confirmation of pregnancy-15	13-16	24-27	35-37		+22-25	+48-56
Weight	X	X	X	X		X	X
Height	X						
HbA1c	X						
Prenatal vitamin dispensing/inventory**		X	X	X		X***	
Vital signs (blood pressure, heart rate)	X	X	X	X		X	X
Neck circumference		X	X	X		X	X
Questionnaires	X	X	X	X		X	X
Body composition (BOD POD/SFT)		X	X	X		X	X
Body composition (MRI scan)		X	X	X			X
Energy Intake (RFPM)		X		X			X
Accelerometry (Actigraph/Sense Wear)		X		X			X
Doubly Labeled Water (DLW)		X		X			X
Metabolic chamber testing with urine collection		X		X			X
Dinner provided		X		X			X
Breakfast provided		X		X			X
Resting Metabolic Rate (RMR)		X		X			X
Fetal size (3D ultrasound)		X	X	X			
Oral glucose tolerance test							X
Blood collection		X	X	X		X	X
Blood collection for future use*		X	X	X		X	X
Urine collection for future use*		X	X	X		X	X
Placental Biospecimen collection*					X		

*Optional **Subject to participant's needs ***Inventory only

Infant Assessments (Table 2) will be completed at the infant's Delivery visit. The Delivery visit will occur before the infants reach 10 days of life. At the visit, the MomEE participant who is the mother of the infant and signs the minor informed consent will be provided with questionnaires to complete related to the infant. After obtaining written parental informed consent for the infant, weight, recumbent length, head circumference, abdominal circumference and body composition by skinfold thickness and air displacement plethysmography

(PEA POD). Weight, length, and head circumference will be plotted on WHO growth charts. The medical investigator or available nurse practitioner will be called for evaluation of the infant to review any reported adverse events and to determine any signs of jaundice, difficulty breathing, or other obvious congenital malformations. If the medical staff members are unavailable at the time of the visit, follow up may be completed by the medical staff members with the parent/legal guardian of the infant as is deemed necessary. At the MomEE participant mother's 12 month postpartum visit, she will be provided with a Request of Medical Records form to complete for the infant participant so that medical data from the infant's pediatrician's office can be extracted from birth to the end of the infant's first year of life.

Table 2. Infant Assessments

	Delivery (<10 days old)
Informed Consent (Minor)	X
Length	X
Weight	X
Head Circumference	X
Abdominal Circumference	X
PEAPOD	X
Skinfold Thickness	X
Questionnaires*	X
*Completed by MomEE participant who is the mother of the infant	

8. MEASURES AND OUTCOME ASSESSMENTS

8.1. Total daily energy expenditure

Total daily energy expenditure (TDEE) will be measured over 7 days by the doubly labeled water method early and late pregnancy and 12 months postpartum. The doubly labeled water method (DLW) is a noninvasive technique for assessing energy expenditure in free-living individuals. This method has been used for measuring energy expenditure in various populations such as pregnant and lactating women, infants and children [24-28].

Subjects will provide two urine samples before being dosed with labeled water (1.25g of 10% enriched $H_2{}^{18}O$ and 0.10g of 99.9% enriched ^2H_2O per kg of body weight). After administering the DLW dose to the subject, staff will add about 50mL of tap water to the container, swirl, and ask participant to consume through a straw. Staff will then pour an approximate additional 50 mL of tap water to the container, swirl and ask participant to consume through a straw. Two urine samples will be collected at approximately 4.5h and 12h after dosing (in the chamber). Subjects will be instructed to empty their bladder about 1.5 and 3 hours post-dose. On days 6 and 7 after dosing, subjects will collect two additional urine samples at home, first thing in the morning.

Analysis: Samples will be measured and analyzed in the Pennington Biomedical Mass Spectrometry Laboratory. Each sample will be analyzed for ^{18}O and ^2H abundance by isotope ratio mass spectrometry [29]. The isotopic enrichments of the post-dose urines compared with the pre-dose samples will be used to calculate elimination rates (k_H and k_O) using linear regression and initial isotope dilution spaces were calculated by extrapolation to time 0. CO_2 production rate (rCO_2) will be calculated using the equations of Schoeller et al. [30] as modified by Racette et al [31]; $rCO2 = (N/2.078)(1.007k_O - 1.041k_H) - 0.0246rH_2O_f$ where, N is the total body water and rH_2O_f is the rate of fractionated evaporative water loss which is estimated to be $1.05N (1.007k_O - 1.041k_H)$. Total daily energy expenditure will be calculated by multiplying rCO_2 by the energy equivalent of CO_2 for the average RQ measured in the metabolic chamber.

8.2. Sleeping energy expenditure and resting metabolic rate

Sleeping energy expenditure and resting metabolic rate as well as substrate oxidation will be measured in a metabolic chamber early and late in pregnancy and 12 months postpartum. The metabolic chambers at Pennington Biomedical are small rooms which measure at least 10'x7' and are designed to provide a pleasant

ambiance for study volunteers [32]. Each chamber has windows, and comfortable furnishings including a bed, desk and chair, cable television, telephone, computer with internet access, sink and toilet with privacy curtain. The accuracy (1-2%) and precision (3%) of the respiratory chambers are assessed monthly by 24-h propane combustion tests.

Subjects will enter the chamber at approximately 6:30 PM and leave the chamber at approximately 6:50 AM the next morning. Sleeping energy expenditure will be assessed between 2:00 and 5:00 AM for those minutes with activity < 1% and extrapolated to 24-hours. Resting metabolic rate will be measured over 30 minutes in the morning after the participant has voided. Resting metabolic rate (RMR) will be measured either by ventilated hood system or the metabolic chamber. Spontaneous physical activity is measured by continuous monitoring of microwave motion detectors. A standard dinner (provided at approximately 7:00 PM) prepared by the metabolic kitchen will provide 30% of the estimated daily calories and a standardized composition (55% carbohydrates, 30% fat, 15% protein). All urine will be collected for nitrogen, creatinine, norepinephrine and epinephrine excretion. The rate of protein oxidation will be determined from the rate of urinary urea production and the rates of carbohydrate and lipid oxidation according to Livesey and Elia [33].

The activity of the sympathetic nervous system will be assessed by 12-h urinary free epinephrine and norepinephrine excretion using the Bio-Rad high performance liquid chromatography with electrochemical detection (Hercules, CA).

8.3. Maternal Physical activity

Sense Wear® armbands (SWA; BodyMedia, Inc.) will be used to measure physical activity and sleep during chamber visits. The SWA is a wireless multi-sensor activity monitor that is lightweight (83-grams) and worn on the upper arm. The SWA records key aspects of daily sedentary and active behaviors including: I) minutes lying down, 2) minutes of sleep, 3) number of steps taken, 4) minutes spent in activities of different intensities (sedenta1y, moderate, vigorous, and very vigorous), 5) total energy expenditure (kcal/day), 6) active energy expenditure (kcal/day), 7) average METS, and 8) time On/Off Body.

The *ActiGraph GT3X+* accelerometer will also be used to measure maternal activity and sleep objectively during chamber visits and for no less than seven days post chamber visit. The monitor records time varying accelerations ranging in magnitude from+/- 6 g's. The accelerometer output is sampled by a twelve-bit Analog to Digital Convertor and the raw acceleration is then stored in flash memory for future analysis. The GT3X + is small and lightweight (19g) and can be worn at the hip, wrist, or ankle. Wrist-placement will be used to minimize participant inconvenience and to better measure sleep.

8.4. Geographic Information Systems (GIS)

Participants' primary address of residence will be recorded to use GIS (Geographic Information Systems) to geocode participants' environments in terms of proximity to parks and recreation facilities, restaurants, mass transit, etc. Primary address will be collected at screening and study staff will confirm address is up to date throughout study. When a participant experiences a change in address during her participation, the new address and move date will be recorded.

8.5. Maternal energy intake

The Remote Food Photography Method will be used to quantify food intake as an outcome variable in all participants at the time points noted in Table 1. Using our previously described and validated procedures [34, 35], participants will use a Smartphone to capture images of their food selection and plate waste in free-living conditions over at least 5 day period. Participants will be instructed how to use the SmartIntake application and respond to Ecological Momentary Assessment (EMA) reminders. Staff members will be responsible for monitoring the images in the Automated Data Management Utility (ADMU) and contacting participants to provide feedback or inquire about further details. As previously demonstrated [34], accurate food intake estimates are dependent upon sound EMA procedures, including quickly providing participants with feedback

(positive and corrective) and quickly responding to participants if data appear to be missing or if food image data is of low quality. Food images will be analyzed to estimate food intake using previously described methods [36].

8.6. Continuous weight monitoring

A bathroom scale that contains a cellular card to automatically and wirelessly transmit body weight data to a server will be provided to all subjects at enrollment. Subjects will be requested to weigh themselves at least once a week during the study. NOTE: Low or non-compliance to this assessment is anticipated and will not be reported as a deviation.

8.7. Questionnaires

The following measures of quality of life, mood, and psychiatric symptoms and forms for the collection of demographics, social and obstetrical history information will be administered to the subjects at various time points. The forms administered via interview are denoted below with an "[i]". When a visit coincides with a chamber stay the questionnaires can be administered in the chamber to reduce burden by decreasing visit length.

Table 3. Questionnaire/Form	Screening Visit	Early Pregnancy	Mid Pregnancy	Late Pregnancy	Delivery (Infant's Visit)	6 months after delivery	12 months after delivery
Body Shape Questionnaire (BSQ)		**X**	**X**	**X**		**X**	**X**
Body Morph Assessment 2.0 (BMA)		**X**	**X**	**X**		**X**	**X**
Body Areas Satisfaction Scale		**X**	**X**	**X**		**X**	**X**
Mindful Eating Questionnaire		**X**		**X**			**X**
Food Craving Inventory		**X**		**X**			**X**
Eating Disorder Examination Questionnaire	**X**						
BDI-II	**X**			**X**		**X**	**X**
Baseline Questionnaire	**X**						
Previous Pregnancy Outcome Form[i]	**X**						
Sleep Questionnaire		**X**	**X**	**X**		**X**	**X**
Frequency of self-weighing		**X**	**X**	**X**		**X**	**X**
Maternal Sedentary Behavior		**X**	**X**	**X**		**X**	**X**
Berlin Questionnaire		**X**	**X**	**X**		**X**	**X**
SF-12		**X**		**X**			**X**
Eating Inventory		**X**		**X**			**X**
Infant Feeding Styles Questionnaire				**X**	**X**	**X**	
Postpartum Medical History Form[i]						**X**	
Child Television Viewing						**X**	**X**
Breastfeeding Questionnaire					**X**	**X**	**X**
Maternal Follow Up Questionnaire							**X**

Baseline Questionnaire: will be used to collect self-reported pre-pregnancy weight, family history of diabetes and hypertension, demographics (maternal and paternal), social history, severity of nausea and vomiting (PUQE) and food security (Household Food Insecurity) at screening. This questionnaire includes:

- *Maternal Demographic Information:* maternal date of birth, race and ethnicity, marital status, family size, household type, income, occupational status, TV, telephone and computer ownership, internet/computer access, cell phone usage and technology, maternity leave, education/literacy.
- *Paternal Demographic Information*: age at maternal enrollment, race, ethnicity and body type as identified by participant using standard silhouettes.
- *Social History*: Alcohol and tobacco use will be collected from the participant or via chart review at screening.
- *Household Food Insecurity*: measured with the first two questions of the USDA Food Security Module subscale.
- *Modified-Pregnancy-Unique Quantification of Emesis and Nausea Index (PUQE)* questionnaire[37]: The PUQE will be given at screening to assess the severity of nausea and vomiting of pregnancy.

Previous Pregnancy Outcome Form[i]: The following will be collected from the participant via interview at screening: to include gravidity, parity, and a description of previous births (year, birth weight, gestational age at delivery, weight gain, gestational diabetes, preeclampsia, and birth trauma).

Eating Disorders Examination Questionnaire- modified (EDE-Q): questions 2-4 will be used to assess frequency of unsafe dieting practices at screening .[38]

Sleep Questionnaire: A sleep questionnaire revised from the original version to include sections A, C, E and F will be administered at each time point excluding screening.. The original questionnaire was from a cohort study of pregnant women and their infants (nuMOM2b), and it includes items regarding sleep schedule, quantity, quality, habits and disorders and sleepiness.

Frequency of self-weighing: Participants will be asked to indicate how frequently they had weighed themselves during the past month using a 7-point scale ranging from *several times a day* to *never*[39, 40]. This will be administered at each time point excluding screening.

Maternal Sedentary Behavior: will be measured with Item 39 from the Nurse's Health Study II Questionnaire (http://www.channing.harvard.edu/nhs/questionnaires/pdfs/NHSII/2009.pdf). This item includes 5 questions that ask respondents to rate the average hours per week that they engaged in certain sedentary behaviors. This will be administered at each time point excluding screening.

Berlin Questionnaire: will be used to assess participant's risk for sleep apnea syndrome[41, 42]. This will be administered at each time point excluding screening.

Breastfeeding: measured using three questions adapted from the Southampton Women's Survey[43] and CDC Infant Feeding Practices study[44]. The questions assess initiation and duration of breastfeeding,[44] duration of exclusive breastfeeding[43], and timing of introduction of water and complementary fluids[43]. The questions will be administered at infant delivery, and 6 months and 12 months postpartum time points.

Medical Outcomes Study Short-Form 12 Health Survey (SF-12): Mental and physical health-related quality of life will be quantified with the reliable and valid Medical Outcomes Study Short-Form 12 Health Survey (SF-12). [45] This will be administered at early pregnancy, late pregnancy, and 12 months postpartum.

Food Craving Inventory II (FCI-II): The FCI-II is a 33 item measure of cravings for specific types of foods [46]. The FCI-II assesses the frequency of cravings for particular foods over the previous month. The FCI-II consists of 4 empirically-derived factors: high fats, sweets, carbohydrate/starches and fast food fats. General cravings are assessed with the FCI-II total score. All items are scored on a Likert-type scale. The FCI-II has been shown to be reliable and support has been found on all aspects of validity. This will be administered at early, late pregnancy, and 12 months postpartum.

The Mindful Eating Questionnaire (MEQ): The MEQ is a 28-item self-report instrument that measures five domains of mindful eating: disinhibition, awareness, external cues, emotional response, and distraction[47]. Mindful eating refers to an unbiased awareness of sensations surrounding eating. Items are scored on a Likert-type scale from 1 (never/rarely) to 4 (usually/always). Items 2, 4, 8, 17, and 23 each have an additional answer options which indicate that none of the item responses are applicable. This will be administered at early, late pregnancy, and 12 months postpartum.

Eating Inventory (EI): The EI measures dietary restraint, disinhibition, and perceived hunger. Higher scores on disinhibition and hunger are indicative of episodic overeating and the subjective sense of hunger,

respectively. Dietary restraint is the intent to restrict food intake. The measure has been shown to be reliable and valid [48]. This will be administered at early, late pregnancy, and 12 months postpartum.

Beck Depression Inventory-II (BDI-II): The BDI-II [49] is one of the most widely used and well validated self-report measures of depressive symptoms and mood. Higher scores indicate more severe symptoms of depression. Scores on the BDI-II can be interpreted within the context of the following categories: 0-13 = minimal depression, 14-19 = mild depression, 20-28 = moderate depression, and >28 = severe depression [49]. This will be administered at screening, late pregnancy, 6 months postpartum, and 12 months postpartum.

Body Shape Questionnaire (BSQ short): The Body Shape Questionnaire – short form [50] was developed to measure concerns about body shape and the antecedents and consequences of this concern. The 8-item questionnaire is particularly concerned with the phenomenon of "feeling fat" and about worries related to how the body is perceived. The questionnaire assesses concerns over the past four weeks. The questionnaire distinguishes between those who are diagnosed with bulimia and those not diagnosed, attesting to its validity. This will be administered at each time point excluding screening.

Body Areas Satisfaction Scale: is a nine-item subscale of the Multidimensional Body Self Relations Questionnaire that assesses perceived body satisfaction. Using a 5-point Likert scale, participants rate their degree of body satisfaction with specified body parts (e.g., thighs, face, or stomach) as well as their height, weight, and overall muscle tone. The reliability and validity of the scale has previously been established. The Body Areas Satisfaction Scale has a test–retest reliability of r=0.86, and the scale's internal consistency reliability in a previous study with pregnant women was good (i.e., alpha=0.84 at trimester 1and alpha=0.84 at trimester 2). The internal consistency reliability alpha for the scale in the current study was also good (alpha=0.79 at trimester 1, alpha=0.83 at trimester 2, and alpha=0.83 at trimester 3)[51-55]. This will be administered at each time point excluding screening.

The Body Morph Assessment 2.0: The BMA 2.0 [56] is a psychometrically validated, *computer-based and self-administered* assessment of body image. The BMA measures estimates of current, ideal, reasonable body size, and body image dissatisfaction. There are one hundred total increments from the extremely thin endpoint on the measure to the obese endpoint. This will be administered at each time point excluding screening.

Child Television Viewing: Child television viewing will be assessed at postpartum times by the following question, which was adapted from another study[57], "Over the past week, how much total time would you say your child spent watching television or videos (including use of Cable, VCR, DVD, computers, mobile phones, and electronic tablets such as iPads)?" Hours of TV viewing will be reported in whole numbers, i.e., 1.0, 1.25, 2.5, etc. This will be administered at postpartum time points.

Infant Feeding Styles: Infant Feeding Styles will be assessed at late pregnancy, infant delivery, and 6 months postpartum time points. The 16 item questionnaire, adapted from Thompson et al.[58], assesses Restrictive Feeding Style (3 items - item #: 7, 9, 15), Pressuring/Overfeeding Style (7 items - item #: 1, 2, 4, 6, 10, 11, 14), Responsive Feeding Style (1 item - item #: 5), and Beliefs in the Benefits of Breastfeeding (5 items - item #: 3, 8, 12, 13, 16).

Maternal Follow Up Questionnaire: will be used to collect self-reported demographics social history, and food security (Household Food Insecurity) at 12 months postpartum. This questionnaire includes *Maternal Demographic Information, Social History*, and *Household Food Insecurity* as previously described.

Postpartum Medical History Form[i]: underlying medical conditions will be collected from the participant via interview at 6 months postpartum.

8.8. Maternal body composition

8.8.1. BOD POD

BOD POD measures body composition using air displacement plethysmography (Life Measurement Inc, Concord, CA) as previously described [60, 61]. Subjects will be clothed in a Lycra-style swim cap and tight fitting underwear or shorts. Body weight will be measured to the nearest 1g. Following standard calibration procedures, body volume of the subject will be measured in the BOD POD and correction made for thoracic gas volume (TGV) which is estimated using the BOD POD breathing circuit system [60]. The final TGV and the average of two body volume measurements that are within 0.2% will be used to calculate body density [60]. In terms of accuracy, the BOD POD has a 2% error relative to hydrostatic weighing and a CV of 2% [61].

8.8.2. Skinfold Thickness

Skinfold thickness will be measured at 6 sites (triceps, biceps, subscapular, iliac crest, mid-calf and mid-thigh) using skinfold calipers.

8.8.3. MRI

Magnetic resonance imaging (MRI) will be performed using a 3.0 T scanner (General Electric, Discovery MR750w, Milwaukee, WI). Subjects will be placed supine on the scanner table with the arms above the head and legs propped up. Subject positioning will be taught and reviewed by a Maternal Fetal Medicine physician to ensure mother and fetal safety. A SPGRE weighted localizer and T1 8 point ME-FSPGRE images will be acquired through the anatomy from the tips of the fingers through the pubic symphysis (waist). The subject will then be repositioned on the table with the head propped up and the pubic symphysis (waist) and legs positioned flat on the table. A SPGRE weighted localizer and T1 8 point ME-FSPGRE images will be acquired from the pubic symphysis (waist) to the bottom of the feet. A total of approximately ~800-1100 T1 8 point ME-FSPGRE images will be acquired on each subject. A frequency of 256, phase of 256, and a field of view of 50 cm will be utilized for the scan. The slice thickness will be 10mm with 10mm interslice gaps. Scan time will be approximately 20-30 minutes with the total mean acquisition time, including positioning of the subject, approximately 30 minutes. Scans will be saved onto CD or DVD and sent to imaging department for analysis, but can also be sent to a third party for analysis. Scans will be performed per Pennington Biomedical SOP 1109. Any abnormal findings identified during scan acquisition or analysis will be documented and reported per this SOP and the incidental findings plan agreed upon by the collaboration of the Principal Investigator, Leanne Redman, Co-Investigator, Marshall St. Amant, and the Pennington Biomedical Imaging Director. These data will also have an additional layer of oversight as Dr. St. Amant will review each scan within 14 days of acquisition. Dr. St. Amant is a Co-Investigator of this study and the Director of the Maternal Fetal Medicine Department at Woman's Hospital and as such will follow his standard procedure for identification of an abnormal finding including fetal anomaly which is his area of expertise. This includes, but is not limited to, contacting the participant to discuss the finding, contacting the participant's physician and/or contacting the participant's obstetrician.

Scans will not be performed during the participant's first trimester (12,6 GA or earlier). Gestational age will be determined based on the gestational age determination algorithm below. The MRI will not be performed until the youngest gestational age determination (either algorithm or ultrasound report from participant's obstetrician) is 13,0 or later.

Gestational Age Determination:

Gestational age is determined as follows, and is denoted "project gestational age". The "project EDC", which is based on the project gestational age, cannot be revised once a determination has been made.

Spontaneous Pregnancies

The following algorithm is based upon a comparison of the last menstrual period (LMP) and gestational age as assessed by the earliest dating ultrasound. The algorithm is also applicable to pregnancies where ovulation induction or artificial insemination was used. If a participant has not received a dating ultrasound by the time she has been determined to meet other eligibility requirements, one must be

conducted before randomization can take place (which will also be used to rule out known fetal anomalies). Abnormal findings found on pre-randomization ultrasounds which are performed outside clinical care for the study should be reported to the participant's physician.

Qualifications of the earliest dating ultrasound:

- *The ultrasound must have been conducted at 6 weeks or later. This means that if the ultrasound was done early in the first trimester, the crown rump length measurement (CRL) must be at least 4.0 mm.*
- *Gestational age cannot be determined by gestational sac measurement.*
- *There must be a report or an image showing the biometric parameter(s). A copy of the ultrasound report or image used for gestational age consideration must be kept in the participant's research folder.*
- *Information from a participant's chart (such as a doctor's note) without an official report is not acceptable for gestational age determination.*
- *The gestational age estimate must be expressed in weeks and days.*

The dating algorithm is as follows:

- *The first day of the LMP is determined, and a judgment made as to whether or not the patient has a "sure" LMP date.*
- *If the LMP date is unsure, the ultrasound measurements obtained at the patient's first dating ultrasound examination are used to determine the project gestational age, by the standard method of ultrasound gestational age determination at that institution. Ultrasound reports often have more than one gestational age estimate. Choose the gestational age estimate based on the biometric parameter(s) (such as CRL) not the estimate that includes LMP in the calculation.*
- *If the LMP date is sure, project gestational age is determined by a comparison between the gestational age by LMP and by the earliest dating ultrasound. Choose the ultrasound gestational age estimate that is based on the biometric parameter(s) (such as CRL) not the estimate that includes LMP in the calculation.*
- *If the earliest dating ultrasound confirms the gestational age by LMP within ± 7 days, the LMP-derived gestational age is used to determine the project gestational age.*
- *If the ultrasound determined gestational age does not confirm the LMP generated gestational age within ± 7 days, the ultrasound estimate is used to determine the project gestational age.*

In-Vitro Fertilization

The only exception to the algorithm above is the case where the patient has undergone in vitro fertilization (IVF) to achieve pregnancy. If in-vitro fertilization is used (standard IVF, IVF with donor egg/sperm, or IVF with ICSI) and

- *the embryo is transferred at three days of age, the project EDC is 263 days after the date of transfer;*
- *the embryo is transferred at five days of age, the project EDC is 261 days after the date of transfer;*
- *the embryo is transferred at six days of age, the project EDC is 260 days after the date of transfer.*

8.9. Fetal size

Fetal size [62], fetal adiposity [63, 64], and placental metrics will be measured using two dimensional (2-D) and three-dimensional (3-D) ultrasound. Fetal size will be measured early (13-16), mid (24-27 weeks GA) and late (35-37 weeks GA) pregnancy from 2-D conventional biometry which includes measurements of biparietal diameter (BPD), head circumference (HC), transverse diameter and circumference of the abdomen(AC) and femur length (FL) and 2) 3-D volumetry of the fetal thigh, upper arm and abdomen [62]. Fetal adiposity will be obtained from measurements of anterior and lateral abdominal subcutaneous fat thickness, mid-humeral and mid-thigh lean mass, mid-humeral and mid-thigh subcutaneous fat thickness [63, 64]. At least three measurements will be taken and the mean value used in the analysis. During measurements, images of the hands, feet, face, etc. of the fetus may be captured and, upon participant request, may be provided to the participant after review by Dr. St. Amant.

Analysis: All scans from all subjects will be read by the same analyst under the direction of Dr. St. Amant.

8.10. Clinical chemistry and biospecimens

For study use: 30mL of blood (at 5 timepoints, 150mL total over 18 months) will be collected to measure the following: glucose, insulin, thyroxine (T4), tri-iodothyronine (T3), thyroid stimulating hormone (TSH), leptin, total ghrelin, peptide YY, and cholecystokinin.
For future use: 20mL of blood (5 timepoints, 100mL total over 18 months) will be collected if the subject provides permission for samples to be stored for future use. 5mL of urine (5 timepoints, 25mL over 18 months) will be collected if the subject provides permission for samples to be stored for future use. Blood archives will be stored for running the following assays: FGF-21, lipids, ADP, HMWADP, FFA, hsCRP, 25 OH VitD, 1,25 dihydroxy VitD, 4-plex cytokines (IL-1, IL-6, IL-8, TNFa), and DHA/EPA. Placental biospecimens and cord blood will be collected if the subject provides permission for samples to be stored for future use.

8.11. Classification of gestational weight gain

Gestational weight gain will be defined, as recommended by the IOM, as the last measured weight during pregnancy minus the self-reported pre-pregnancy weight [10]. Self-reported pre-pregnancy weight will be validated against chart extracted weights recorded within 6 months of the current pregnancy. If the self-report and the chart extract weight differ too greatly, adjustment of pre-pregnancy weight used will be at the discretion of the principal investigator. The 2009 IOM guidelines for weight gain during pregnancy[10], will be used to categorize subjects on the basis of gestational weight gain. Subjects with gestational weight gain above the 2009 IOM guidelines (at the time of delivery) will be defined as '*High Gainers*'. Subjects with gestational weight gain within the 2009 IOM guidelines (at the time of delivery) will be defined as '*Normal Gainers*'. Subjects with gestational weight gain below the 2009 IOM guidelines (at the time of delivery) will be defined as '*Low Gainers*' and excluded from the primary analysis.

8.12. Measurement of maternal body composition

MRI will be used to quantify total adipose tissue mass, skeletal muscle mass and non-adipose tissue mass volumes in the mother during pregnancy and at 12 months postpartum. To investigate the contribution of the fetus to energy metabolism, we will estimate maternal fat mass and fat-free mass both with and without the inclusion of fetal size.

Total adipose tissue volumes (called fat mass, FM) will be measured by MRI and converted to kilograms as previously described [65-67]. Non-adipose tissue volume (called fat-free mass, FFM) will be calculated as;

FFM_{TOTAL} (kg) = Total maternal mass including fetus (kg) - fat mass (kg)

$FFM_{Maternal}$ (kg) = [maternal mass – fetal size* (kg)] - fat mass (kg)

*The mass of the fetus will be measured with 3-D ultrasound as described above.

8.13. Measurement of energy intake by energy balance method

Energy intake will be calculated from total daily energy expenditure measured by DLW and the changes in body energy stores (ΔES) using the following equations: Energy intake (kcal/d) = Total daily energy expenditure + ΔEnergy Stores where, Δ energy stores, was calculated using the actual change in fat mass (kg) and total fat-free mass (kg) between the late pregnancy (weeks 35 to 37) and early pregnancy testing (weeks 13 to 16) multiplied by the energy coefficients for fat mass and total fat-free mass. The energy density of fat mass has been well-established as approximately 9300 kcal/kg and has been validated in pregnant women [68]. Energy density of fat-free mass differs during pregnancy [69] and we will use 771 kcal/kg as previously determined for pregnant women [70]. Therefore: ΔEnergy stores (kcal/d) = (9300 x Δfat mass, kg/d) + (771 x Δtotal fat-free mass, g/d).

8.14. Changes in energy expenditure during pregnancy

We will measure each component of energy expenditure. Sleeping metabolic rate and resting metabolic rate will be measured directly in the metabolic chamber as described above. Total daily energy expenditure will be

measured directly by doubly labeled water as described above. Each component of energy expenditure will be studied according to following steps:

- Absolute change in energy expenditure (unadjusted). The absolute change in energy expenditure will be derived by taking the differences between two measurements of energy expenditure:
- Unadjusted = Late pregnancy energy expenditure (kcal/d) – Early pregnancy energy expenditure (kcal/d) Change in energy expenditure (adjusted). To adjust energy expenditure measures for changes in maternal and fetal mass, linear regression equations will be created for each measure of energy expenditure with fat mass, fat-free mass (FFM_{TOTAL}) and maternal age as independent variables (data from baseline will be used). We will use the actual measurement of fat mass, fat-free mass and maternal age late pregnancy to predict energy expenditure at this time point. The difference between actual and predicted energy expenditure will be calculated and defined as Adjusted. We will also run these models with maternal fat-free mass ($FFM_{Maternal}$) and fetal mass independently.

8.15. Measurement of changes in physical activity during pregnancy

Physical activity will be estimated from total daily energy expenditure by doubly labeled water and resting metabolic rate using two different calculations. First the physical activity level (PAL) will be calculated by the widely accepted method of dividing total daily energy expenditure (TDEE) by sleeping metabolic rate (RMR) as; PAL = TDEE/RMR. Because of the inherent problem of using ratios when the two variables have an intercept not equal to zero [71], we will also express physical activity as the residual value of the regression between measured TDEE and measured RMR [72]. This value, termed Activity Related Energy Expenditure (AREE), is positive for subjects with higher physical activity than average and negative for subjects with lower physical activity than average independent of metabolic body size. Because AREE is adjusted for metabolic body size (RMR), this value is directly proportional to the amount of physical activity. We have previously used both approaches to study changes in physical activity during calorie restriction [73].

8.16. Placenta biospecimens

For participants who provide permission, as soon as possible (preferably within 60 minutes of delivery), the delivered placenta will be weighed, photographed and the following biospecimens will be collected:

- **Basal plate tissue**: samples of the basal plate (the maternal side) will be collected and stored at PBRC.
- **Villous tissue**: samples of the villous tissue (inner placental layer) will be collected and stored at PBRC.
- **Chorionic plate tissue**: samples of the chorionic plate (the fetal side) will be collected and stored at PBRC.
- **Cord Blood**: Attempted collection will be completed with as much blood as possible that can be extracted from the placenta (average extraction is 50 mL). **Note:** as cord blood supply is only to the fetus and not the mother, an attempted maximum collection implies no increased risk to baby (as s/he is already separated from the cord) or the mother.
- **Umbilical cord tissue**: small (approximately than 1 inch in length) sections of the umbilical cord will be collected and stored at PBRC.
- **Fetal membranes**: a small section of membrane (approximately 10 cm x 10 cm) will be collected and stored at PBRC.

In the case where a request for placental examination is made by the physician, study staff will attempt to coordinate with pathologists to obtain samples in order to preserve the tissue for histological analysis. Placental biospecimens will be snap frozen in liquid nitrogen, preserved for immunohistochemistry or the fresh tissue will be prepared for isolation of trophoblast cells.

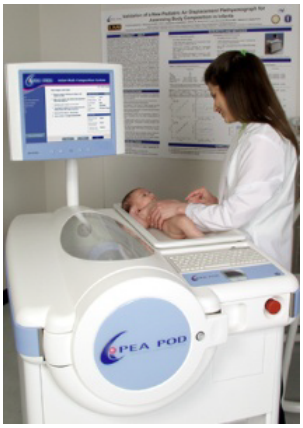
The specimens collected at delivery will be collected at community hospitals where the hospital has approved this procedure, as defined by the community hospital policies and procedures. If the PI does not have permission from the hospital or it is not possible to collect the specimen for any other reason, regardless of the subject's consent to the procedure, the specimen collection procedure will not be done.

8.17. Infant Assessments

Infant Anthropometrics

Infant body weight will be obtained using a standard electrical infant scale with the infant undressed. Recumbent infant length will be measured using an infantometer with a stationary head-board, a moveable footboard and a built-in centimeter scale. Maximal head circumference will be measured by a standard measuring tape around the child's head, and abdominal circumference will be measured by a standard measuring tape around the child's abdomen. Length, weight, head circumference, and abdominal circumference will be performed by a trained research team member before 10 days of life.

PEAPOD



Body composition will be assessed in infants with air displacement plethysmography (Life Measurement Inc, Concord, CA), as previously described [28, 29]. The infant will be placed in a special chamber called a PEA POD (pictured). The infant will lay naked in the supine position on a flat tray that slides into a transparent plastic chamber. The amount of volume (space) occupied by the infant will be measured. The staff will be able to monitor the child during the test through the transparent top.

Infant Skinfold Thickness

Infant skinfold thickness will be measured at four sites (tricep, subscapular, iliac crest and thigh) by a trained research team member before 10 days of life using Harpenden skinfold calipers.

9. PARTICIPANT SAFETY AND CONFIDENTIALITY

This study does not involve major risk to participants. Efforts to minimize the potential risks of the assessment methods and outcome variables include frequent monitoring by the investigators to assure that no volunteer suffers any adverse effects from participating in the research. Risks of complications will be reduced by carefully selecting participants for treatment. Other than subjects being obese at the time of enrollment, the inclusion and exclusion criteria were created to ensure women with low risk for pregnancy complications are included. The subjects' primary care obstetrician will be informed of the study procedures and purpose and will be required to provide medical clearance for the subject to participate in the proposed study. Medical clearance will also be obtained by the Medical Investigator. The Medical Investigator will communicate all adverse and serious adverse events to the primary care obstetrician and the development of any pregnancy-induced contraindications will be disclosed by the primary care physician to the Medical Investigator. Patients and the primary care obstetrician will be made aware of any abnormal findings determined throughout the course of the proposed study.

9.1. Risks to participants

- Accelerometry. There is no risk associated with measuring activity with accelerometers. The accelerometers used for outcome measurements in the proposed research (SenseWear and ActiGraph) fit comfortably on the participant's arm and wrist. In rare cases, the device(s) may irritate the skin; if this occurs, the device(s) can easily be removed should they become uncomfortable.

- Archive of Biological Samples: The primary risk to participants who donate biospecimens to be banked for future research is the risk of loss of confidentiality and/or privacy. Participants will be given a chance to approve/disapprove on the consent form the taking of extra blood for further research purposes. Storage and disposal of tissue will be conducted in a manner conforming to the appropriate care and handling of biological specimens as outlined through the Institutional Biohazard Committee Guidelines.
- Blood draw: There is the possibility of pain and bruising at the vein on the arm where the needle is inserted. Aseptic (sterile) technique and trained personnel minimize these risks.
- Body weight: There is no known risk to participants who measure/record their body weight.
- BOD POD: Air displacement plethysmography by BOD POD® (Life Measurement Inc, Concord, CA) will be used to measure body composition in the participants. Measurement of whole body adiposity with the BOD POD® is non-invasive and completely safe for use during pregnancy [74].
- Collection of urine: There is no known risk to participants in collecting their urine.
- Doubly labeled water: The safety and ethical considerations for the use of stable isotopes in humans and specifically in studies of special populations including pregnant women and infants is reviewed in an International publication from the International Atomic Energy Agency [75]. Stable isotopes have been used in studies of human metabolism for more than 50 years and no significant adverse event has been documented [76]. Stable isotopes of several elements such as hydrogen, oxygen and carbon have widespread use in clinical research. Deuterium and oxygen-18 or ^{18}O are stable isotopes for hydrogen and oxygen, respectively. The two atoms occur naturally in the body and the dose given in the water is not dangerous. The dosage of deuterium is typically 20-80 mg per kg of body weight which results in a maximum concentration in body water of ~0.16% [76, 77]. Deuterium has been given to humans including pregnant women up to a stable concentration of 0.5% for several days [78]. The threshold of deuterium toxicity is 15.0% and this concentration far exceeds doses administered to humans [79, 80]. The doubly labeled water we will use in this study in pregnant women will raise the deuterium concentration in body water to about 1/50th of the threshold for toxicity. There is no evidence regarding untoward effects of deuterium for the measurement of total energy expenditure in humans including pregnant women [75]. The biological effects of oxygen-18 are small. For example, replacement of up to 60% of body oxygen with oxygen-18 did not result in any adverse effects [80]. Given the low risk associated with the use of stable isotopes in humans, deuterium and oxygen-18 are considered safe for use in humans across the lifespan. The use of doubly labeled water for measuring energy expenditure has been used in various special populations such as pregnant and lactating women, infants (including premature infants) and children [24-28]. Measuring energy expenditure in pregnant women with doubly labeled water at the planned doses carries no known risk.
- Indirect calorimetry in a metabolic chamber: Measuring energy expenditure in the metabolic chamber carries no known risk. Some participants may experience claustrophobia or discomfort from staying in the chamber however they will have close contact with metabolic core and inpatient staff during the chamber stay.
- Infant assessments. There are no known risks to infants from participation in this study. Some newborns will begin to cry during the gentle handling necessary to undress the infant and to perform measurements such as length and weight. This is a normal occurrence. If infants cry while being measured, he or she will be comforted by holding, covering with a warm blanket, gentle rocking, or a diaper change, if necessary.
- Maternal food intake: Food intake will be estimated with the Remote Food Photography Method (RFPM). The RFPM involves taking pictures of food selection and plate waste and is not associated with significant risk. Considerable effort has been expended to make these procedures as user-friendly and unobtrusive as possible.
- MRI: The risks associated with MRI include the discomfort of being in a small space, noise from the scanner, and extraction of metal lodged in the body. Subjects with any implanted metal device will be excluded. Subjects will be offered lumbar and/or leg support if uncomfortable. We have significant experience performing whole-body MRI scans on adults and have not experienced any issues thus far. While there are no known risks of MRI on the pregnant woman or fetus, the National Radiological Protection Board advises that these tests be done after the first trimester. Therefore, the first MRI will be scheduled at or after 13

weeks, and only after there has been an ultrasound confirming gestational age. The study equipment contains magnets that are at or below the strength of the magnets used in safety studies. A standard MR pre-study questionnaire will be filled out to screen for: presence of a pacemaker, presence of metallic objects, either surgically placed or otherwise, history of claustrophobia. An investigator or MR technician will ensure that no metallic objects are in the subject's possession before they enter the MR suite.

- Oral glucose tolerance test: The glucose drink may cause nausea, vomiting, abdominal bloating, or a headache. The blood draw may be associated with pain or bruising where the needle is inserted. Trained personnel and aseptic (sterile) techniques are used to minimize these risks.
- Self-report Questionnaires: There are no anticipated risks from completing self-report questionnaires. If signs of minor stress or fatigue are apparent, participants will be given time to take a break from completing the questionnaires. It is estimated that the questionnaires will take from 35 to 50 minutes to complete. The questions contained in some of the questionnaires may make people feel uncomfortable since they ask about topics such as how they feel about their body size. Responses to the questions will be coded to protect confidentiality, and participants may choose to not answer questions.
- Ultrasound: There are no known risks associated with obstetric ultrasound.

9.2. Safety Monitoring/Adverse Events

The following findings would generate a safety alert and study staff should be notified immediately:

1. High Score on the Beck Depression Inventory II
   - Actively suicidal
     - BDI-II question 9 value of 2 or 3 (regardless of BDI-II total score) requires notification of the Principal Investigator (PI) or a designated clinician with appropriate training in mental health assessment. The research staff should wait with the patient until the PI or designated clinician (Pennington psychiatric staff with coordination by the social work department) has the opportunity to assess the participant. The PI or designated clinician should assess the patient for risk of imminent harm to self and contact emergency services if needed. If the PI or designated clinician is not immediately available, notify emergency services according to local standards. Research staff should wait with the participant until emergency services has assumed care.
   - Severe depression
     - BDI-II score of 29-63 (and BDI-II question 9 value of 0 or 1) requires that research staff inform the participant about her elevated score and notify the PI or designated clinician who should assess the patient for safety and/or possible psychiatric referral. If the PI or designated clinician is not immediately available, the participant may leave unattended, but the PI/ designated clinician should contact the participant within 24 hours to assess her for risk of imminent harm and should contact emergency services if needed. The participant should be told to expect such contact from study personnel. The patient's prenatal care provider also should be informed within 2 business days.
   - Moderate depression
     - BDI-II score of 20-28 (and BDI-II question 9 values of 0 or 1) requires that the participant be informed and that it is recommended she see her physician or mental health professional for further evaluation. Participants may be provided with materials on available mental health resources.
2. High Blood pressure
   - If measured blood pressure is ≥ 160/110, i.e. systolic blood pressure ≥ 160 mm Hg and/or diastolic blood pressure ≥ 110 mm Hg, study staff will call the participant's prenatal care provider immediately and have a Pennington Biomedical physician or nurse practitioner meet with participant and confirm measures. The physician or NP will initiate the action plan.
   - If measured blood pressure is ≥ 140/90, i.e. systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg, study staff will instruct participants to contact their provider regarding the

elevated measure to arrange appropriate follow-up. Additionally, study staff will notify the prenatal care provider by phone before the end of the study visit.
- At least one blood pressure reading must meet criteria when two readings are taken, and at least two blood pressure readings must meet criteria when three readings are taken.

3. Weight loss from enrollment weight during pregnancy
   - Alert values will be weight loss of 6% or greater of enrollment weight for women who were obese at enrollment.
   - For the first weight loss alert, the research staff will notify the patient, discuss her energy balance activities.
   - The patient will be asked to notify prenatal care provider and will be given weight measurement to report to prenatal care provider.
4. Fetal Growth
   - Ultrasound alert abnormalities will be determined by physician Marshall St. Amant
5. Infant Growth
   - If the infant weight measured is indicative of a ≥ 12% weight loss from birth weight (reported by the parent/legal guardian at the visit), the research staff will notify the infant participants' parent/legal guardian and a referral will be made to the infant's pediatrician with the assistance of the study's medical investigator.
   - If the infant growth safety alert is triggered, the medical investigator or IPU nurse practitioner will be called prior to the conclusion of the study visit for further evaluation of the infant.

An **adverse event** is any untoward medical occurrence including but not limited to:
- Musculoskeletal injury that requires medical attention
- Fetal growth restriction (<5th percentile with abnormal umbilical artery Dopplers)
- Unanticipated untoward medical events

Pennington Biomedical Research Center is an AAHRPP accredited institution and, above all else, is committed to ensuring and maintaining the safety of its participants. Study co-investigator, Dr. Marshall St. Amant, directs the Maternal Fetal Medicine Department at Woman's Hospital in Baton Rouge, Louisiana. Dr. St. Amant will review all MRI scans within 14 days and will follow standard institutional reporting procedures for reporting any abnormal findings. We will use the provided definitions of Adverse Events and Serious Adverse Events. Events will be reported according to our institutional reporting policy.

Although MRI scans and ultrasound images are collected during the study for research purposes only, it is possible that research physician or radiology staff may notice something that could be important to the health of the participant. Should such findings arise from these procedures, the research physician or research staff will contact the participant and/or the participant's primary healthcare provider with information about the findings. The participant and/or their healthcare provider may also receive a copy of the images pertaining to these findings.

A **serious adverse event** (SAE) is defined as an unanticipated medical occurrence that is deemed associated with study participation by the study Medical Investigator that results in one of the following:
- Death
  - Maternal death
  - Fetal or infant death, including miscarriage, therapeutic abortion because of increasing signs of maternal or fetal compromise, and stillbirth.
- Life-threatening event
  - Life threatening events in the mother or fetus are defined as those that in the view of the research staff and PI put the individual patient at imminent substantial risk of dying, or if continued participation in the study might have resulted in death.
- Hospitalization (initial or prolonged)

- Maternal hospitalization or acute outpatient evaluation (e.g. in an emergency room or labor and delivery triage unit) alone is not sufficient to qualify as a serious adverse event. Hospitalization or acute outpatient evaluation for the following would not be considered a serious adverse event: term delivery, preterm premature rupture of membranes (pPROM), pyelonephritis, bedrest, contractions, ruling in or out preeclampsia, and preterm labor.
- Any infant hospitalization after neonatal discharge and during the infant's participation in the study is reported as a serious adverse event.
- Any medical or surgical procedure performed (e.g., surgery, transfusion) itself is not the adverse event; instead, the condition that leads to the procedure is the adverse event.

- Disability or permanent damage
  - If the adverse event resulted in a substantial disruption of the mother or infant's ability to conduct normal life functions, i.e., the adverse event resulted in a significant, persistent or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life. The recommendation for bedrest does not constitute an SAE.
- Medical Intervention to prevent permanent impairment or damage

Other serious important medical events also qualify. When an event does not fit the other outcomes, but may jeopardize the patient and may require medical or surgical intervention or treatment to prevent one of the other outcomes, this should also be reported as an SAE.

8.1.5. Surveillance and Reporting Procedures

All AEs (except for expected) will be reported. Adverse events will be documented during the scheduled visits. For each sign, symptom or adverse event, the following information will be recorded:

- A brief descriptor of the adverse event
- Start and stop dates
- Intensity (mild / moderate / severe)
- Whether the AE was "serious" or not (as defined below)
- Causal association with the intervention assigned (none / doubtful / possibly / probably / very likely)
- Outcome (resolved / resolved with sequelae / improving / still present and unchanged / death)
- Action taken with respect to the intervention (none / intervention temporarily discontinued / medical therapy required / intervention permanently discontinued / other).

Serious adverse events that are deemed unanticipated and related and therefore required to be reported to the IRB will be telephoned, faxed, or emailed *within 48 hours* of ascertainment by the center PI or study coordinator to the local Institutional Review Board. Because a written record of notification is required, telephone notifications must be followed with written documentation that the IRB has been informed. Unanticipated and related SAEs not resolved by the end of the study or that have not resolved upon discontinuation of the subject's participation in the study will be followed by the clinical site until the event resolves, stabilizes or returns to baseline.

9.3. Stopping rules

This study does not involve major risk to participants. The most likely scenario that would indicate a cessation of the study would be failure to recruit participants or implement the study as planned. Nevertheless, in addition to monitoring recruitment and compliance to the study, we also will monitor the rates of injury in our participants. The study investigators will alert the IRB, if a larger than reasonably expected injury rate occurs in the treatment groups. Other issues that are related to the stopping rules include:

- New information – It is unlikely that new information will become available during this study that would result in discontinuing the trial.
- Limits of assumption – It is possible that the value of data analysis will be limited by differences because of study dropouts or missing data. Baseline differences will be analyzed and effects on the power to detect differences in the outcome measures will be evaluated and discussed with the PI, and the NIH Project Officer. Although an excessive number of dropouts could occur, this has not been our past

experience. In CALERIE study (24 weeks), the dropout rate was only 2% (1 out of 36 enrolled). If the dropout rate for the proposed study exceeds 15%, the study coordinator will initiate a meeting with the PI to discuss strategies to increase retention. If the dropout rate exceeds 25%, the safety officer will meet with the study investigators to determine whether or not the study should continue.

- Limit of rules – We acknowledge that circumstances, other than what are listed, may justify stopping the study.

9.4. Confidentiality

The study investigators will treat participant identity with professional standards of confidentiality. Each participant will be assigned an identification number and all participant protected health information/records/questionnaires/data will be coded and identified using these numbers rather than participant names. The coded participant list identifying participants by name will be stored separately from the number coded records/questionnaires/data. All participant records/data will be stored in locked file cabinets. Study results will be reported as group data and/or by participant identification number only. Study results may be presented in publications or at meetings/conferences, however participant identity will not be disclosed. A participant may request their personal health information or their personal study data/results at any time during the study and will be given copies. All volunteers are assured of their confidentiality both verbally and in the informed consent form. The clinical facilities are strictly limited to the staff of the research institution and to research volunteers. This is accomplished by a variety of stringent security measures. All medical records are stored in locked areas. Access to these areas is limited to the clinical support staff, director of the clinical facilities, and the PIs. Electronic data storage is similarly restricted with only the PIs and authorized persons having access to databases containing confidential clinical records, i.e. those containing name OR other identifying information.

10. DATA MANAGEMENT

10.1. Statistical power and sample size

The primary outcome is energy intake during pregnancy. We were able to apply the energy-balance model to estimate energy intake in previously reported data on energy requirements during pregnancy [82]. The standard deviation of energy intake in the third trimester was conservatively estimated to be 700 kcal/d using Butte's data on total energy expenditure and energy deposition of fat mass and fat-free mass.

Table 4. Minimum sample size for power at .80 and .90, ratio between groups 1:2

Standard Deviation kcal/d	Mean Difference kcal/d	Power (β)	Group 1 N	Group 2 N
700	541	.90	27	54
700	468	.80	27	54
700	*600*	*.90*	*22*	*44*
700	520	.80	22	44
700	688	.90	17	34
700	*590*	*.80*	*17*	*34*

We hypothesize that women who will exceed 2009 IOM recommendations for weight gain during pregnancy, the 'High Gainers', will have a larger energy intake than those who will keep their weight gain within IOM guidelines, the 'Normal Gainers'. Because of the intervention, we predict to have a decrease in the incidence of 'High Gainers' from 66% to a rate below 50%. Therefore we predict that the ratio "High Gainers" to "Normal Gainers" will likely be 1:2. Our power/sample size analysis (Table 2) assumed: 1) power ≥ 0.90, 2) alpha = 0.05, 3) the null hypothesis will be tested against a two-directional alternative, 4) the common standard deviation for energy intake is 700 kcal/d and 5) conservative estimates that the ratio between the two groups, 'High Gainers' to 'Normal Gainers' is 1:2.

As shown in Table 2, with subject assignment to the 2009 IOM guidelines being 1:2, we require a minimum of 51 subjects, to detect a between group difference of 590 kcal/d with 90% power or 520 kcal/d with 80% power. To allow for early deliveries, the inability to perform MRI in some subjects late pregnancy, due to limitations with the field of view, and that 10% of subjects will gain less weight than recommended; we aim to complete at least 60 subjects.

10.2. Data analysis plan

Analyses will be performed in SAS version 9.3 (SAS Institute, Cary, NC). Statistical significance will be set at $p \leq 0.05$.

Aim 1: Mixed models will be used to analyze differences between 'High Gainers' and 'Normal Gainers' with respect to energy intake during pregnancy estimated with the energy balance method. Adjustments for covariates, such as maternal age, fetal size and parity will be made if appropriate. Least square means will be compared with the mixed models contrasts. Energy intake from the energy balance model will be analyzed analogously in the context of repeated measurements. We expect that energy intake by the two methods will all be significantly higher in 'High Gainers' versus 'Normal Gainers'. In addition, the two methods for measuring energy intake will be cross validated with each other and compared with Bland-Altman analysis. Regression analyses will be used to assess the relationship between energy intake during pregnancy and postpartum weight loss after 1 year. We predict that lower energy intake during pregnancy is associated with better postpartum weight loss results after 1 year.

Aim 2: Analysis of covariance will be used to assess significance of differential changes ('High Gainers' versus 'Normal Gainers') in absolute energy expenditure. Differential changes in energy expenditure after adjustment for changes in maternal and fetal size will be tested analogously. For secondary hypotheses, change in energy expenditure attributable to accelerometer-measured physical activity (steps per min) will be analyzed to investigate its role in differential energy balance in 'High Gainers' versus 'Normal Gainers'.

Analyses of exploratory aims: The change in determinants of energy intake and energy expenditure in blood and urine from early to late pregnancy and 12 months postpartum will be analyzed by repeated measures with IOM classification and time interactions. Adjustments for covariates, such as maternal age, fetal size, parity and early pregnancy values will be made if appropriate. To control for type I error, statistical significance for all multiple comparisons will be adjusted using the Tukey-Kramer method.

11. SUBJECT PAYMENT

MomEE subjects will be compensated $600 for completing the study; $200 after completing the early pregnancy assessments, $200 after delivery, and the remaining $200 at the completion of the study. In addition, up to $25 will be compensated to infant participants for completion of the infant delivery visit before the infant reaches 10 days of life. If the parent/legal guardian reports that the infant participant has a social security number at the time of the infant delivery visit, the infant social security number will be obtained for check processing, and the infant participant will be paid. If the parent/legal guardian reports that the infant participant has not yet obtained a social security number at the time of the study visit which is likely for infants of this age, the parent/legal guardian of the infant participant will be allowed to receive payment on behalf of the infant participant only if the parent/legal guardian agrees to provide his/her own social security number as it is required for check processing.

12. REFERENCES

1. Ogden, C.L., et al., *Prevalence of obesity in the United States, 2009-2010*, in *NCHS Data Brief*2012, National Center for Health Statistics: Hyattsville, MD.
2. Flegal, K.M., et al., *Prevalence and trends in obesity among US adults, 1999-2008.* JAMA, 2010. **303**(3): p. 235-41.
3. Viswanathan, M., et al., *Outcomes of maternal weight gain: evidence report/technology assessment No. 168*, P.b.R.I.U.o.N.C.E.-B.P. Center, Editor 2008, Agency for healthcare research and quality: Rockville, MD.
4. Gore, S.A., D.M. Brown, and D.S. West, *The role of postpartum weight retention in obesity among women: a review of the evidence.* Ann Behav Med, 2003. **26**(2): p. 149-59.
5. Rooney, B.L. and C.W. Schauberger, *Excess pregnancy weight gain and long-term obesity: one decade later.* Obstetrics and gynecology, 2002. **100**(2): p. 245-52.
6. Crane, J.M., et al., *The effect of gestational weight gain by body mass index on maternal and neonatal outcomes.* J Obstet Gynaecol Can, 2009. **31**(1): p. 28-35.

7. Beyerlein, A., N. Lack, and R. von Kries, *Within-population average ranges compared with Institute of Medicine recommendations for gestational weight gain.* Obstetrics and gynecology, 2010. **116**(5): p. 1111-8.
8. Frederick, I.O., et al., *Pre-pregnancy body mass index, gestational weight gain, and other maternal characteristics in relation to infant birth weight.* Maternal and child health journal, 2008. **12**(5): p. 557-67.
9. Whitaker, R.C., *Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy.* Pediatrics, 2004. **114**: p. e29-36.
10. IOM and NRC, *Weight Gain During Pregnancy* ed. K.M. Rasmussen and A.L. Yaktine2009, Washington D. C.: The National Academies Press.
11. Dalenius, K., et al., *Pregnancy Nutrition Surveillance 2010 Report*, D.o.H.a.H. Services, Editor 2012: Atlanta, GA.
12. Asbee, S.M., et al., *Preventing excessive weight gain during pregnancy through dietary and lifestyle counseling: a randomized controlled trial.* Obstetrics and gynecology, 2009. **113**(2 Pt 1): p. 305-12.
13. Guelinckx, I., et al., *Effect of lifestyle intervention on dietary habits, physical activity, and gestational weight gain in obese pregnant women: a randomized controlled trial.* The American journal of clinical nutrition, 2010. **91**(2): p. 373-80.
14. Jeffries, K., et al., *Reducing excessive weight gain in pregnancy: a randomised controlled trial.* Med J Aust, 2009. **191**(8): p. 429-33.
15. Phelan, S., et al., *Randomized trial of a behavioral intervention to prevent excessive gestational weight gain: the Fit for Delivery Study.* The American journal of clinical nutrition, 2011.
16. Polley, B.A., R.R. Wing, and C.J. Sims, *Randomized controlled trial to prevent excessive weight gain in pregnant women.* International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity, 2002. **26**(11): p. 1494-502.
17. Wolff, S., et al., *A randomized trial of the effects of dietary counseling on gestational weight gain and glucose metabolism in obese pregnant women.* International journal of obesity, 2008. **32**(3): p. 495-501.
18. Delahanty, L.M. and D.M. Nathan, *Implications of the diabetes prevention program and Look AHEAD clinical trials for lifestyle interventions.* Journal of the American Dietetic Association, 2008. **108**(4 Suppl 1): p. S66-72.
19. Group, T.D.P.P.D.R., *The Diabetes Prevention Program. Design and methods for a clinical trial in the prevention of type 2 diabetes.* Diabetes Care, 1999. **22**(4): p. 623-34.
20. Group, T.D.P.P.D.R., *The Diabetes Prevention Program (DPP): description of lifestyle intervention.* Diabetes Care, 2002. **25**(12): p. 2165-71.
21. Raynor, H.A., et al., *Weight loss strategies associated with BMI in overweight adults with type 2 diabetes at entry into the Look AHEAD (Action for Health in Diabetes) trial.* Diabetes care, 2008. **31**(7): p. 1299-304.
22. de Jonge, L., et al., *Validation study of energy expenditure and intake during calorie restriction using doubly labeled water and changes in body composition.* Am J Clin Nutr, 2007. **85**(1): p. 73-9.
23. Racette, S.B., et al., *Approaches for quantifying energy intake and %calorie restriction during calorie restriction interventions in humans: the multicenter CALERIE study.* Am J Physiol Endocrinol Metab, 2012. **302**(4): p. E441-8.
24. Butte, N.F., et al., *Energy requirements during pregnancy based on total energy expenditure and energy deposition.* The American Journal of Clinical Nutrition, 2004. **79**(6): p. 1078-1087.
25. Heini, A., et al., *Free-living energy expenditure measured by two independent techniques in pregnant and nonpregnant Gambian women.* Am J Physiol, 1991. **261**(1 Pt 1): p. E9-17.
26. Prentice, A.M., et al., *Energy requirements of pregnant and lactating women.* Eur J Clin Nutr, 1996. **50 Suppl 1**: p. S82-110; discussion S10-1.
27. Roberts, S.B., et al., *Comparison of the doubly labeled water (2H2(18)O) method with indirect calorimetry and a nutrient-balance study for simultaneous determination of energy expenditure, water intake, and metabolizable energy intake in preterm infants.* Am J Clin Nutr, 1986. **44**(3): p. 315-22.

28. Butte, N.F., W.W. Wong, and J.M. Hopkinson, *Energy requirements of lactating women derived from doubly labeled water and milk energy output.* J Nutr, 2001. **131**(1): p. 53-8.
29. Martin, C.K., et al., *Effect of calorie restriction on resting metabolic rate and spontaneous physical activity.* Obesity (Silver Spring), 2007. **15**(12): p. 2964-73.
30. Schoeller, D.A., *Measurement of energy expenditure in free-living humans by using doubly labeled water.* J Nutr, 1988. **118**(11): p. 1278-89.
31. Racette, S.B., et al., *Relative dilution spaces of 2H- and 18O-labeled water in humans.* Am J Physiol, 1994. **267**(4 Pt 1): p. E585-90.
32. Nguyen, T., et al., *Chamber for indirect calorimetry with accurate measurement and time discrimination of metabolic plateaus of over 20 min.* Med Biol Eng Comput, 2003. **41**(5): p. 572-8.
33. Livesey, G. and M. Elia, *Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels.* Am J Clin Nutr, 1988. **47**(4): p. 608-28.
34. Martin, C.K., et al., *The effect of ecological momentary assessment methods on the validity of the Remote Food Photography Method (RFPM) for measuring energy intake in near real-time: A pilot study.* Obesity (Silver Spring), 2009. **17**(2): p. S111-S112.
35. Martin, C.K., et al., *A novel method to remotely measure food intake of free-living individuals in real time: the remote food photography method.* Br J Nutr, 2009. **101**(3): p. 446-56.
36. Martin, C.K., S. Kaya, and B.K. Gunturk, *Quantification of food intake using food image analysis.* IEEE Engineering in Medicine and Biology Magazine, 2009. **1**: p. 6869-6872.
37. Lacasse, A., et al., *Validity of a modified Pregnancy-Unique Quantification of Emesis and Nausea (PUQE) scoring index to assess severity of nausea and vomiting of pregnancy.* American journal of obstetrics and gynecology, 2008. **198**(1): p. 71 e1-7.
38. Fairburn CG, B.S., *Assessment of eating disorders: Interview or self-report questionnaire?* Int J Eat Disord 1994. **6**: p. 1-8.
39. Wing RR, T.D., Gorin AA, et al. , *STOP regain: are there negative effects of daily weighing?* . J Consult Clin Psychol, 2007. **75**(4): p. 652-656.
40. Phelan S, L.T., Gorin A, et al. , *What distinguishes weight-loss maintainers from the treatment-seeking obese? Analysis of environmental, behavioral, and psychosocial variables in diverse populations.* Ann Behav Med, 2009. **38**(2): p. 94-104.
41. Olivarez, S.A., et al., *Obstructive sleep apnea screening in pregnancy, perinatal outcomes, and impact of maternal obesity.* Am J Perinatol, 2011. **28**(8): p. 651-8.
42. Olivarez, S.A., et al., *Prospective trial on obstructive sleep apnea in pregnancy and fetal heart rate monitoring.* Am J Obstet Gynecol, 2010. **202**(6): p. 552 e1-7.
43. Marriott, L.D., et al., *What do babies eat? Evaluation of a food frequency questionnaire to assess the diets of infants aged 12 months.* Pub Health Nutr, 2008: p. 1-6.
44. Fein, S.B., et al., *Infant Feeding Practices Study II: study methods.* Pediatrics, 2008. **122 Suppl 2**: p. S28-35.
45. Ware, J., Jr., M. Kosinski, and S.D. Keller, *A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity.* Med Care, 1996. **34**(3): p. 220-33.
46. White, M.A., et al., *Development and validation of the food-craving inventory.* Obes Res, 2002. **10**(2): p. 107-14.
47. Framson, C., et al, *Development and validation of the mindful eating questionnaire.* Journal of the American Dietetic Association, 2009. **109**(8): p. 1439-1444.
48. Stunkard, A.J. and S. Messick, *Eating Inventory Manual (The Psychological Corporation)*1988, San Antonio, TX: Harcourt Brace & Company.
49. Beck, A.T., R.A. Steer, and G.K. Brown, *BDI-II Manual*1996, San Antonio, TX: Harcourt Brace & Company.
50. Cooper, P.J., et al., *The development and validation of the Body Shape Questionnaire.* International Journal of eating disorders, 1987. **6**: p. 485-494.

51. Downs, D.S. and H.A. Hausenblas, *Exercising for two: examining pregnant women's second trimester exercise intention and behavior using the framework of the theory of planned behavior.* Women's health issues : official publication of the Jacobs Institute of Women's Health, 2003. **13**(6): p. 222-8.
52. Cash, T.F., *The multidimensional body-self relations questionnaire user's manual.* 1994, Norfolk: Old Dominion University.
53. *Body images: Development, deviance, and change.*, ed. T.S. Giovannelli and P. T. Vol. 51. 1990, New York: Guilford.
54. Rauff, E.L. and D.S. Downs, *Mediating effects of body image satisfaction on exercise behavior, depressive symptoms, and gestational weight gain in pregnancy.* Annals of behavioral medicine : a publication of the Society of Behavioral Medicine, 2011. **42**(3): p. 381-90.
55. Giovannelli, T.S., et al., *The measurement of body-image dissatisfaction-satisfaction: is rating importance important?* Body image, 2008. **5**(2): p. 216-23.
56. Stewart, T.M., et al., *The development of the Body Morph Assessment version 2.0 (BMA 2.0): tests of reliability and validity.* Body Image, 2009. **6**(2): p. 67-74.
57. Burdette HL, W.R., *A national study of neighborhood safety, outdoor play, television viewing, and obesity in preschool children.* Pediatrics, 2005. **116**(3): p. 657-662.
58. Thompson AL, M.M., Borja JB, Adair LS, Zimmer CR, Bentley ME, *Development and validation of the Infant Feeding Style Questionnaire.* Appetite 2009. **53**(2): p. 210-221.
59. Wolfe, L.A. and M.F. Mottola, *PARmed-X for pregnancy: physical activity readiness medical examination* Can Soc Exerc Physiol & Health Canada, 2002: p. 1-4.
60. Dempster, P. and S. Aitkens, *A new air displacement method for the determination of human body composition.* Medicine and science in sports and exercise, 1995. **27**(12): p. 1692-7.
61. McCrory, M.A., et al., *Evaluation of a new air displacement plethysmograph for measuring human body composition.* Medicine and science in sports and exercise, 1995. **27**(12): p. 1686-91.
62. Schild, R.L., R. Fimmers, and M. Hansmann, *Fetal weight estimation by three-dimensional ultrasound.* Ultrasound Obstet Gynecol, 2000. **16**(5): p. 445-52.
63. Bernstein, I.M., et al., *Differential growth of fetal tissues during the second half of pregnancy.* Am J Obstet Gynecol, 1997. **176**(1 Pt 1): p. 28-32.
64. Khoury, F.R., et al., *Comparison of estimated fetal weights using volume and 2-dimensional sonography and their relationship to neonatal markers of fat.* J Ultrasound Med, 2009. **28**(3): p. 309-15.
65. Gallagher, D., et al., *Organ-tissue mass measurement allows modeling of REE and metabolically active tissue mass.* Am J Physiol, 1998. **275**(2 Pt 1): p. E249-58.
66. Gallagher, D., et al., *Weight loss in postmenopausal obesity: no adverse alterations in body composition and protein metabolism.* Am J Physiol Endocrinol Metab, 2000. **279**(1): p. E124-31.
67. Gallagher, D., et al., *Adipose tissue in muscle: a novel depot similar in size to visceral adipose tissue.* The American journal of clinical nutrition, 2005. **81**(4): p. 903-10.
68. Butte, N.F., et al., *Composition of gestational weight gain impacts maternal fat retention and infant birth weight.* American Journal of Obstetrics and Gynecology, 2003. **189**(5): p. 1423-32.
69. Kopp-Hoolihan, L.E., et al., *Fat mass deposition during pregnancy using a four-component model.* J Appl Physiol, 1999. **87**(1): p. 196-202.
70. Thomas, D.M., et al., *Dynamic energy-balance model predicting gestational weight gain.* The American journal of clinical nutrition, 2012. **95**(1): p. 115-22.
71. Allison, D.B., et al., *Statistical considerations regarding the use of ratios to adjust data.* Int J Obes Relat Metab Disord, 1995. **19**(9): p. 644-52.
72. Frisard, M.I., et al., *Physical activity level and physical functionality in nonagenarians compared to individuals aged 60 74 years.* J Gerontol A Biol Sci Med Sci, 2007. **62**(7): p. 783-8.
73. Redman, L.M., et al., *Metabolic and behavioral compensations in response to caloric restriction: implications for the maintenance of weight loss.* PLoS One, 2009. **4**(2): p. e4377.
74. McCarthy, E.A., et al., *Determination of maternal body composition in pregnancy and its relevance to perinatal outcomes.* Obstet Gynecol Surv, 2004. **59**(10): p. 731-42; quiz 745-6.

75. IAEA, *Assessment of body composition and total energy expenditure in humans using stable isotope techniques*. Human Health Series, No. 32009, Vienna, Austria: Division of human health, International Atomic Energy Agency.
76. Koletzko, B., T. Sauerwald, and H. Demmelmair, *Safety of stable isotope use.* Eur J Pediatr, 1997. **156 Suppl 1**: p. S12-7.
77. Koletzko, B., et al., *The use of stable isotope techniques for nutritional and metabolic research in paediatrics.* Early Hum Dev, 1998. **53 Suppl**: p. S77-97.
78. van Thien, H., et al., *Influence of prolonged starvation on glucose kinetics in pregnant patients infected with Plasmodium falciparum.* Clin Nutr, 2004. **23**(1): p. 59-67.
79. Klein, P.D. and E.R. Klein, *Stable isotopes: origins and safety.* J Clin Pharmacol, 1986. **26**(6): p. 378-82.
80. Jones, P.J. and S.T. Leatherdale, *Stable isotopes in clinical research: safety reaffirmed.* Clin Sci (Lond), 1991. **80**(4): p. 277-80.
81. ACOG, *ACOG committee opinion. Exercise during pregnancy and the postpartum period. Number 267, January 2002. American College of Obstetricians and Gynecologists.* International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics, 2002. **77**(1): p. 79-81.
82. Butte, N.F., et al., *Energy requirements during pregnancy based on total energy expenditure and energy deposition.* The American journal of clinical nutrition, 2004. **79**(6): p. 1078-87.