Measurement of Body Fat in Infants

"Baby Fat Pilot"

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PROTOCOL

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TABLE OF CONTENTS

1. SUMMARY

In the 21st century, children are born into an obesity prone environment where consumption of nutrient-deplete and calorie-dense diets and sedentary behaviors are the norm. Mothers (and fathers) are more likely to be obese prior to conception and parental health at conception, particularly obesity, is linearly associated with obesity in children. Independent of obesity prior to pregnancy, excessive weight gain throughout pregnancy leads to higher adiposity in infancy. There are very few studies that have attempted to understand the role of the intrauterine environment and the impact of early life exposures on development of adiposity in humans. Understanding the amount and type of adipose tissue present early in life is important to understand how obesity develops in childhood and determines metabolic risk throughout one's lifetime. We have been successfully deploying methodology in infant subjects at Pennington Biomedical to quantify measurements of energy metabolism and eating behavior. Our current measures of body composition include an indirect method of adiposity from a measurement of body volume using air displacement plethysmography. We are seeking to expand our methodological capability with direct measurements of adiposity including the quantification of both white and brown adipose tissues using dual energy x-ray absorptiometry and magnetic resonance imaging, respectively. The aim of this study therefore is to use state-of-the-art equipment and established methods supported by appropriate validation studies to develop methods at Pennington Biomedical that assess whole body adiposity (fat mass) and brown adipose tissue in infant subjects. This study will obtain important data pertaining to the accuracy and precision of the procedures and methods that is required to inform the development of future studies including these parameters as endpoints.

2. STUDY AIMS

The overarching goal of this study is to develop a technique for measuring whole body adiposity (fat mass) and supraclavicular brown adipose tissue in infant subjects. To this end the study will:

1. Develop a protocol for measurement of whole body adiposity in infant subjects using dual energy x-ray absorptiometry (DXA).

2. Develop a protocol for measurement of brown adipose tissue in infant subjects using a published, non-invasive measurement by magnetic resonance imaging.

3. Evaluate the accuracy and precision of the measurement of brown adipose tissue as well as feasibility of protocol completion in infant subjects measured twice within 1 to 10 days apart.

3. BACKGROUND AND SIGNIFICANCE

One of the most troubling aspects of the current epidemic of obesity is the increasing prevalence of obesity and type 2 diabetes among children [1, 2]. Childhood obesity is a very serious problem because adiposity can be traced between childhood and adulthood [3], and children with one or two obese parents are at a higher risk of weight gain during childhood and adolescence than children with both parents normal weight [4]. Furthermore, duration of obesity is an independent predictor of health outcomes [5]. The lifelong risk of obesity is now believed to begin in the womb. Children born to mothers who enter pregnancy overweight or obese have

significantly more adiposity at birth [6, 7], and longitudinal studies indicate that maternal adiposity has a positive influence on adiposity in offspring at 1 [8], 2 [9], 9 [10], and even 30 [11] years later.

Reliable measurements of adipose tissue from early life are critical to further knowledge regarding the development of obesity in humans. While non-invasive methods exist for measurement of body composition in infant subjects, these methods provide an indirect measurement of body fat and current equipment limit continued evaluation across childhood. For example, the PEA POD, which we frequently use at Pennington Biomedical uses air displacement technology which provides a measure of body volume. From a volume measurement, old equations are used to determine body density, and from and in conjunction with weight, fat mass and fat-free mass are assumed. The current devices for measurement of body volume in children are limited to birth – 6 months with the PEA POD and ~5 years of age and older with the BODPOD. Therefore, considerable and thought to be important data on body composition from 6 months to 4 years of age is not possible with this technique.

Dual energy x-ray absorptiometry (DXA) provides a direct measurement of body composition in three compartments; bone, fat and lean and is not limited to the age of the test subject. DXA is the gold standard methodology however the exposure to radiation emitted during the scan is a potential concern, particularly in vulnerable subjects. There are increasing numbers of reports of measurements of body composition in infants using DXA in the literature[12-23], including numerous studies that use DXA as a validation against other devices[17, 19, 20] and that describe best practices[13, 17, 18, 21]. Furthermore, while several studies use DXA to undertake cross-sectional comparisons of infant body composition across various groups[12, 22, 23], there are also prospective studies in infants to show changes in body composition across infancy[14-16]. The more widespread use of DXA in healthy populations demonstrates increased acceptability of DXA in infant subjects.

Not all adipose tissue is equal. For example, we know that the deposit of lipid in different regions of the body yields varying risks for metabolic disease. Furthermore, the morphology of adipose tissue also renders different metabolic functions. The most abundant adipose tissue and primary store of lipid is white adipose tissue. Brown adipose tissue (BAT) on the other hand is specialized for energy expenditure by dissipating energy as heat. BAT is able to convert nutrient-derived chemical energy into heat. The thermogenic capacity of BAT is due to its high content of mitochondria and expression of uncoupling protein 1 (UCP1), which dissipates the mitochondrial proton gradient as heat [24]. In rodents and human infants, BAT-mediated heat production is important for maintenance of normal body temperature in newborns after birth [24]. Recent studies in animals and humans further indicate that BAT plays a crucial role in the regulation of body weight and insulin sensitivity. While mice with impaired BAT thermogenesis are more susceptible to diet-induced obesity, BAT activation reduces dietary and genetic obesity [25, 26]. Moreover, the ability of BAT to take up high amounts of lipids and glucose from the blood alleviates hyperlipidemia and hyperglycemia [25, 26]. Although human BAT diminishes with age, metabolically active BAT persists into adulthood. Cold-induced activation of human BAT, as determined by ^{18}F-FDG uptake using PET/CT, is closely associated with an increase in energy expenditure and a concomitant decrease in body fat mass [27, 28]. Moreover, BAT mass and activity are inversely correlated with BMI and body fat mass [27, 28], indicating that decreased BAT mass and activity may be implicated in the development of obesity and metabolic disorders.

The prevalence of childhood obesity is increasing worldwide. A number of human epidemiological studies and animal models link maternal obesity during pregnancy with an increased risk of obesity in offspring [29-31]. However, the underlying mechanisms by which maternal physiology predisposes offspring to obesity are not fully understood. Growing evidence suggests that the intrauterine nutritional and hormonal environments play a crucial role in metabolic programming of offspring obesity by permanently altering appetite control,

neuroendocrine functioning and energy expenditure in the developing fetus [29-31]. Therefore understanding the physiological regulation of BAT is thus important for developing strategies to prevent or treat obesity and metabolic diseases and quantification of BAT from early in life is an important area of research.

In adults, PET/CT has been utilized due to its ability to measure active BAT metabolism in combination with radiotracer uptake. PET/CT however, is not suitable for research in infants. Several groups [32-34] have developed and validated protocols for the measurement of BAT in infant subjects using magnetic resonance imaging (MRI), which is non-invasive and presents minimal risk to infant subjects. In collaboration with Dr. Owen Carmichael, we will develop and optimize a MRI technique to quantitatively measure BAT mass in human infants at Pennington Biomedical.

As part of a translational research project funded by Pennington Biomedical, the aim of the study is to establish a technique for measuring whole body adiposity (fat mass) and supraclavicular brown adipose tissue in infant subjects using DXA and MRI, respectively.

4. RESEARCH DESIGN

This is an observational method development study. Up to 10 infants will be enrolled in this study which includes two study visits to be completed within 1-10 days apart.

5. STUDY POPULATION

5.1. Participants

Up to 10 healthy, full-term infants aged 14 to 28 days old at enrollment with no contraindications to MRI will be recruited by Pennington Biomedical Research Center to participate in this study.

5.2. Eligibility Criteria

- Healthy, full-term infant
- Aged 14 to 28 days at Visit 1
- Willingness of parents to be notified of incidental findings from study procedures

5.3. Exclusion Criteria

- Born preterm (< 37 weeks gestation)
- Implanted metal or electronic objects that render MRI unsafe
- Unable to complete 2 clinic visits 1-10 days apart at Pennington Biomedical Research Center

6. RECRUITMENT

Parents of potential study participants will be recruited from within PBRC and the Greater Baton Rouge area via Institutional Review Board (IRB) approved posters and flyers and advertisements posted on the PBRC website (http://www.pbrc.edu/clinicaltrials) and through PBRC approved social media outlets including but not limited to Facebook, Twitter, blogs, and Craigslist. There is also the possibility to recruit parents of potential study participants who have participated in previous or current studies conducted at PBRC. Physician referrals from local midwives, obstetricians or pediatricians will also be used in recruitment efforts.

7. SCREENING

Interested parents or legal guardians will respond to targeted recruitment materials and will be directed to PBRC for initial screening via phone or email. Following initial contact by interested parents/legal guardians, a brief interview (via phone) will be conducted to describe the study and determine basic eligibility criteria. If the infant

has been born, basic eligibility criteria will be obtained through the initial screening survey for the infant who would potentially qualify to participate in the study. If the infant has not been born but is expected, basic contact information for the parent or legal guardian will be obtained so that they can be re-contacted about their interest after the infant is born. After the infant is born, the initial screening questions will be obtained to determine eligibility. Potential infants that do not meet criteria will be excluded. All eligible participants will be assigned a unique study ID number and referred to study staff for further screening. Email may be used for initial contact and other correspondence (e.g. study description, scheduling), but no protected health information will be contained in email correspondence.

8. ASSESSMENT SCHEDULE AND PROCEDURES

The first study visit will be completed when the infant reaches 2 – 4 weeks (14 - 28 days) of age. Study visits will be scheduled according to the ability of the parent/legal guardian to attend the second visit within 1 to 10 days after Visit 1 is completed. Before initiation of the first study visit (Visit 1), the study and study procedures will be explained to parents/legal guardians, and informed consent and HIPAA authorization will be obtained. An outline of the study visits and procedures to be performed is summarized in the Table 1.

TABLE 1. Schedule of Procedures

Visit Assessment*	Visit 1 (14-28 days)	Visit 2 (1-10 days after Visit 1)
Informed Consent (Minor)	**X**	
Recumbent Length	**X**	**X**
Weight	**X**	**X**
PEA POD	**X**	
DXA	**X**	
MRI	**X**	**X**
Questionnaires[#]	**X**	
[#]Completed by a parent or legal guardian that signs the study informed consent and HIPAA authorization and accompanies the infant to the visit		

8.1. Visit 1

The parent/guardian and infant will be escorted to the Maternal and Infant Phenotyping Room for informed consenting. The minor informed consent form and HIPAA authorization will be provided for review and completion after all questions are answered. After obtaining written parental informed consent for participation, the following procedures will be performed: infant weight, recumbent length, body composition by PEA POD, DXA and MRI. Questionnaires will be administered to a parent/guardian who attends the visit. The DXA and MRI procedures will be completed while the infant is sleeping. The order of the procedures at the visit will be conducted in a manner that is most respectful to the schedule and temperament of the infant. For example, if the infant arrives at Pennington Biomedical and is soundly sleeping, once the parents/legal guardians sign the informed consent, the infant may proceed directly to imaging for DXA and MRI. Visit 2 will be scheduled to occur 1-10 days later.

8.2. Visit 2

The following procedures will be performed: infant weight, recumbent length, and MRI. The order of the procedures at the visit will be conducted in a manner that is most respectful to the schedule and temperament of the infant. Infants will be fed and calmed before preparation for MRI. The MRI procedures will be completed while the infant is sleeping.

9. MEASURES AND OUTCOME ASSESSMENTS

9.1. Infant Anthropometrics

Infant body weight will be obtained using a standard electrical infant scale with the infant undressed. Recumbent length will be measured using an infant length board with a stationary head-board, a moveable footboard and a built-in centimeter scale. Length and weight will be performed in accordance with laboratory SOPs by trained research team members at both Visits 1 and 2.

9.2. Infant Body Composition

9.2.1. PEA POD

Body composition will be assessed in infants weighing less than or equal to 8 kilograms with air displacement plethysmography (Life Measurement Inc, Concord, CA), as previously described [35, 36] at Visit 1. 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.

9.2.2. DXA

9.2.2.1. Background

A total of 6 studies reporting DXA measurement on 971 neonates and infants are summarized in the table below (Table 2). Two of these studies (771 neonates) included measurements at birth (within 4 days), two studies (156 infants) completed measurements within 2-4 weeks of birth and two studies (44 infants) completed measurements at 1 month after birth. A review of the inclusion, exclusion criteria suggested that healthy infants were enrolled and studied. Three of the published studies were longitudinal in nature and obtained DXA measurements at multiple time points throughout childhood[14, 37, 38] and two of these studies included two DXA measurements within the first 6 months of life[14, 37].

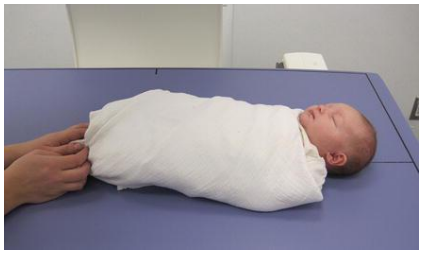
Table 2. DXA Literature Summary

Reference, Country	Purpose	Study Design	Sample Size with DXA	Age at DXA Assessment	Participants' Health Status	DXA Procedure Details	Safety Information/ AEs Reported
Crozier et al 2010, United Kingdom	To examine relationship between pregnancy weight gain and neonatal and childhood body composition	Longitudinal	564	As soon as possible after birth 4 years 6 years	Term neonates with no major congenital growth abnormalities	Whole body scan using Lunar DPX-L instrument in pediatric scan mode	Total x ray dose was ~10.5 µSv ~1-2 days background radiation from natural sources
Godang et al 2010 and Friis et al 2013, Norway	To investigate the reliability of body composition measurements by DXA	Cross - sectional	207	Within 4 days of birth	Term neonates with no major congenital growth abnormalities	Whole body scan by thin fan-beam GE Lunar Prodigy densitometer in pediatric scan mode First 50 neonates had 2 DXA scans for reliability testing	DXA scan of the total body of a neonate results in an effective dose of ~1 µSv Negligible amount of radiation ~1 day background effective dose from natural sources
Gallo et al 2012, Canada	To create a normative reference data set for infants between birth to 12 months of age for whole body bone mineral content by DXA	Longitudinal	52	2-4 weeks after birth 6 months 12 months	Term neonates at AGA with no congenital malformations	Whole body scan by QDR 4500A Elite Hologic in array mode	Authors reported that their data is not reliable beyond 6 months because of the limited sample size due to movement artefact in older infants who have more difficulty staying asleep on the DXA instrument.
Fields et al 2012, United States	To assess the association of hormones and inflammatory factors in human breast milk with infant size and adiposity	Cross - sectional	19	1 month	Term infants with no known congenital birth defects and no exposure to type 1, type 2, or gestational diabetes	Whole body scan by Lunar iDXA in infant whole body analysis mode	Not available
Bisson et al 2016, Canada	To evaluate the association between maternal physical activity intensity and neonatal body composition	Cross - sectional	104	Within 2 weeks of birth	Term neonates with no congenital heart defects	Whole body scan by fan beam Hologic Discovery in infant whole body mode	Not available
Goran et al 2017, United States	To examine relationship between fructose in breast milk and infant weight and body composition	Longitudinal	25	1 month 6 months	Term infants with no known congenital birth defects and no exposure to type 1, type 2, or gestational diabetes	Whole body scan by Lunar iDXA in infant whole body analysis mode	Not available

**Table References: Crozier et al 2010[38], Godang et al 2010[21], Friis et al 2013[22], Gallo et al 2012[37], Fields et al 2012[39], Bisson et al 2016[12], Goran et al 2017[14]

9.2.2.2. Procedure

Body composition will be assessed using dual-energy x-ray absorptiometry (DXA) on GE Lunar iDXA at Visit 1. In accordance with previous studies[16, 21, 37], DXA scans will be acquired while the infant is resting and calm. The infant will be swaddled in a blanket (pictured) with clothes (excluding a clean diaper) removed and placed on the scanning table. The scanner emits low energy x-rays and a detector will pass along the body to measure the amount of bone, muscle, and fat. Scan time will be about 5 minutes, but there is a possibility of rescanning if the technician detects too much movement or misplacement of the infant on the scanning table. Gallo et al 2012



reported that when making up to two scanning attempts, movement free whole body scans were acquired on 99% of infants at 1 month of age [37].

9.2.2.3. Safety and Radiation Exposure

9.2.2.3.1. GE Lunar iDXA Radiation Report from the Department of Pediatrics at the University of Oklahoma

Dr. Fields and his collaborators at the Department of Pediatrics at the University of Oklahoma conduct DXA assessments using the GE Lunar iDXA equipment (same as in this study). The radiation report for the GE Lunar iDXA (provided as Protocol Supplementary Info) was obtained since we do not have a similar report from the iDXA at PBRC. The report documents the radiation exposure of the iDXA scanner for an infant phantom (13.3 lbs, 25.5" long). As shown, the measured radiation absorption of the infant scan was 0.893 mR or 0.00893 mSV, dose equivalent radiation where hourly average background radiation is 38.83 µSV (Reference https://www.convert-me.com/en/convert/radiation/rrmrem.html?u=rrmrem&v=0.893).

9.2.2.3.2. Phantom Study to Assess Radiation Dose and Risk

A report by Damilakis et al 2013[40] using a GE Lunar Prodigy scanner, estimated that the effective radiation dose for a female and male neonate is 0.00027 mSV and 0.00023 mSV, respectively. The paper also estimated the patient cancer risk associated with acquiring DXA scans in neonates/infants. It is reported that the cancer risk for whole body DXA scans of individuals aged 0 years at the time of exposure to be 0.13 in 1,000,000 and 0.06 in 1,000,000, for female and male neonates, respectively. In the discussion of the paper, the authors note in relation to these findings; "…The Health Protection Agency of the UK (formerly the National Radiological Protection Board) has categorized X-ray examinations into four risk bands according to risk range, i.e., negligible (<1 in 1,000,000), minimal (1 in 1,000,000 to 1 in 100,000), very low (1 in 100,000 to 1 in 10,000) and low (1 in 10,000 to 1 in 1,000). According to this classification, the lifetime risks of radiation-induced cancer from the DXA examinations investigated in this work using the Prodigy system are negligible."

9.3. Measurement of Brown Adipose Tissue

9.3.1. BAT measurement by MRI

9.3.1.1. Background

Gale et al reported performing whole body MRI on over 450 infants without the use of sedation on infants up to 3 months of age with 94% success rate and 0.8% prevalence of incidental findings[32]. Gale et al describes that whole body MRI without sedation may have greater success rates in younger infants because they are usually less alert and active than older infants, and their approach has been shown to be safe, efficient, and effective [32]. Recently, Rasmussen et al reported characterizing BAT in 22 infants by measuring the BAT depot volume and fat fraction in the spine, supraclavicular, and axillary regions with high intra- and inter-rater reliability [33]. Similarly, Hu et al used water-fat MRI to measure fat fraction and T2* values to characterize BAT in the supraclavicular fossa and subcutaneous depots in 12 infants [34]. Gale et al, Rasmussen et al, Hu et al have performed MRI on infants with success, and their methodologies have contributed to the development of the infant BAT procedure planned for this study.

9.3.1.2. Procedure

Magnetic resonance imaging (MRI) will be performed using a 3.0 T scanner (General Electric Healthcare, Signa Exite HDxT, Chicago, IL). Preferably after feeding, clothes will be removed and diaper changed. Then, infants will be swaddled in a blanket. Infants will be soothed to the point of sleep, and, once soundly asleep, the infant will be moved to the MRI scanner room to finish preparation and testing. Disposable neonatal noise guards (Latex-free, Newmatic Medical) specifically designed for infants in MRI environments will be applied to the infants' ears

for noise shielding. In preparation for MRI positioning, the infant will be placed in scanner with padding positioned around the infant to provide a comforting swaddle without allowing movement during the test. The pulse oximeter will be applied to the foot, and heart rate and oxygen saturation will be continuously measured throughout the test. In addition, a study staff member who is trained in neonatal BLS will be present at all times during the preparation and will remain next to the scanner during testing. Scan time will be about 10 minutes, but there is a possibility of rescanning if the infant wakes, the technician detects too much movement, or there was misplacement of the infant on the scanning table. If the infant seems to be waking through movement detection, noises, or increased heart rate from the pulse oximeter, scanning will be interrupted so that the baby can be settled and scanning can resume. In addition, the test may be stopped if the baby is not resting quietly and appears uncomfortable or distressed by the procedure.

9.3.1.3. Incidental Findings

MRI has the potential to detect previously unknown findings of uncertain significance. Any abnormal findings identified during scan acquisition or analysis will be documented and reported per *PBRC SOP 1103 Reporting Abnormal BioImaging Results.* In the event that study personnel identify MR abnormalities, they will consult with a radiologist who will determine the clinical relevance of the abnormalities. Participant identity will not be shared with the radiologist in this event. If the radiologist determines that an MR abnormality is relevant to personal health, the radiologist will then determine whether the finding is clinically actionable. In the event that the finding is clinically actionable, or if the participant consented to be informed of non-clinically actionable incidental findings, study personnel will discuss the relevance of the finding with the participant.

9.4. Infant Health and Medical History Questionnaire

Parents/legal guardians will be asked to complete a questionnaire to collect information on infant health, first degree relatives' health related to the infant, infant feeding and infant lifestyle measures.

10. PARTICIPANT SAFETY AND CONFIDENTIALITY

10.1. Risks to participants

This Human Subjects Research meets the definition of a Clinical Trial. 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.

Potential Risks associated with the study procedures include (presented in alphabetical order):

- Body composition by DXA. The amount of radiation used for this procedure is very small. The measured radiation exposure to an infant using the Lunar iDXA, the scanner being used in our study (performed by colleagues at The University of Oklahoma-report included as Protocol Supplementary Info), was found to be 0.893 mR (0.00893 mSV). This level of radiation is 75% less than a bitewing dental x-ray (0.38 mSV) reported by the American Dental Association which is also permitted in pregnant women and the radiation exposure received during airplane travel across the United States (0.4 mSV) and is less than 2% of the background radiation associated with altitude such as living in Denver, CO (0.51 mSV). Radiation risk is cumulative over a lifetime. Although information regarding radiation exposure is not specifically available for children, the exposure for children is less because they are smaller in size. Radiation risk is cumulative over a lifetime. It is reported that the cancer risk for whole body DXA scans of individuals aged 0 years at the time of exposure to be 0.13 in 1,000,000 and 0.06 in 1,000,000, for female and male neonates, respectively[40] which concludes that the lifetime risks of radiation-induced cancer are negligible according to the Health Protection Agency of the UK.

- Body composition by PEA POD. Air displacement plethysmography will be used to measure body composition in infants. The PEA POD for infants up to 8 kg (Life Measurement Inc, Concord, CA). Measurement of whole body adiposity with the PEA POD is non-invasive and completely safe for use in infants[41]. We have measured infant body composition with the PEA POD in approximately 150 infants without any adverse events noted.
- Brown Adipose Tissue measurement by 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. Participants with any implanted metal device will be excluded. To help with the possible discomfort, padding will be placed around the baby to provide a comforting swaddle. This will also help with reducing movement and therefore reducing the time of the procedure. 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 participant's possession before they enter the MR suite.
- Incidental Findings. It is possible that one of the scanning procedures (DXA or MRI) could find an abnormal result that may be relevant to the study participants' health. Study staff will consult with a radiologist who will determine the relevance of any such incidental finding. Participants' identity will not be shared with the radiologist in this event. The radiologist determines whether an incidental finding is relevant to study participants' health, and if so whether the imaging finding suggests an underlying, treatable medical condition. Participants' parents will be kept informed of all incidental findings via the study staff over the course of this study.
- 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. We are experienced in conducting anthropometrical assessments in infants and have completed the weight and length in approximately 150 infants ranging from 1 day to 12 months of age.
- Questionnaires. There are no anticipated risks from completing questionnaires. If signs of minor stress or fatigue are apparent, parents or guardians of study participants will be given time to take a break from completing the questionnaires. If uncomfortable, participants may choose to not answer questions.

10.2. Adverse events

Serious adverse events in this study are defined to include: death, a life-threatening adverse experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity.

In this study, an **adverse event or experience** is defined as any health-related unfavorable or unintended medical occurrence that happens throughout study participation. Examples of adverse events include but are not limited to the following:

- A clinically significant laboratory or clinical test result.
- An event that results in missing a study visit.
- An event that requires a visit to a physician.
- An event that occurs as a result of a study procedure.
- Unanticipated or untoward medical events that may be study related.

Adverse event data will be collected from the date of consent until the final visit. Adverse event data will be analyzed quarterly, but serious or life-threatening adverse events require immediate reporting and follow-up. We anticipate most adverse events will be mild and the participant will be able to resume activities within a day or two of reporting the event. Adverse Event reporting will follow the requirements of the IRB of the Pennington Biomedical Research Center. Only adverse events that qualify as unanticipated problems will be reported to the IRB. Unanticipated problems involving risks to participants or others include incidents only if the incident is unexpected, related or possibly related to participation in the research, and indicated that subjects or others are at a greater risk of harm than was previously known or recognized. Any action resulting in a temporary or permanent suspension of this study (e.g., IRB actions, or actions by the PI and/or co-investigators) will be reported to the appropriate officials.

10.3. Stopping rules

There is minimal risk for participating in this trial. Given the young age of the study participants, it might be necessary to permanently discontinue one of the imaging procedures. We will make every effort to ensure participants are comfortable during scanning. For participants who are not able to rest quietly during a scanning procedure, the procedure will be aborted after three attempts at a single study visit. It is unlikely that stopping rules will need to be invoked to discontinue the study. The most likely scenario that would indicate a cessation of the study would be failure to recruit participants. Nevertheless, in addition to monitoring recruitment, we also will monitor the rates of adverse events in our participants. The study investigators will alert the IRB, if a larger than reasonably expected adverse event rate occurs in our participants. 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.
- Limit of rules—we acknowledge that circumstances, other than what are listed, may justify stopping the study.

10.4. Confidentiality

All volunteers are assured of their anonymity and 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. Volunteers' medical records are filed according to ID numbers. All forms on the chart, with the exception of consent form, display only the ID number. 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, Social Security Number or other identifying information. Data will be collected from participants. Data are confidentially collected from study participants and are only used for research purposes. All records are kept in locked file cabinets, and participant data can be identified only by number. Data are used only in aggregate, and no identifying characteristics of individuals are published or presented.

11. DATA MANAGEMENT

11.1. Sample Size Consideration

As noted in Section 2. Aims, the primary aim is to develop techniques for measuring whole body adiposity (fat mass) and brown adipose tissue in infant subjects. Given the pilot nature of this research and the plan to learn important information regarding our protocols for phenotyping of infants, we did not conduct a formal power calculation for this study. It is unlikely that this data will be published as 1) the study will help to develop the

techniques for future powered studies and 2) the data from this pilot study will be used for sample size estimates in future grants and projects.

11.2. Data Analysis Plan

As noted in Section 2. Aims, the primary aim is to develop techniques for measuring whole body adiposity (fat mass) and brown adipose tissue in infant subjects. We intend on understanding the repeat test accuracy of the measurement of infant BAT by calculating % BAT and comparing the test-retest reliability between Visit 1 and Visit 2 measurement within various equivalency intervals (e.g. within 15%, 10%, 5%). The coefficient of variation for the BAT measurement will be computed from the two measures performed under the same conditions 1 to 10 days apart.

% BAT will be calculated: $\% \, BAT = \frac{BAT \; mass \; (by \; MRI)}{FAT \; mass \; (by \; DXA)}$

Depending on the characteristics of the recruited cohort, exploratory analyses maybe undertaken to determine differences in measures between boys and girls, if energy expenditure is different on the account of maternal BMI, birth weight, breast milk versus formula-fed infants.

12. SUBJECT PAYMENT

Participants will receive up to $100 for participation in the study to help offset transportation costs and their time. Participants will receive $50 for completing the Visit 1 testing and $50 for completing the Visit 2 testing. One check will be requested at the conclusion of the study. If a participant completes Visit 1 testing but does not complete Visit 2 testing, one check of $50 for completing the Visit 1 testing will be requested. Checks will be requested from the Pennington Biomedical (LSU) payroll department. If the parent/legal guardian reports that the infant participant has a social security number at the time of the study 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 or her own social security number as it is required for check processing.

13. REFERENCES

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