SLEEPING METABOLIC RATE

IN EARLY INFANCY

by

Lindsay E. Domino

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Lindsay E. Domino

Approved: _____
Jillian C. Trabulsi, Ph.D., RD
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____
P. Michael Peterson, Ed.D.
Chair of the Department of Behavior Health and Nutrition

Approved: _____
Kathleen S. Matt, Ph.D.
Dean of the College of Heath Sciences

Approved: _____
Ann L. Ardis, Ph.D.
Senior Vice Provost for Graduate and Professional Education

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ABSTRACT

In the United States, nearly two-thirds of infants receive infant formula by 3 months of age, either in combination with or fully replacing breast milk, the gold standard for infant nutrition growth. Studies have shown that formula fed infants, the majority of whom are fed cow's milk formula (CMF), gain weight more rapidly than breastfed (BF) infants. Accelerated weight gain in early infancy is of concern as numerous studies have found an association between rapid infant weight gain and increased risk for overweight and obesity later in life. Not all infant formulas are alike in terms of composition and growth outcomes. Infants fed an extensive protein hydrolysate formula (EHF), which is comprised mainly of free amino acids and small peptides and has a slightly higher protein content than cow's milk formula (CMF), have been found to gain weight similarly to BF infants. The energy balance mechanisms underlying the differences in weight gain by infant formula type are not known. However, it is possible that the different protein composition of the infant formulas (CMF versus EHF) impacts energy expenditure via differences in metabolic rate. This study had two overarching aims. First, we sought to determine the effect of formula type (CMF vs. EHF) on sleeping metabolic rate (SMR) in healthy, exclusively formula-fed infants. Second, we sought to utilize the measures of SMR to determine which of several available empirical equations for the calculation of metabolic rate in infants, was most accurate.

A total of 141 mother-infant dyads were recruited from the greater Philadelphia area. At 0.75 months of age (baseline) when all infants were receiving CMF and again 3.5-months old, when all infants had been receiving their randomized formula for nearly three months, SMR was measured via indirect calorimetry. There were 102 infants with successful SMR at 0.75- months and SMR did not differ ($p=0.148$) by eventual formula randomization group. Eighty-three infants had successful SMR measurements at 3.5 months and formula type did not have a significant effect ($p=0.9633$) on SMR. At 0.75 months, we found that the Schofield weight only performed best for at the individual level at 0.75-months; it had the highest R^2, suggesting good individual level agreement. At 3.5-month, the Schofield weight only and Oxford weight only equations performed best at the individual level. Since clinicians aim to calculate energy needs of individual patients, these analyses suggest the Schofield weight only is most accurate for estimating energy needs of 0.75 and 3.5 month old infants. These results are preliminary and will be repeated when the data set is complete.

Chapter 1

INTRODUCTION

While national guidelines and healthcare efforts seek to encourage exclusive breastfeeding for the first six months of life, nearly two-thirds of infants in the United States (U.S.) receive infant formula by three months of age, either as sole source of nutrition or in combination with breast milk.[1-3] Research has shown that formula fed infants gain weight at a significantly accelerated rate compared to breastfed infants.[4,5] While age appropriate weight gain is desired in the first year of life, accelerated weight gain has been associated with increased risk for childhood overweight and obesity.[6-8] The macronutrient composition of infant formula differs by formula type and brand; recent research uncovered differences in weight gain between infants fed standard cow's milk formula (CMF), the most commonly consumed formula in the U.S.), and an extensive protein hydrolysate formula (EHF), a formula in which the protein is mainly in the form of free amino acids and small peptides and hence less allergenic.[9,10] Infants fed CMF gained significantly more weight compared to infants fed EHF, whose weight gain was similar to that of breastfed infants.[11] Since the macronutrient composition of the diet influences energy expenditure (thermic effect of feeding), it is possible that the peptides and free amino acids in the EHF lead to an increase in energy expenditure in EHF fed infants, thus leading to lower weight gain. No research to date has evaluated the effect of the macronutrient composition of the diet in infancy and sleeping metabolic rate (SMR) or sleeping energy expenditure (SEE).

The measurement of SMR) is considered the gold standard for determining metabolic needs while at rest or sleep for an infant.[12,13] While SMR is conducted in research studies and in some clinical settings, its use in clinical settings is very limited. Clinicians often use empirically derived predictive equations, instead of actual SMR, as a basis to calculate energy needs.[14] Predictive equations from the World Health Organization (WHO) and researcher W.N Schofield, have been used in clinical practice for the past several decades, however the accuracy of such predictive equations has recently come into question.[15-17] Newer equations, such as the Oxford equation, have been introduced to elevate the accuracy of these empirical equations.[18] However, to our knowledge, no studies have evaluated the accuracy of the WHO, Schofield, or Oxford equation against actual SMR measurements.

Chapter 2

REVIEW OF LITERATURE

2.1 Energy Balance

Energy balance is a fundamental principle of all living organisms. The constant exchange and transfer of energy sustains life by supporting numerous physiologic functions. [14] Energy balance has two main components, the energy required or expended by an organism, and the energy taken in or added to the organism. An organism is in energy balance when energy intake is equal to energy expenditure. Positive energy balance occurs when energy intake exceeds energy expenditure. Infants and children must be in positive energy balance in order to support age appropriate growth, meaning their energy intake must exceed their total energy expenditure (TEE) by a certain allowance. For infants, the allowance for growth is 175 kcal/d for 0-3 month olds, 56 kcal/d for 4-6 month olds, and 22 kcal/d for 7-12 month olds. Negative energy balance occurs when energy intake is less than expenditure. [14] In humans, determination of energy (caloric) needs is an essential component of nutritional care.

2.2 Energy Expenditure

Total energy expenditure (TEE) is comprised of three major components: basal metabolic rate (BMR), the thermic effect of food (TEF), and physical activity. The combination of these three factors reflects the total amount of energy expended by the human body. [14] Doubly labeled water (DLW) is an indirect calorimetry method that can be used to measure TEE in free-living human subjects. [19] The method is based upon differential kinetics of oxygen and hydrogen in body water. Subjects drink a known volume of water that contains enriched quantities of the stable isotopes

oxygen-18 (O-18) and deuterium. These isotopes equilibrate with total body water and measuring the elimination rates of these isotopes (deuterium and O-18) over time through repeated sampling of body water (e.g., urine or blood) provides a measure of carbon dioxide production, which in turn can be used to calculate energy expenditure using standard indirect calorimetric equations. [16,19] The DLW method has been used repeatedly in healthy children and adults (from pre-term infants to elderly individuals[2]), as well as those with acute and chronic disease for the determination of total energy expenditure.[19-21] However, there is very limited research involving DLW in healthy term infants, particularly those early in infancy.

2.2.1 Basal, Resting, and Sleeping Metabolic Rate

Basal metabolic rate (BMR) is the energy needed to support metabolic processes at a fundamental level. These fundamental processes include, brain, respiration, blood circulation, gastrointestinal processing and renal filtration, and other required organ function. BMR requires that energy expenditure is measured in a thermoneutral room, after an overnight fast, and ideally prior to the subject getting out of bed in the morning to control for known diurnal variation (although transportation to the testing facility is permissible). The goal is to minimize diurnal variation and increased energy expenditure associated with physical activity and food intake. [22] Food intake triggers metabolic processes of digestion and absorption, which increase energy expenditure. This boost in energy expenditure is known as TEF and accounts for approximate 7-10% percent of total energy expenditure. [23] Recent physical activity can also have an effect on metabolic rate, even in a rested state. This phenomenon is known as the delayed effect of physical activity, and represents the increased metabolic rate after the physical activity has been completed. Together, TEF and

delayed effect of physical activity contribute to basal metabolic rate (BMR), increasing BMR by approximately 10 to 30 percent. [22] During the BMR measure, the individual is awake (at rest) and lying in a supine position with no movement. The energy cost of the awake state is the main factor that differentiates BMR from sleeping metabolic rate (SMR).

SMR measures the same fundamental processes as BMR, however it is measured during sleep, and thus the energy cost of SMR is lower than BMR. The intra-individual variability of SMR versus BMR is approximately 5 to 10 percent. [22,26]

Resting metabolic rate (RMR) is similar to BMR in that the measurement is conducted in a thermoneutral room, with the subject in a supine position. The definition for measuring RMR can vary slightly throughout the literature; from study to study the period of rest sometimes varies, as does the time since the last meal. During an RMR measure the subject may have fasted, but not necessarily overnight, and the subject is rested, but the measurement is not taken immediately following an overnight rest (with no movement at all). [12] Therefore a measure of RMR in an individual will be slightly higher than the measure of BMR.

When BMR is extrapolated over a 24-hour period and expressed as kilocalories over a 24-hour period (kcal/24 hour) it is called basal energy expenditure (BEE). Similarly sleeping energy expenditure (SEE) and resting energy expenditure (REE) are both extrapolated from SMR and RMR, respectively, and express energy expenditure over a 24-hour period. Understanding the distinctions of each value can help when performing measurements and interpreting assessments across various populations.

2.2.2 Measuring Basal, Resting, and Sleeping Metabolic Rate

The current gold standard for measuring REE is indirect calorimetry as measured via a metabolic cart. [12] Indirect calorimetry is a non-invasive procedure used to calculate BMR, RMR, or SMR based on rates of oxygen (O_2) consumption and CO_2 expiration in a rested state. The practice of indirect calorimetry in research today is based on the same principles developed and performed as early as the 1930 and 1940s. [12,15,16] With advances in technology, computerized systems have increased measurement accuracy and ease of administration, making it possible to perform these techniques on virtually all populations of varying body size and health status.

Due to the fact that oxygen is the currency for all energy-releasing reactions in the body, measuring oxygen uptake is an indirect measure of energy expenditure. [22] There are two different approaches to capturing resting/basal/sleeping metabolism by indirect calorimetry: an open-circuit system and a closed-circuit system. Closed-circuit spirometer systems were primarily used to capture metabolic rate as early as the 1800s. [22] In a closed-circuit, the subjects breathes 100% oxygen from a spirometer containing an CO_2 absorber, and no outside air is introduced to the system. [18] As the subject rebreathes air, CO_2 production is captured by the absorber and quantified. Oxygen (O_2) consumption is determined from the difference between the initial and final volume of oxygen in the spirometer. The rate of O_2 consumption (which is used to determine energy expenditure using the modified de Weir equation [25]) is then derived from the average rate of decrease in volume from the spirometer. A drawback to the closed-circuit is that requires large volumes of air to be exchanged and cannot be used to capture energy expenditure during exercise.[18,22] Open-circuit spirometer systems are the more common method used throughout the literature today. In this system, when a subject inhales ambient air of constant composition (20.9% oxygen,

0.03% carbon dioxide, 79.04% nitrogen), the ratio of oxygen to carbon dioxide percentages in the expired air is compared to inspired ambient air. The composition of exhaled air and volume of air breathed are used to measure oxygen uptake[22], which can then be converted to energy expenditure using the modified de Weir equation. [25] The equipment commonly used to measure basal, resting, or sleeping energy expenditure is a computerized metabolic cart. It is a portable device that is considered to be the gold standard for assessing metabolic rate in humans in research and clinical populations. [14] It consists of a computer on a cart, which is connected by a plastic hose to a small chamber (e.g., a hood, canopy, facemask) for gas exchange. The computer system measures the volume of air breathed and also assesses the concentration of carbon dioxide and oxygen in the air in one-second to one-minute intervals. [13] Optimally, it is performed when a person fasted and rested. [12]

Infant populations pose particular challenges when measuring metabolic rate. Their small body size which is often too small to meet technical specifications of many metabolic carts. Their inability to consciously control body movements, and requirement for frequent feedings making fasting not possible are additional reasons measuring RMR is not feasible. [13] . Therefore, SMR is commonly used as a proxy for BMR in infants; this methodology is seen repeatedly in the literature for this age group. [13,17] Infants less than one month old are able to sleep and have SMR measured by an open-circuit calorimeter. A clear ventilated canopy-like chamber is placed over the infant subject's entire body once they have fallen asleep in a supine position. [12,13] In most cases, a parent or guardian is nearby at all times and available to calm the infant down if he/she begin to fidget or fuss. Most study protocols aim to take measurements for a goal of 60 minutes or until the infant wakes up, with a minimum

of 30 minutes total. The first 5-10 minutes are discarded to allow gas equilibration in the hood and achievement of steady-state conditions. [12]

2.2.3 Predicting Metabolic Rate

Measurement of energy expenditure by indirect calorimetry has been and continues to be, the gold standard method for capturing resting, sleeping, or basal metabolic rate in clinical and research settings. [12] Unfortunately however, not all hospitals, clinics, universities have the equipment, personnel, or expertise to conduct BMR by indirect calorimetry. Predictive equations are the alternative for assessing BMR. Predictive equations can be utilized for patient care at almost no cost, and as such the use of predictive equations is a common practice across many healthcare facilities. These equations have been empirically derived and require anthropometric measures (i.e. length/height and weight) for age and sex calculation of metabolic rate. [13,15,17]

In the early 1980s, a joint work group composed of the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), and the United Nations University (UNU) conducted an in-depth literature review of energy and protein requirements across the life span (infant, child, adult). [15] The workgroup evaluated and consolidated data, including 6000 data points from males and 6500 data points from females to develop empirical equations for the estimation of BMR based on infant body weight. These equations are referred to as the WHO BMR equations.

Shortly thereafter, W.N. Schofield reviewed all published literature measuring BMR dating back over 60 years.[16] His literature review followed more rigorous guidelines to derive age and sex specific equations. Equations for 0-3-year old

children were based on data from 10 separate studies, totaling 162 males and 137 females. In the majority of the studies, infants were either sleeping or sedated and about one-third of the measurements were within the infants first week of life. [16] Schofield published sex specific empiric equations for the estimation of BMR based on subject age and weight only ($Schofield_{wo}$ equation) and other equations based on age, weight and length/height ($Schofield_{wh}$ equation) for six age groups; 0-3 years; 3-10 years; 10-18 years; 30-60 years; and over 60 years of age. [16] The equations were intended to predict BMR at a group level rather than an individual level. [16]

While there are several other equations published for predicting BMR, few include specified equations from those 0-3 years of age (i.e. Harris-Benedict). [26] Therefore, the WHO and Schofield equations are used predominantly throughout the literature in pediatric populations. [18] However, over the last few decades, the accuracy of these equations has come into question, specifically in regards to infant populations. The methods and techniques used over 80 years ago to obtain the metabolic measurements have changed with advances in knowledge and technology. The WHO, and Schofield equations were derived from data collected as early as the 1930's. [15,18] With such large technological advances it reasonable to question if these equations still hold true today, especially because the equations have wide spread use with healthy and ill populations. Additionally, the generalizability of the WHO and Schofield equations may be limited because the studies from which they are based did not represent all ethnicities (mainly subjects of European and North Americans decent). As a result, the Oxford Equations were published in 2005, from a research group at Oxford University. [18]

In 2005, after reviewing the historical development and accuracy of the BMR measurements, Oxford University researchers critically reviewed the literature under strict guidelines to develop a series of new BMR equations. [18] To maintain high quality, studies were included only if they: 1. Provided age, weight and sex of subjects, 2. Explicit description of experimental condition and BMR equipment, 3. Conducted measurement when subjects were post-absorptive and rested, 4. Performed measurements on healthy subjects, and 5. Provided information on geographic location and/or ethnicity of subjects. Similar to the Schofield equations, the Oxford equations are sex specific for six age brackets (0-3 years, 3-10 years, 10-18 years,18-30 years, 30-60 years and >60 years), and can be used with weight only ($Oxford_{wo}$) or weight and length/height ($Oxford_{wh}$).[18] The weight only equations used to predict BMR of children 0-3 years of age were derived from 277 data points from males with an average age of 0.4 years of age (±0.62 year) and 215 data points from females with an average age of 0.5 years (±0.71 year). The 0-3 year predictive equations using weight and length/height were derived from 246 data points for males and 201 data points for females. Authors of the Oxford equations compared estimated RMR derived from the WHO equations to estimated RMR from the Oxford equation, and found (at the 0-3 year old age bracket) there was a large difference in predicted RMR in subjects with low body weights for both male and females, with the Oxford equations producing lower, but closer to measured BMR values. [18]

For example, in a study by Wells et al[27], both the $Schofield_{wo}$ and $Schofield_{wh}$ were found to be unsuccessful at predicting measured-SMR in a study of 40 healthy infants, 6 weeks to 1 year of age in England. [27] SMR measurements were taken at 6 weeks, 12 weeks, 6 months 9 months and 12 months by indirect calorimetry through

use of the Douglas Bag method, a method in which the subject breathes via a mouthpiece or facemask and a one way value captures expired air into a large bag. Despite a lack of significant difference between $Schofield_{wo}$ and the measured-SMR at the group level, the equation was ultimately unsuccessful in predicting SMR, at the individual level. An age bias was also evident, with underestimation of measured-SMR by 4% at 6-weeks and an overestimation by 6% at 12 months. In the same population, the $Schofield_{wh}$ generated a significantly different predicted BMR compared to the measured-SMR ($p<0.0001$). [27]

In another study, by Thomson et al[28], the $Schofield_{wo}$ and $Schofield_{wh}$ and WHO all significantly over estimated REE compared to actual measurement taken of 36 healthy infants ($p<0.05$). [28] The average age of the infants was 0.47 yrs. old (~5.5 months). Unlike Wells et al[27], which found no significant differences between measured SMR versus $Schofield_{wo}$, this study found the $Schofield_{wo}$ equation overestimated metabolic rate by 16% for the 6-12 month group. [28] Furthermore, the 95% confidence limits were wide (12%) which suggest large individual variation among this population, making it difficult to generalize the accuracy of these predictive equations.

Similarly, another study of healthy infants aged 6 weeks to 1 year found that the Schofield equation for 0-3 y-olds was inaccurate when compared with SMR measurements. [13] Bland-Altman analysis showed the $Schofield_{wo}$ over predicted BMR by 11% on average and the $Schofield_{wh}$ equation under predicted the actual by only 1% on average. Though differences appear relatively small, the 95% confidence limits of agreement were considerably wide (28% and 27% respectively) for both equations, again suggesting there was large variation between measure and predicted metabolic

rate and a bias with increasing metabolic rate. When considering the 95% confidence intervals, 52% of the infants' would fall outside of the clinical acceptable range for accuracy (90%-110% of actual according to the authors) for the $Schofield_{wo}$. For the $Schofield_{wh}$ 46% of infants' predicted REE fell outside the range. Nearly, 20% of the estimations fell outside of the 80-120% of actual SMR. Thus, the authors concluded the Schofield equations lacked accuracy and may be misleading in a clinical setting. [13]

Taken together, these studies suggest the Schofield equations and the WHO equation may not be as suitable as once thought for the prediction of in SMR/BMR, in healthy infants under one year of age. [13,27,28] Although there are differing opinions on how to assess accuracy (equation must be within 90-110% of measured or within or within 95% confidence interval of measured), statistically significant discrepancies between measured versus predicted values have been reported.[13,27] Whether the discrepancy has clinical significance also must be considered. Most equations for predicting BMR are intended for male and female infants 0-3 years of age, and it has been suggested that the age range is too wide, and may not be accurate for estimating BMR for 0 to 1 year olds. [13] The growth rate and changes in body composition experienced by infants from birth to one year is considerably different than the growth rate and body composition changes from one to three years of age. [29] The change in the ratio of metabolically active organ mass to muscle mass throughout the first year of life compared to the year second and third year of life is considerably different as well. [29] Knowing that body size and composition influence metabolic rate, it is reasonable to consider that one equation for children 0 to 3 years of age, may not suffice children less than one year of age. [13,29]

2.3 Factors that Affect Basal, Resting, and Sleeping Metabolic Rate

Many factors affect metabolic rate including age, sex, genetics, body composition, and physical activity. The most influential of these is body composition and more specifically fat free mass (FFM). [30,31]

2.3.1 Body Composition and Body Size

The relationship with BMR and FFM is well established. FFM is metabolically active tissue and is the largest contributor to metabolic rate, explaining captures upwards of 73% of RMR variation. [14,23,24] From late infancy into child- and adulthood, BMR per kilogram (kg) body weight decreases; this is due to slower growth of high energy-producing organ cell mass relative to total body weight. In infants, organ cells contribute greatly to the active tissue percentage in the body. The most metabolically active organs are the brain, liver, heart and kidneys. For infants, the sum of active organ mass account for more than 60% of BMR, compared to male adults whose sum accounts for less than 6%. [29] Similar to adult studies, FFM has been shown to influence RMR in infants [17]

2.3.2 Age

Age has often been identified as a factor that can impact energy metabolism and expenditure, as age increases BMR decreases. [30,32] A 20-yr longitudinal study found that age-related reduction in BMR may be a small as 1-2% per decade from 20-75years old. These decreases in energy metabolism in aging adults may actually be attributed to the change in body composition over time and not the aging process itself, because FFM typically declines with age. [32] When age is included in the model, FFM overrides age as a predictive measure of REE. [32]

2.3.3 Diet and Food Consumption

Digestion of macronutrients (carbohydrate, protein and fat) elicits varying degrees of oxygen consumption, which indicates that diet composition may influence energy expenditure as well. One of the largest differences in infant diet is whether is infant is breastfed (BF) versus formula fed (FF).[4] With respect to macronutrient composition, breast milk contains a higher protein concentration early in infancy and a lower protein concentration later in infancy, versus infant formula, which contains a steady concentration of protein whenever fed.[4,9,10] The transition to lower protein concentration in human milk happens rather quickly, such that on average, formula fed infants have a greater total protein intake than BF infants. A study evaluating SMR measured by indirect calorimetry in 40 breastfed and 36-formula fed healthy infants at 3-month intervals for two years, found SMR differed between sex and between feeding groups at 3 and 6 months of age, with formula-fed infants having consistently higher SMR. [33] Significance remained even when adjusted for weight, length or FFM and FM. [33] Interestingly, another study, which compared 25 breastfed infants and 27 infants who were fed a combination of breast and formula (average age 8.7 months), found that those receiving CMF had higher SMR. [34] Though the mechanism is still unknown, these results may indicate a relationship between cow's milk formula, more specifically protein intake, and SMR in infancy. [34] To our knowledge, there are no studies that have investigated the effect of exclusively formula fed infants, feeding different types of infant formula, and the impact on SMR.

2.3.4 Sex

The sex of an individual has often been identified as a factor that can impact energy metabolism and expenditure. In adults, the majority of studies have found a

slight increase in either, RMR, BMR, or SMR in pre-menopausal women during the luteal phase of menstruation compared to the follicular phase. [35-37] The exact mechanism for these observed differences remains unclear. Hormonal differences, differences in organ tissues and FFM are considered most likely to be responsible.

In regards to children, there is limited research. Two studies of pre-pubertal children found that females had lower REE than males, even when adjusted for differences in body composition. [38] Another study found similar results in 203 obese Caucasian and African American children (ages 7-15). When using predictive modeling, females had lower REE than boys with both races included, even after adjusting for FFM ($P<0.001$). [39] There are limited studies in the literature focusing on sex and SMR in early infancy. One study measured SMR in 73 healthy infants ranging from 1-12 months of age and found no significant difference in SMR between males and females. However, they did report large inter-individual variation for SMR measurements of infants of similar age. [40]

2.3.5 Race

Race appears to be another factor influencing energy expenditure. Overall, studies show adult African Americans have significantly lower RMR, BMR and SMR compared to adult Caucasians when adjusted for body composition. [41,42] Similar results are seen in pediatric populations. Studies have shown African American children have lower RMR and BMR than Caucasian children, even when adjusted for body composition. [43,44] Another study found similar results in 203 obese children (ages 7-15). When using predictive modeling, African Americans had lower REE than Caucasians ($p<0.001$). [39] Interestingly, when adjusted for trunk lean body mass differences based on race were lessened. [39] However, one study found no differences

in adult or child RMR based on race [45,46] Additionally, no statistically significant differences in measured RMR were found between Caucasian and Pima Indian children (mean age 9.9 yrs.), or between Caucasian and Mohawk Indian children (mean age 5.3 yrs.) after adjusting for FFM. [47,48] At this time, there are no separate predictive equations based on race. Overall, more research is needed to truly understand the role of race in relation to energy expenditure.

2.4 Infant Growth

Infancy is a critical and unique period of growth during which weight gain is more rapid than any other period in the lifespan. Infants double birth weight by 6 months and triple it by 12 months. [33] Newborns are approximately 11% body fat at birth and deposition of fat tissue progressively occurs through infancy, such that infants have 31% body fat at 3 to 6 months and then gradually decline to 27% body fat at 12 months. [33] These changes can alter energy expenditure due to changes in organ size and metabolic active tissues. [29] It is normal for infants to experience considerable changes in body composition, especially in the first 3 months. [29] Research also has shown diet composition in early infancy affects weight status in childhood and into adulthood.[6-8]

2.4.1 Impact of infant diet composition on growth

One of the first studies to document a difference in infant growth based on diet composition, was the Davis Area Research in Lactation, Infant Nutrition, and Growth (DARLING Study). This study, followed a cohort of breast fed (BF, n=46) and formula fed (FF, n=41) infants throughout the first year of life found that FF infants grew differently than BF counterparts, especially after the first 3 months of life. [5] BF

gained less weight than FF infants between 3 and 12 months resulting in a significant 0.65 kg weight difference at 12 months. Additionally, weight for length z-scores were significantly higher in FF infants compared to BF infants from 4 to 18 months. Finally, no significant differences in length were seen between the BF and FF cohorts. These findings suggested that BF infants were generally leaner than FF infants. [5] Other studies since have reported that FF infants are heavier and gain weight more rapidly in infancy compared to BF infants, including a study done of 40 BF and 36 FF healthy infants. [33] Infants were followed for the first two years of life and measured at 3-month intervals. This study found weight velocity was higher in FF infants compared to BF from 3-6 months of both sexes, and from 6-9 months in females only. [33]

The WHO and American Academy of Pediatrics (AAP) recommend exclusive breastfeeding for 6 months and continuation though 12 months.[2] Despite the strong national encouragement, the CDC reports only 43% of mothers in the United States (US) exclusively breastfeed through 3 months and 22% of mothers exclusively through 6 months (CDC).[1,3] Therefore, by 3 months of age, nearly 60% of U.S. infants receive infant formula in combination with breast milk or as sole source of nutrition. There are a large variety of infant formulas on the market, such as cow's-milk based formulas (CMF)[9], soy-based formula, and protein hydrolysate formula (EHF)[10]. EHF is often prescribed to infants who experience difficulties digesting/absorbing intact protein based formula. In EHF formula the proteins have been enzymatically digested and ultra-filtered to remove large peptides, resulting in mainly small peptides and free amino acids. [10,49] Being that EHF formula differ not only in flavor, but macronutrient composition, it is reasonable to assume they may influence feeding and growth

patterns differently as well.[49] This notion was supported by a study that followed growth patterns from 0.5 to 7.5 months of age in infants randomized to either CMF or EHF starting at 0.5 months.[11] The study found that EHF fed infants consumed less formula to satiation during laboratory ad libitum feeding sessions, and EHF fed infants had significantly lower weight-for-age z-scores from 3.5 to 7.5 months and weight-for-length z-scores from 2.5 to 7.5 months, compared to their CMF counterparts. EHF infants also experienced significantly less change for weight-for-age z-score period ($p=<0.0001$), as well as, less change in weight-for-length z-score ($p<0.01$) from baseline to 7.5 months. When assessing growth trajectory, EHF fed infants had z-scores closer to 0, indicating EHF infants grow more similarly to breast fed infants compared to the CMF infants, which had accelerated weight gain with z-scores greater than 0, starting at 2.5 and continuing through the end of the study (7.5-months).[11]

There are several hypotheses, which may explain the mechanisms underlying such results. First, it is possible that EHF infants consumed less because the EHF formula is known to have a stronger or bitter taste due to the free amino acids in the formula; EHF is considered unpleasant when tested with adults.[50] However, the credibility of this hypothesis has weakening with previous research that found infants introduced EHF within the sensitive taste period (0-3 months of life) were not deterred by taste and mothers reported infants enjoyed the formula. Second, EHF formula transits the gastrointestinal tract at a faster rate than CMF, and this may lead to greater energy loss in stool.[51] Third, is that the protein structure of the two formulas differs, and this may alter nutrient metabolism.[52] Proteins in EHF have been enzymatically hydrolyzed and are predominately free amino acids and small peptides. In contrast, CMF proteins are predominately intact protein from whey and nonfat milk. In adults,

hydrolyzed protein elicits a biochemical-signaling cascade, which results in earlier stimulation of gut receptors, such as cholecystokinin, and earlier satiation response. [53-55] In addition, research suggests gut receptor stimulated by free amino acids may also stimulate an increase in energy expenditure in adult males, which would result in slower weight gain over time. [56] Taken together, it is reasonable to postulate that diet composition, specifically the form of the protein ingested, can influence energy expenditure.

In summary, infancy is a unique period in the lifespan, in which weight gain is desired and rapid. The growth of BF infants is considered the gold standard, and FF infants experience accelerated weight gain compared to breastfed infants.[4] Rapid weight gain in infancy has been linked with increased risk of obesity, metabolic syndrome, cardiovascular related mortality and diabetes in adulthood. [57-60] Unfortunately, national data shows that nearly 60% of infants receive infant formula by three months of age. Infant formulas greatly vary in macronutrient composition, specifically protein content and structure. Infants fed EHF, which contains higher concentrations of free amino acids, grew more similarly to breast fed infants, than those receiving standard CMF. The energy balance mechanisms that led to differential growth are not know. It is possible that the peptides and free amino acids in the EHF lead to increased SMR. The present study provides the opportunity to determine if resting metabolic rate differs by the type of formula fed to the infant.

Additionally, in clinical settings, predictive equations are most commonly used to estimate BMR in infants. The WHO and Schofield equations have been used in clinical practice for the past several decades, however, their accuracy in predicting BMR in infant population (<1 year of age) is questionable. Newer equations, such as

the Oxford equation, have sought to elevate the accuracy of the WHO and Schofield. However, to our knowledge, no studies have evaluated the accuracy of WHO, Schofield, or Oxford equation against actual SMR measurement in infants receiving formulas, which differ in protein content. The purpose of the present study is to evaluate the effect of formula composition on SMR measurements. Additionally, we sought to compare the accuracy of three predictive equations for the estimation of BMR to SMR measurements.

Chapter 3

AIMS

The overall aim of this thesis was to evaluate sleeping metabolic rate (SMR) in a contemporary cohort of healthy term formula fed (FF) infants within the first month of life when infants were fed the same formula (CMF) and again after infants have been randomized to one of two formulas, which are isocaloric but differ in the protein concentration and form (CMF versus EHF). We also sought to utilize the SMR measurements to evaluate the accuracy of formulas used to estimate metabolic rate versus measured SMR.

3.1 Specific Aims

Aim 1: Describe SMR of 0.75 month old infants and determine if SMR differs by race and sex. Given reported differences in SMR by sex[26,93] and race[41-44] we hypothesize SMR will be higher in male compared to female infants and lower in African American versus Caucasian infants.

Aim 2: Describe SMR of 3.5 month old infants, and determine if SMR differs by diet (CMF vs. EHF), race, sex. Because differences in metabolic rate have been observed in infants receiving infant formula, which is higher in protein compared to breast milk[33,34], and because EHF is greater in protein concentration than CMF, we hypothesize that EHF fed infants will have higher SMR at 3.5 months compared to CMF fed infants.

Aim 3: Determine the accuracy of three empirical equations commonly used to calculate BMR. Given the strict criteria used to develop the Oxford equations[18], we hypothesize the Oxford weight height (OX-wh) equation will most accurately predict SMR.

Chapter 4

METHODS

4.1 Subjects

Parturient mothers in the greater Philadelphia area who planned to exclusively formula feed their infants were recruited from local hospitals, the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC), and via print and web advertisements to participate in the study. Inclusion criteria specified that: infants were healthy, born full term (≥ 37 weeks to ≤ 42 weeks), with appropriate birth weight for gestational age (2500-4500 grams), ≤2 weeks of age at enrollment, the mother's independent decision to formula feed was firmly established and the infant received infant formula exclusively for at least two days, and mothers were older than 18 years of age. Exclusion criteria were: infants who were being breastfed, were preterm or had medical conditions that might interfere with feeding or eating (infectious or systemic diseases, documented systemic congenital infections, or evidence of significant cardiac, respiratory, endocrinologic, hematologic, gastrointestinal disease), mothers with gestational diabetes or are diagnosed with a major illness requiring treatment or surgery, mothers who planned to go back to work full-time before the infant was four months old.

4.2 Research Design

This research was part of an ongoing NIH-funded, randomized, controlled trial on the effect of diet composition on energy balance and growth. Mothers-infant dyads were enrolled in the study at ≤ 2 weeks post-partum and continued in the study until the infant was approximately 18.5 months of age. At enrollment, all infants were provided with a standard CMF formula (Enfamil™, Mead Johnson Nutrition,

Evansville, IN)[9] to feed for approximately one week. After one week, mother-infant dyads returned to the study center and received their randomized formula, either CMF (Enfamil, Mead Johnson Nutrition, Evansville, IN) or an EHF formula (Nutramigen™, Mead Johnson Nutrition, Evansville, IN)[10] and infants were fed this formula for the remainder of the first year of life. The formulas were isocaloric and contained a similar fat blend (see Table A.1); however, EHF contains a higher protein concentration and protein in the form of small peptides and free amino acids, while CMF contain intact protein. At monthly visits, mothers were provided a month-long supply of the appropriate formula and instructed to refrain from giving the child any other formula.

Anthropometric data for both the mother and infant were collected at each months study visit. At three time points in the first year of life (0.5 months, 3.5 months, 12.5 months), all measures of energy balance were assessed (TEE, SMR, energy intake, and energy loss in stool). For the purpose of this thesis, we focused on measures of sleeping energy expenditure or sleeping metabolic rate at 0.5 and 3.5 months.

4.3 Study Visit Procedures

During **Visit 1** (infants 1-2 weeks of age) mothers-infant dyads arrived at the study site. The informed consent was reviewed and signed by the mother. Anthropometric, demographic, health history, diet history, and feeding practices data were collected. Mothers were provided with CMF and instructed to exclusively feed their infants CMF until the next visit one week later.

Visit 2 (infants 2-3 weeks of age)**:** Measures pertinent to this study included anthropometric and SMR measurements. Afterwards, mothers were provided with

their randomized formula (EHF or CMF feeding group) and instructed to exclusively feed their infants randomized formula for the duration of the study. Mothers were unaware of the study hypothesis and research personnel did not provide any additional infant feeding instructions.

Visit 5 (infant approximately 3.5 months of age)**:** Measures pertinent to this study included anthropometric and SMR measurements.

4.3.1 Demographics

At study entry (Visit 1), demographic data including race/ethnicity, income, parity, and education level of the mother, and sex of child were collected.

4.3.2 Infant Anthropometrics

Trained personnel measured infant weight, recumbent length, and head circumference, in triplicate using standard anthropometry techniques.[61] Weight was measured with a digital scale accurate to 0.001 kg, recumbent length was measured with a length board accurate to 0.1 cm and head circumference was measured with a non-elastic tape measure accurate to 0.1 cm.

4.3.3 Sleeping Metabolic Rate

The sleeping metabolic rate (SMR) measurement was conducted at the Clinical and Translational Research Center at the Children's Hospital of Philadelphia. A computerized metabolic cart (model 2900 Z; Sensor Medics, Yorba Linda, CA) was used to measure post-prandial SMR by open-circuit indirect calorimetry. The cart was located in a quiet and thermally neutral room. Infants were placed in a supine position under a large, clear ventilated hood. SMR was measured for a goal of 60 minutes and a minimum of 30 minutes. The first 10 minutes of the measurement period were

discarded to allow the metabolic cart to equilibrate. Respiratory gases were continuously sampled and documented every second, and 1-minute averages were computed for the duration of the measurement. Infant's movements were also documented throughout. In instances where infant movement altered SMR readings and were associated with infant movement, the associated time points were excluded. SMR was calculated using the de Weir equation.[25]

4.4 Data Analysis and Statistics

For data at each time point (0.75 and 3.5) missing data and out-of-range values were identified using univariate statistics (means, standard deviations, ranges and frequencies). Data were assessed for normality utilizing a Shapiro-Wilk test, and reported using parametric (mean, standard deviations) or non-parametric techniques (median, interquartile ranges) depending on the distribution. The dependent variable within this study is SMR (kcal/day). The independent variables include formula type (CMF versus EHF), fat free mass (FFM), race (African American, Caucasian, other/mixed) and sex (male, female). Baseline differences for all demographic and child characteristics at 0.75 months and 3.5 months by formula type (CMF vs. EHF) were examined using a chi-square test for categorical variables (sex and race) and a t-test for continuous variables (FFM and SMR).

Aim 1: Describe SMR of 0.75 month old infants and determine if SMR differs by race and sex. To assess Aim 1, two separate multiple linear regression models were fit. The first model included SMR as the dependent variable and sex as the independent variable; the second model included SMR as the dependent variable and race as the independent variable. Fat free mass (FFM) was included in both models as a covariate

since FFM is known to be associated with SMR and differs by race and sex of the infant. Sex was a dichotomous variable (male or female) and race (African American, Caucasian, other) was included in the model as a 3-level dummy variable with Caucasian as the reference group.

Aim 2: Describe SMR of 3.5 month old infants, and determine if SMR differs by diet (CMF vs. EHF), race, sex. To assess Aim 2, a multiple linear regression model with SMR at 3.5 months as the dependent variable and formula group (CHF vs. EHF) as the independent variable was fit. FFM was included within this model as well as potential covariates such as race and sex.

Aim 3: Determine the accuracy of three empirical equations commonly used to calculate BMR. To test Aim 3, for both time points, the difference between calculated BMR and measured SMR as well as the ratio of calculated BMR relative to measured SMR was calculated. Next, three separate regression models were fit to examine the performance of each equation (WHO, Schofield, Oxford) for predicting SMR within a common framework. Criteria for formula evaluation and model comparison include: the root mean square error (RMSE), Wald-statistic p-value, and squared multiple coefficient of determination (R^2 (individual level agreement))

Chapter 5

RESULTS

5.1 Normality and Distributions of Variables

A Shapiro-Wilk W test was used to examine normality for all continuous variables. For infant characteristics and anthropometric measures, the variables age (months), weight (kg), length (cm), weight for age z-score (WAZ), length for age z-score (LAZ), weight for length z-score (WLZ) and body mass index (BMI) z-score (BMIZ) were normally distributed ($p>0.05$). Infant sleeping metabolic rate (SMR) and fat free mass (FFM) were also normally distributed ($p>0.05$). Means and standard error of the mean (SEM) are presented for all variables.

5.2 Completion of Study Visits

The completion of study visits from enrollment (0.5 months) through the 3.5-month visit is provided in **Figure B.1** One hundred and forty-one healthy, formula fed infants were enrolled in the study. Of the 141 infants, 28 infants dropped out of the study after the enrollment visit, leaving 113 infants who were randomized to one of two commercially available formula groups (CMF vs. EHF) at the end of the 0.75-month visit. At the 0.75-month visit, 102 infants had a successful sleeping metabolic rate (SMR) measurement. At the 3.5-month visit, 83 infants had a successful SMR measurement, data on seven additional infants is anticipated, but not included in this analysis.

5.3 Infant Demographic and Anthropometric Characteristics

5.3.1 Intent to Treat (Randomized Infants)

Infant demographics and anthropometric characteristics for the one hundred and thirteen infants that were randomized to one of the two formula groups are summarized in **Table A.2a.** There were 59 infants in (31 males, 28 females) in the CMF (Group A) group and 54 infants (28 males, 26 female) in EHF group (Group B). The mean age in months for CMF group was 0.65 months (±0.01 SEM) and 0.63 months (±0.01 SEM) for EHF group. In the CMF group, 59% of subjects were African American, 29% Caucasian, and 12% were other/mixed races. Similarly, in the EHF, 59% African American, 15% Caucasian, and 20% other/mixed race. Ninety-two percent of CMF infants and 85% of EHF infants were non-Hispanic, respectively. There were no significant differences by eventual randomized formula group, for sex (p=0.507), age (p=0.329), or race (p=0.141).

Mean weight was 3.70 kilograms (kg) (±0.06 SEM) for infants in CMF group and 3.73 kg (±0.05 SEM) for infants in EHF group. Mean length was 51.80 centimeters (cm) (±0.30 SEM) for CMF group and 51.47 cm (±0.28 SEM); there were no significant difference by eventual formula randomization group, for weight (p=0.716) and length (p=0.424). WAZ, LAZ, and WLZ were generated using the WHO Multicenter Growth Reference Standard.[62] For the CMF group, the mean WAZ at baseline was -0.41 (±0.11 SEM), the mean LAZ was -0.56 (±0.13 SEM), and the mean WLZ was -0.12 (±0.11 SEM). Infant BMIZ was -0.16(±0.10 SEM) for CMF fed infants. For the EHF group, the mean WAZ was -0.27 (±0.10 SEM), the mean LAZ was -0.58 (±0.14 SEM), and the mean WLZ was 0.12 (±0.99 SEM). Infant BMIZ was 0.06 (±0.10 SEM). There were no significant differences by eventual formula

randomization group for WAZ (p=0.378) and LAZ (p=0.891), WLZ (p=0.162), or BMIZ (p=0.126).

5.3.2 0.75 Month Visit (Baseline)

Infant demographic and anthropometric characteristics for the 0.75-month baseline visit are summarized in **Table A.2b**. One hundred and thirteen healthy infants were randomized to a formula group, however only one hundred and two infants (n= 52 males, 50 females) had successful SMR measurements and were included in the 0.75 months visit (baseline) analysis. To determine if baseline differences existed in subject characteristics by eventual formula randomization group, demographic and anthropometric characteristics were stratified by randomization group and differences between groups were test using an independent t-test for continuous variable and Chi-square test for categorical variables. There were 54 infants (29 males, 25 female) in the CMF group and 48 infants (23 males, 25 females) in EHF group. The mean age in months for CMF group was 0.65 months (±0.01 SEM; range 0.43-0.95 months) (approximately 19 days) and 0.63 months (±0.01 SEM; range 0.46-0.89 months) for EHF group. In the CMF group, 59% of subjects were African American, 30% Caucasian, and 11% were other/mixed races. Similarly, in the EHF, 67% African American, 12% Caucasian, and 21% other/mixed race. Ninety-two percent of CMF infants and 85% of EHF infants were non-Hispanic. There were no significant differences by eventual formula randomization group, for sex (p=0.559), age (p=0.336), or race (p=0.243).

Mean weight was 3.76 kg (±0.05 SEM) for infants in CMF group and 3.74 kg (±0.06 SEM) for infants in EHF group. Mean length was 51.9 cm (±0.29 SEM) for

CMF group and 51.5 cm (±0.29 SEM); there were no significant difference by eventual formula randomization group, for weight (p=0.807) and length (p=0.377). WAZ, LAZ, and WLZ were generated using the WHO Multicenter Growth Reference Standard.[55] For CMF group, the mean WAZ at baseline was -0.30 (±0.10 SEM), the mean LAZ was -0.48 (±0.13 SEM), the mean WLZ was -0.03 (±0.16 SEM), and the mean BMIZ was -0.05 (±0.10 SEM). For the EHF group, the mean WAZ was -0.27 (±0.11 SEM), the mean LAZ was -0.55 (±0.16 SEM), the mean WLZ was 0.08 (±0.14 SEM), and the mean BMIZ was -0.04 (±0.11 SEM). There were no statistically significant differences by eventual formula randomization group for WAZ (p=0.859) and LAZ (p=0.742), WLZ (p=0.494), or BMIZ (p=0.495).

5.3.3 3.5 Month Visit

Infant demographic and anthropometric characteristics for the 3.5-month visit by randomization group, are summarized in **Table A.2c**. Eighty-three formula fed infants (n= 46 males, 37 females) remained in the study at the 3.5-month visit. Data for an additional 7 infants was not complete at the time of this analysis, but will be included for the final analysis. In the CMF group there was 43 infants (26 males, 17 females) and 40 infants (20 males, 20 females) in EHF formula group. The mean age in months for CMF group was 3.52 months (±0.02 SEM; range 3.25-3.95 months) and 3.54 months (±0.02 SEM; range 3.29-3.98 months) for EHF group. In the CMF group 63% were African American, 21% Caucasian, and 16% were other/mixed races. Similarly, in the EHF, 65% African American, 10% Caucasian, and 25% other/mixed race. Ninety-one percent of CMF infants and 80% of EHF infants were non-Hispanic, respectively. There were no significant differences by formula randomization group, for sex (p=0.337), age (p=0.576), or race (p=0.306).

Mean weight was 6.46 kg (±0.11 SEM) for infants in CMF group and 6.00 kg (±0.14 SEM) for infants in EHF group. Mean length was 61.9 cm (±0.69 SEM) for CMF group and 61.3 cm (±0.46 SEM). Mean weight was significantly lower in EHF fed infants (p=0.012) while mean length did not differ between formula groups (p=0.459).

For CMF group, the mean WAZ was -0.09 (±0.13 SEM), the mean LAZ was -0.39 (±0.15 SEM), and the mean WLZ was 0.31 (±0.14 SEM). Infant BMIZ was 0.17 (±0.13 SEM). For EHF group, the mean WAZ was -0.66 (±0.17 SEM), the mean LAZ was -0.36 (±0.20 SEM), and the mean WLZ was -0.48 (±0.16 SEM). Infant BMIZ was -0.63 (±0.16 SEM). EHF fed infants had significantly lower WAZ (p=0.0088), WLZ (p=0.0004), and BMIZ (p=0.0002) compared to CMF fed infants.

5.4 Infant Sleeping Metabolic Rate and Fat Free Mass

A multiple linear regression model with formula group, and adjustment for FFM, was fit to determine if SMR differed by formula group at baseline (0.75-month visit). The same model was fit for the 3.5-month visit. However, since this is an interim analysis and not all infants had a successful or completed measure of FFM (by isotope dilution), FFM values for those with missing data were determined by multiple linear regression imputation. Weight was regressed onto measured FFM utilizing data from subjects with successful FFM measures, and the resultant equation was used to determine FFM for those with missing FFM values. A separate equation was run for each time point (0.75 and 3.5 months). **Table A.3** summarizes the number of infants that had measured versus imputed FFM at 0.75-month and 3.5 month. At 0.75-month visit, 40 infants in the CMF group had measured FFM and FFM was imputed for the remaining 14 infants. For EHF group, there were 30 infants with measured FFM and

18 infants with imputed FFM values. At the 3.5-month time-point, 36 infants in the CMF group had measured FFM and 7 infants had imputed FFM. For EHF group 25 infants had measured and 15 infants had imputed FFM. These counts were not significantly different between formula groups at 0.75-months (Chi-square p=0.208, **Table A.3**). There was a significant difference in proportion of infants at 3.5-months with measured versus imputed FFM, with a greater proportion of CMF infants having measured FFM, which is preferred. However, additional FFM data on 7 infants at the 3.5-month time point is forthcoming and we will repeat the analysis to determine if the proportion with measured versus imputed FFM differs by formula group (Chi-square p=0.028, **Table A.3**).

At baseline all infants were receiving CMF exclusively. There was no statistically significant difference (p=0.148) in measured SMR, adjusted for FFM, by eventual formula randomization group at baseline. Mean SMR for CMF groups was 205.5 kcal/d (±3.28 SEM) and was 198.5 kcal/day (±3.48 SEM) for EHF group as seen in **Table A.4**. At 3.5 months, when infants were receiving different formulas, there was still no statically significant difference (p=0.880) between measured SMR, adjusted for FFM, by formula type. Mean SMR for CMF was 325.4 kcal/d (±14.09 SEM) and was 323.18 kcal/d (±21.15 SEM) for EHF group (See **Table A.4**)

5.4.1 Specific Aim 1

To test Aim 1 (describe SMR of 0.75 month old infants and determine if SMR differs by race and sex), a multiple linear regression model was employed with SMR as the dependent variable and sex as the independent variable; FFM was included as a covariate. This model yielded an R^2=0.28 (overall p-value p<0.0001); sex was a

significant factor in the model (p=0.003). After adjusting for FFM, male infants had an SMR that was 7.0 kcal/day greater than female infants. Next, a second multiple linear regression model was employed with SMR as the dependent variable and race as the independent variable; with FFM included as a covariate. This model yielded an R^2=0.23 (overall p-value p<0.0001) however race was not a significant factor in the model (see **Table A.5a)**.

5.4.2 Specific Aim 2

To test Aim 2 (describe SMR of 3.5 month old infants, and determine if SMR differs by diet (CMF vs. EHF), race, sex), a multiple linear regression model was employed with SMR as the dependent variable and sex as the independent variable; FFM was included as a covariate. This model yielded an R^2=0.21 (overall p-value p<0.0001); sex was a significant factor in the model (p=0.05). After adjusting for FFM, male infants had an SMR that was 9.6 kcal/day greater than female infants. Next, a second multiple linear regression model was employed with SMR as the dependent variable and race as the independent variable; with FFM included as a covariate. This model yielded an R^2=0.19 (overall p-value p=0.0007) however race was not a significant factor in the model (see **Table A.5b**). Finally, a third linear regression model was employed with SMR as the dependent variable, sex, formula group, and a sex by formula group interaction term as the independent variable; with FFM included as a covariate. This model yielded an R^2=0.24 (overall p-value

p=0.0002). Sex was a significant factor in the model (p=0.05) however formula group was not (p=0.933).

5.4.3 Specific Aim 3: Evaluation of Predictive Equations to calculate BMR

Since SMR did not differ by formula, all infants from both time points (0.75-month and 3.5-month) were grouped together to assess the accuracy of predictive equations to calculate basal metabolic rate (BMR). A total of five equations were evaluated (**Table A.6**). The difference between calculated BMR and measured SMR as well as the ratio of calculated BMR relative to measured SMR was determined. To evaluate group level agreement, the ratio of calculated BMR to measured SMR was obtained. To evaluate individual level agreement, five separate regression models were conducted to obtain information on the performance of each equation for predicting measured SMR.

5.4.3.1 0.75-month time point

The evaluation of the equations for calculating BMR compared to measured SMR at 0.75 months is summarized in **Table A.7a**. At 0.75-month all equations slightly under predicted actual SMR. At the group level three equations, Schofield weight only (SCHwo), Oxford weight only (OXwo), Oxford weight and height (OXwh) predicted metabolic rate within 90% of measured SMR, with the OXwo equation being closest to measured SMR (98%). At the individual level, the SCHwo equation yielded the highest R^2 (0.40) and the lowest RMSE (21.32). The WHO and OXwo also performed very well (R^2=0.35) at the individual level.

5.4.3.2 3.5-month time point

The evaluation of the equations for calculating BMR compared to measured SMR at 3.5 months is summarized in **Table A.7b**. At 3.5-month all, but one equation over predicted actual SMR. SCHwh was the only equation that under predicted at 3.5 months, but was still very accurate. At the group level, the SCHwh and the WHO equation calculated energy requirements closest to measured SMR (99% and 102%, respectively). At the individual level, SCHwo and OXwo yielded the highest R^2 (0.35, and 0.35, respectively).

5.4.3.3 Overall Evaluation (all time points together)

The evaluation of the equations for calculating BMR compared to measured SMR when data from the 0.75 month and 3.5-month time points were combined (n=185) is shown in **Table A.7c**. All five equations predicted metabolic rate within the within 92-102% of the measured SMR. At the group level, the SCHwo and OXwo equations calculated energy requirements closest to measured SMR (99% and 102%, respectively). At the individual level, the WHO, SCHwo, OXwo had the highest R^2 (0.80 for all three equations).

Chapter 6

DISCUSSION

The overall aim of this study was to evaluate the effect of diet composition, more specifically the effect of infant formulas differing in protein concentration and form, on sleeping metabolic rate (SMR), which in turn may impact infant weight gain. Although these data are preliminary, our analysis found the following. At baseline (0.75 months), there were no significant differences in weight for age (WAZ), length for age (LAZ), weight for length (WLZ), and body mass index z-score (BMIZ) by eventual formula randomization group. Since all infants were receiving cow milk formula (CMF) at baseline, the effect of race and sex only on SMR was evaluated, adjusting for fat free mass (FFM). While it has been established that African Americans have significantly lower metabolic rate than Caucasians in adult and some pediatric populations[41-44], our study found that race was not a significant predictor of SMR in young infants; sex was a significant factor at this age as reported by Butte et al.[26] Though preliminary, these results suggest that differences in metabolic rate by race were not present in infancy, and develop later in life.

Consistent with the finding of Menella et al[11], by 3.5 months, CMF fed infants had significantly higher in WAZ, WLZ, and BMIZ compared to EHF fed infants, but length for age (LAZ) did not differ significantly between the groups.[11] This indicates that CMF fed infants had significantly greater weight gain. By 3.5 months, CMF fed infants had a WLZ and BMIZ that were 0.79 and 0.80 greater than EHF infants. By this visit, all infants had been receiving their randomized formula (either EHF or

CMF) for 11 weeks. We found no significant differences in SMR based on formula type at this time-point, however consistent with the literature, SMR differed by sex with males having higher SMR than females at both study visits.[26] This significance remained after adjusting for FFM. Given there were no differences in SMR by formula groups at baseline and 3.5 months, it appears that SMR does not differ by infant formula composition.

The final aim of this research was to determine which of the empiric equations available for the calculation of BMR in infants, is most accurate. While it is well-understood that SMR measured via indirect calorimetry is the preferred practice for determining metabolic rate in clinical and research settings, predictive equations are frequently used clinically, as many facilities to not have access to an indirect calorimeter or do not use it regularly for clinical care.[14] Over the past several decades, clinicians have used the WHO and Schofield equations to calculate BMR in infants and children. In an effort to increase accuracy of predictive equations, Henry et al., developed critical criteria for studies to be included in the development of a new equation, the Oxford equations.[18] The validity and accuracy of the Oxford equations have not been extensively tested. To our knowledge, this is the first study to test the accuracy of the Oxford equations against measured SMR in young formula-fed infants.

Initially, we tested each equation by age group. At 0.75 months, we found that SCHwo (Schofield weight only) performed best for at the individual level at 0.75-months; it had the highest R^2, suggesting good individual level agreement. The WHO

(World Health Organization) and OXwo (Oxford weight only) equations had the next highest R^2. At the group level, the OXwo calculated SMR within 98% of measured SMR. At 3.5-month, the SCHwo and OXwo performed best at the individual level. At the group level the SCHwh and WHO performed best. Since clinicians aim to calculate energy needs of individual patients, these analyses suggest the SCHwo is most accurate for estimating energy needs of 0.75 and 3.5 month old infants. These analyses will be repeated when the data set is complete.

Given that all of these equations are for children zero to three years of age, and infants in our study were 0.75 to 3.5 months of age, we then combined measures from both time points (n=185 data points) and again tested each equation. When using all SMR measures for both time-points, SCHwo (Schofield weight only) performed best for group and individual agreement, with the OXwo having the next best group and individual level agreement. The WHO equations were also very strong at an individual level as well, however were not as accurate at the group level.

It is important to remember many of the predictive equations were created to predict mean BMR in a population, not at an individual level. Additionally, there is current debate in the literature with some researchers/clinicians favoring the creation of new predictive equations for infants.[13,27,28] Many researchers claim these equations fail to produce consistently accurate energy expenditure predictions in healthy infants and children. Traditionally, one equation was created for 0-3 years old. Reichman et al suggested the creation of new equations for those 0-1 years old given that infancy is

such a unique period of growth.[13] Others have suggested race specific equations as well.[28,63] However, our results do not suggest race is a factor at this young age.

A strength of our study include the large sample size and racial diversity of infants less than one month of age. Due to the challenges of measuring metabolic rate in such a young infant population, many studies reported successful SMR measurements utilizing smaller sample sizes. A limitation of this study is the preliminary nature of the data. Many of the subjects had a completed SMR measure, however data on fat free mass (FFM) as determined by hydrometry, was the incomplete. As such we used multiple linear regression imputation, to estimate FFM for those with incomplete body composition data. We did examine for differences between measured versus imputed FFM at each time point, and found no significant differences, however these analyses will be repeated in their entirety with all subjects having a their measured, versus imputed FFM data. Lastly, the present study also has SMR measures when subjects are 12.5 months of age and these data are forthcoming. These data will be included in the repeat analysis as well provide SMR measures throughout the first year of life (0.75, 3.5, and 12.5) that can be used to test the accuracy of predictive equations.

Chapter 7

CONCLUSION

Early infancy (0-6 months) is a unique and sensitive period in development; infant feeding practices can have a significant and lasting impact on health. Infants with rapid or accelerated weight gain in infancy are more likely to develop overweight or obesity later in life.[6-8] Research has shown that diet composition in infancy is a factor that affects weight gain trajectory. Infants who are exclusively breast fed (BF) exhibit less rapid weight gain and thus are at lower risk for overweight and obesity in adulthood compared to their formula fed (FF) counterparts.[5] However, FF infants are not a homogenous group. Studies have shown a difference in weight gain among FF infants based on the type, and more specifically protein composition, in various formulas.[11] Infants fed cow's milk formula (CMF), the most commonly consumed infant formula, which contains intact proteins were found to experience accelerated weight gain, while infants receiving a formula with extensively hydrolyzed forms of proteins (EHF), gained weight more similarly to BF infants.

These findings have significant public health impact, given that nearly 60 % of infants in the United States receive infant formula by 3 months of age, either exclusively or in combination with breast milk feeding.[1,4] Therefore, it is important to understand the effect of the different types of infant formula on energy balance and weight gain.

In the present study we sought to determine if an increase in metabolic rate was a potential mechanism by which infants feeding EHF gain less weight. Studies have demonstrated differences in sleeping metabolic rate (SMR) between BF and FF infants.[26,27] The present study was the first to explore SMR between two groups of FF

infants, receiving formula that differed in protein structure and content. We hypothesized that infants consuming EHF would have a greater SMR than infants who fed CMF, however these preliminary data suggest SMR does not differ between infants fed CMF versus EHF.

Additionally, while indirect calorimetry is the widely recognized gold standard for measuring SMR in both clinical and research settings, it is common in practice due to feasibility and cost.[7,14] For the past several decades the WHO/FAO/UNU and Schofield Predictive equations have been used to estimate basal metabolic rate in infants, and more recently the Oxford equations have been used as well.[15,16,18] However, there is debate over the true accuracy of any of these equations for birth to one year-old infants, given the age range for all equations is birth to three years of age. The results to date found that the Schofield weight only (SCHwo) equations performed very well at the individual level for the estimation of metabolic rate.

Though preliminary, our results suggest that SMR is not influenced by diet composition of formula at this age. Additionally, the results provide support for the continued use of the Schofield weight only equation for the calculation of metabolic rate in this age group. Further analysis utilizing the full data set (0.75-12.5 months) is needed to better understand the effect of formulas with differing protein content and structure on SMR, as well as to confirm the findings regarding accuracy of predictive equations for this unique period of life.

REFERENCES

1. Centers for Disease Control and Prevention. Breastfeeding report card. http://www.cdc.gov/breastfeeding/pdf/2014breastfeedingreportcard.pdf. Published August 25, 2015. Updated 2015. Accessed January, 2016.

2. Kleinman RE. American academy of pediatrics recommendations for complementary feeding. *Pediatrics*. 2000;106(5):1274.

3. World Health Organization. The world health organization's infant feeding recommendation. Nutrition Web site. http://www.who.int/nutrition/topics/infantfeeding_recommendation/en/. Updated 2001. Accessed January, 2016.

4. Section on Breastfeeding. Breastfeeding and the use of human milk. *Pediatrics*. 2012;129(3):e827-41.

5. Dewey KG, Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B. Breast-fed infants are leaner than formula-fed infants at 1 y of age: The DARLING study. *Am J Clin Nutr*. 1993;57(2):140-145.

6. Gungor DE, Paul IM, Birch LL, Bartok CJ. Risky vs rapid growth in infancy: Refining pediatric screening for childhood overweight. *Arch Pediatr Adolesc Med*. 2010;164(12):1091-1097.

7. Ong KK, Emmett P, Northstone K, et al. Infancy weight gain predicts childhood body fat and age at menarche in girls. *J Clin Endocrinol Metab*. 2009;94(5):1527-1532.

8. Stettler N, Kumanyika SK, Katz SH, Zemel BS, Stallings VA. Rapid weight gain during infancy and obesity in young adulthood in a cohort of african americans. *Am J Clin Nutr*. 2003;77(6):1374-1378.

9. Enfamil infant. Enfamil Web site. http://www.enfamil.com/products/routine-feeding/enfamil-infant. Published 4/23/14. Updated 2014. Accessed January, 2016.

10. Nutramigen with enflora LGG. Enfamil Web site. http://www.enfamil.com/products/solutions-feeding-issues/nutramigen-enflora-lgg. Accessed January, 2016.

11. Mennella JA, Ventura AK, Beauchamp GK. Differential growth patterns among healthy infants fed protein hydrolysate or cow-milk formulas. *Pediatrics*. 2011;127(1):110-118.

12. Fullmer S, Benson-Davies S, Earthman CP, et al. Evidence analysis library review of best practices for performing indirect calorimetry in healthy and Non–Critically ill individuals. *Journal of the Academy of Nutrition and Dietetics*. 2015;115(9):1417-1446. e2.

13. Reichman CA, Shepherd RW, Trocki O, Cleghorn GJ, Davies PS. Comparison of measured sleeping metabolic rate and predicted basal metabolic rate during the first year of life: Evidence of a bias changing with increasing metabolic rate. *Eur J Clin Nutr*. 2002;56(7):650-655.

14. Mahan LK, Escott-Stump S, Raymond JL. *Krause's food & the nutrition care process*. Elsevier Health Sciences; 2012.

15. Joint F, World Health Organization. Energy and protein requirements: Report of a joint FA. . 1985.

16. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr Clin Nutr*. 1985;39 Suppl 1:5-41.

17. Wells JC, Davies PS. Sleeping metabolic rate and body size in 12-week-old infants. *Eur J Clin Nutr*. 1995;49(5):323-328.

18. Henry C. Basal metabolic rate studies in humans: Measurement and development of new equations. *Public Health Nutr*. 2005;8(7a):1133-1152.

19. Schoeller DA, van Santen E. Measurement of energy expenditure in humans by doubly labeled water method. *J Appl Physiol Respir Environ Exerc Physiol*. 1982;53(4):955-959.

20. Jensen CL, Butte NF, Wong WW, Moon JK. Determining energy expenditure in preterm infants: Comparison of 2H(2)18O method and indirect calorimetry. *Am J Physiol*. 1992;263(3 Pt 2):R685-92.

21. Blanc S, Schoeller DA, Bauer D, et al. Energy requirements in the eighth decade of life. *Am J Clin Nutr*. 2004;79(2):303-310.

22. McArdle WD, Katch FI, Katch VL. *Essentials of exercise physiology*. Lippincott Williams & Wilkins; 2006.

23. Institute of Medicine (US). Panel on Dietary Reference Intakes for Electrolytes, Water. *DRI, dietary reference intakes for water, potassium, sodium, chloride, and sulfate*. National Academy Press; 2005.

24. Garby L, Astrup A. The relationship between the respiratory quotient and the energy equivalent of oxygen during simultaneous glucose and lipid oxidation and lipogenesis. *Acta Physiol Scand*. 1987;129(3):443-444.

25. WEIR JB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol*. 1949;109(1-2):1-9.

26. Harris JA, Benedict FG. A biometric study of human basal metabolism. *Proc Natl Acad Sci U S A*. 1918;4(12):370-373.

27. Wells JC, Joughin C, Crisp JA, Cole TJ, Davies PS. Comparison of measured sleeping metabolic rate and predicted basal metabolic rate in the first year of life. *Acta Paediatr*. 1996;85(9):1013-1018.

28. Thomson M, Bucolo S, Quirk P, Shepherd R. Measured versus predicted resting energy expenditure in infants: A need for reappraisal. *J Pediatr*. 1995;126(1):21-27.

29. Holliday MA. Metabolic rate and organ size during growth from infancy to maturity and during late gastation and early infancy. *Pediatrics*. 1971;47(1):Suppl 2:169+.

30. Ravussin E, Bogardus C. Relationship of genetics, age, and physical fitness to daily energy expenditure and fuel utilization. *Am J Clin Nutr*. 1989;49(5 Suppl):968-975.

31. Cunningham JJ. Body composition as a determinant of energy expenditure: A synthetic review and a proposed general prediction equation. *Am J Clin Nutr*. 1991;54(6):963-969.

32. Keys A, Taylor HL, Grande F. Basal metabolism and age of adult man. *Metab Clin Exp*. 1973;22(4):579-587.

33. Butte NF, Wong WW, Hopkinson JM, Heinz CJ, Mehta NR, Smith EO. Energy requirements derived from total energy expenditure and energy deposition during the first 2 y of life. *Am J Clin Nutr*. 2000;72(6):1558-1569.

34 Haisma H, Wells JC, Coward WA, et al. Complementary feeding with cow's milk alters sleeping metabolic rate in breast-fed infants. *J Nutr*. 2005;135(8):1889-1895.

35. Bisdee J, James W, Shaw M. Changes in energy expenditure during the menstrual cycle. *Br J Nutr*. 1989;61(02):187-199.

36. Heymsfield SB, Gallagher D, Poehlman ET, et al. Menopausal changes in body composition and energy expenditure. *Exp Gerontol*. 1994;29(3-4):377-389.

37. Solomon SJ, Kurzer MS, Calloway DH. Menstrual cycle and basal metabolic rate in women. *Am J Clin Nutr*. 1982;36(4):611-616.

38. Goran MI, Poehlman ET, Johnson RK. Energy requirements across the life span: New findings based on measurement of total energy expenditure with doubly labeled water. *Nutr Res*. 1995;15(1):115-150.

39. Tershakovec AM, Kuppler KM, Zemel B, Stallings VA. Age, sex, ethnicity, body composition, and resting energy expenditure of obese african american and Caucasian children and adolescents. *Am J Clin Nutr*. 2002;75(5):867-871.

40. Hull D, McArthur AJ, Pritchard K, Goodall M. Metabolic rate of sleeping infants. *Arch Dis Child*. 1996;75(4):282-287.

41. Albu J, Shur M, Curi M, Murphy L, Heymsfield SB, Pi-Sunyer FX. Resting metabolic rate in obese, premenopausal African American women. *Am J Clin Nutr*. 1997;66(3):531-538.

42. Carpenter WH, Fonong T, Toth MJ, et al. Total daily energy expenditure in free-living older african-americans and caucasians. *Am J Physiol*. 1998;274(1 Pt 1):E96-101.

43. Kaplan AS, Zemel BS, Neiswender KM, Stallings VA. Resting energy expenditure in clinical pediatrics: Measured versus prediction equations. *J Pediatr*. 1995;127(2):200-205.

44. Treuth MS, Butte NF, Wong WW. Effects of familial predisposition to obesity on energy expenditure in multiethnic prepubertal girls. *Am J Clin Nutr*. 2000;71(4):893-900.

45. Weyer C, Snitker S, Rising R, Bogardus C, Ravussin E. Determinants of energy expenditure and fuel utilization in man: Effects of body composition, age, sex, ethnicity and glucose tolerance in 916 subjects. *Int J Obes*. 1999;23(7):715-722.

46. Trowbridge CA, Gower BA, Nagy TR, Hunter GR, Treuth MS, Goran MI. Maximal aerobic capacity in african-american and caucasian prepubertal children. *Am J Physiol*. 1997;273(4 Pt 1):E809-14.

47. 3. Fontvieille AM, Dwyer J, Ravussin E. Resting metabolic rate and body composition of pima indian and caucasian children. *Int J Obes Relat Metab Disord*. 1992;16(8):535-542.

48. Goran MI, Kaskoun M, Johnson R. Determinants of resting energy expenditure in young children. *J Pediatr*. 1994;125(3):362-367.

49. Mennella JA, Beauchamp GK. Developmental changes in the acceptance of protein hydrolysate formula. *Journal of Developmental & Behavioral Pediatrics*. 1996;17(6):386-391.

50. Mennella JA, Forestell CA, Morgan LK, Beauchamp GK. Early milk feeding influences taste acceptance and liking during infancy. *Am J Clin Nutr*. 2009;90(3):780S-788S.

51. Mihatsch W, Högel J, Pohlandt F. Hydrolysed protein accelerates the gastrointestinal transport of formula in preterm infants. *Acta Paediatrica*. 2001;90(2):196-198.

52. Mennella JA, Beauchamp GK. Developmental changes in the acceptance of protein hydrolysate formula. *Journal of Developmental & Behavioral Pediatrics*. 1996;17(6):386-391.

53. Diepvens K, Häberer D, Westerterp-Plantenga M. Different proteins and biopeptides differently affect satiety and anorexigenic/orexigenic hormones in healthy humans. *Int J Obes*. 2008;32(3):510-518.

54. Foltz M, Ansems P, Schwarz J, Tasker MC, Lourbakos A, Gerhardt CC. Protein hydrolysates induce CCK release from enteroendocrine cells and act as partial agonists of the CCK1 receptor. *J Agric Food Chem*. 2008;56(3):837-843.

55. Choi S, Lee M, Shiu AL, Yo SJ, Aponte GW. Identification of a protein hydrolysate responsive G protein-coupled receptor in enterocytes. *Am J Physiol Gastrointest Liver Physiol*. 2007;292(1):G98-G112.

56. Kondoh T, Torii K. MSG intake suppresses weight gain, fat deposition, and plasma leptin levels in male Sprague–Dawley rats. *Physiol Behav*. 2008;95(1):135-144.

57. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: Systematic review of size and growth in infancy and later obesity. *BMJ*. 2005;331(7522):929.

58. Chomtho S, Wells JC, Davies PS, Lucas A, Fewtrell MS. Early growth and body composition in infancy. In: *Early nutrition programming and health outcomes in later life*. Springer; 2009:165-168.

59. Ekelund U, Ong KK, Linné Y, et al. Association of weight gain in infancy and early childhood with metabolic risk in young adults. *The Journal of Clinical Endocrinology & Metabolism*. 2007;92(1):98-103.

60. Gluckman P, Hanson M. Developmental and epigenetic pathways to obesity: An evolutionary-developmental perspective. *Int J Obes*. 2008;32:S62-S71.

61. Lohman TG. Assessment of body composition in children. *Pediatr Exerc Sci*. 1989;1(1):19-30.

62. WHO Multicentre Growth Reference Study Group. Assessment of differences in linear growth among populations in the WHO multicentre growth reference study. *Acta Paediatr Suppl*. 2006;450:56-65.

63. Carpenter A, Pencharz P, Mouzaki M. Accurate estimation of energy requirements of young patients. *J Pediatr Gastroenterol Nutr*. 2015;60(1):4-10.

64. Ventura AK, Inamdar LB, Mennella JA. Consistency in infants' behavioural signalling of satiation during bottle-feeding. *Pediatr Obes*. 2015;10(3):180-187.

Appendix A

TABLES

Table A.1. - Macronutrient composition of study formulas

	CMF	EHF
Total calories (kcal/100 ml)	67.7	67.7
Carbohydrates (g/100 kcal)	7.4	7.0
Fat (g/100 kcal)	3.6	3.6
Protein or protein equivalent (g/100 ml)	1.4	1.9
Essential FAA: (umol/L)		
Histidine	9	1880
Isoleucine	tr	5327
Leucine	nd	11886
Lysine	22	8254
Methionine	nd	2854
Phenylalanine	11	4283
Threonine	5	4653
Tryptophan	nd	1348
Valine	10	7038
Semi-essential FAA:		
Arginine	10	3489
Cystine	nd	542
Taurine	529	496
Tyrosine	9	1170
Nonessential FAA:		
Alanine	31	4905
Asparagine	tr	3705
Aspartic acid	9	1535
Glutamic acid	109	7472
Glutamine	nd	nd
Glycine	38	1658
Proline	63	2667
Serine	9	5213
Total FAAs	864	80375

Tr: trace amount; nd: not detected; CMF, cow milk formula; EHF, extensively hydrolyzed formula. Values from Ventura et al. 2012[64].

Table A.2a - Demographic characteristics at 0.75 month visit (baseline, N=113) by eventual randomization group

	CMF group N=59	**EHF group N=54**	**p-value**
Infant characteristics			
Sex, male, % (n)	53 (31)	47 (28)	0.507
Age, months, mean ± SEM	0.65±0.01	0.63±0.01	0.329
Racial category, % (n)			0.141
African American	59 (35)	59 (35)	
Caucasian	29 (17)	15 (8)	
Other/mixed	12 (7)	20 (11)	
Ethnicity, %(n)			0.291
Hispanic	8 (5)	15 (8)	
Not Hispanic	92 (54)	85 (46)	
Weight, kg, mean± SEM	3.70±0.06	3.73±0.05	0.716
Length, cm, mean± SEM	51.80±0.30	51.47±0.28	0.424
Infant Z-scores			
WAZ, mean ± SEM	-0.41±0.11	-0.27±0.10	0.378
LAZ, mean ± SEM	-0.56±0.13	-0.58±0.14	0.891
WLZ, mean ± SEM	-0.12±0.11	0.12±0.99	0.162
BMIZ, mean ± SEM	-0.16±0.10	0.06±0.10	0.126

CMF=Cow Milk Formula, EHF=Extensively Hydrolyzed Formula, WAZ= weight-for-age z-score, LAZ= length-for age z-score, WLZ= weight-for-length z-score, BMIZ= body mass index z-score.

An independent t-test (continuous variables) or Chi-square test (categorical variables) was used to determine baseline differences in infant characteristics between eventual randomization groups. There were no significant differences between groups for any of the above variables.

NOTE: Data are preliminary, additional data are forthcoming.

Table A.2b - Demographic characteristics of infants with successful SMR measurements at 0.75-month visit (N=102) by eventual randomization group

	CMF group N=54	**EHF group N=48**	**P-value**
Infant characteristics			
Sex, male, % (n)	54 (29)	48 (23)	0.559
Age, months, mean ± SEM	0.66±0.01	0.64±0.01	0.336
Racial category, % (n)			0.243
African American	59 (32)	67 (32)	
Caucasian	30 (16)	12 (6)	
Other/mixed	11 (6)	21(10)	
Ethnicity, % (n)			0.241
Hispanic	8 (4)	15 (7)	
Not Hispanic	92 (50)	85 (41)	
Weight, kg, mean± SEM	3.76±0.05	3.74±0.06	0.807
Length, kg, mean± SEM	51.9±0.29	51.5±0.29	0.377
Infant Z-scores			
WAZ, mean ± SEM	-0.03±0.10	-0.27±0.11	0.859
LAZ, mean ± SEM	-0.48±0.13	-0.55 ±0.16	0.742
WLZ, mean ± SEM	-0.03±0.16	0.08±0.14	0.494
BMIZ, mean ± SEM	-0.05±0.10	-0.04±0.11	0.495

CMF=Cow Milk Formula, EHF=Extensively Hydrolyzed Formula, WAZ= weight-for-age z-score, LAZ= length-for age z-score, WLZ= weight-for-length z-score, BMIZ= body mass index z-score.
An independent t-test (continuous variables) or Chi-square test (categorical variables) was used to determine differences in infant characteristics between eventual randomization groups.
NOTE: Data are preliminary, additional data are forthcoming.

Table A.2c - Demographic characteristics of infants with successful SMR measurements at 3.5 month visit (N=83) by randomization group

	CMF group N=43	**EHF group N=40**	**P-value**
Infant characteristics			
Sex, male, % (n)	60 (26)	50 (20)	0.337
Age, months, mean ± SEM	3.52±0.02	3.54±0.02	0.576
Racial category, % (n)			0.306
African American	63 (27)	65 (26)	
Caucasian	21 (9)	10 (4)	
Other/mixed	16 (7)	25 (10)	
Ethnicity, % (n)			0.166
Hispanic	9 (4)	20 (8)	
Not Hispanic	91 (39)	80 (32)	
Weight, kg, mean± SEM	6.46±0.11	6.00±0.14	0.012
Length, kg, mean± SEM	61.9±0.69	61.3±0.46	0.459
Infant Z-scores at 3.5-month visit			
WAZ, mean ± SEM	-0.09±0.13	-0.66±0.17	0.0088
LAZ, mean ± SEM	-0.39±0.15	-0.36 ±0.20	0.921
WLZ, mean ± SEM	0.31±0.14	-0.48±0.16	0.0004
BMIZ, mean ± SEM	0.17±0.13	-0.63±0.16	0.0002

CMF=Cow Milk Formula, EHF=Extensively Hydrolyzed Formula, WAZ= weight-for-age z-score, LAZ= length-for age z-score, WLZ= weight-for-length z-score, BMIZ= body mass index z-score.
An independent t-test (continuous variables) or Chi-square test (categorical variables) was used to determine differences in infant characteristics between eventual randomization groups.
Data are preliminary, additional data are forthcoming.

Table A.3 - Count of FFM derived from TBW or derived by multiple linear regression imputation at 0.75 and 3.5 months by formula group

Age	Formula Group	**FFM from TBW**	**Imputed FFM**	**p-value**
0.75 months	CMF, n	40	14	0.208[a]
	EHF, n	30	18	
	FFM, kg, mean ± SEM	3.40 ± 0.06	3.42±0.06	0.806[b]
3.5 months	CMF, n	36	7	0.028[a,c]
	EHF, n	25	15	
	FFM, kg, mean ± SEM	5.15±0.10	4.95±0.08	0.156[b]

CMF= Cow's Milk Formula, EHF= Extensively Hydrolyzed Formula, FFM= Fat free mass, TBW= Total body water.
[a] chi-squared statistic
[b] independent samples t-test comparing mean FFM from TBW to mean imputed FFM
[d] significant difference in proportion of subjects that required FFM imputation by formula group, however awaiting data on seven additional subjects at 3.5 months time point.
NOTE: Data are preliminary, additional data are forthcoming.

Table A.4 - Sleeping Metabolic Rate adjusted for Fat Free Mass and Fat Free Mass at 0.75 and 3.5 months

Measure	Formula Group	0.75 months[a] (LSM ± SEM)	(p-value)	3.5 months[b] (LSM ± SEM)	(p-value)
SMR (kcal/d)	CMF	205.5 (3.28)	0.148[c]	325.40 (14.09)	0.880[c]
	EHF	198.5 (3.48)		323.18 (21.15)	
FFM (kg)	CMF	3.40 (0.06)	0.806[d]	5.15 (0.10)	0.156[d]
	EHF	3.42 (0.06)		4.95 (0.08)	

SMR= Sleep metabolic rate, CMF= Cow's Milk Formula, EHF= Extensively Hydrolyzed Formula
LSM=least square mean, SEM= standard error of the mean, FFM= Fat free mass,
[a]CMF: n=54, 54% male, 59%African American; EHF: n=48 48% male, 67% African American
[b]CMF: n=43, 60% male, 63%African American; EHF: n=40 50% male, 65% African American
[c]ANCOVA for SMR adjusted for FFM between formula type at each time-point
[d]Independent samples t-test for FFM between formula type at each time-point
NOTE: Data are preliminary, additional data are forthcoming.

Table A.5a - Factors predictive of SMR at 0.75 month (N=102)

Term	Estimate	Standard Error	t Ratio	Prob >|t|
Model 1				
Intercept	121.57	17.00	7.15	<0.0001
Sex	7.05	2.37	2.98	0.0037
FFM	23.60	4.94	4.78	<0.0001
Model 2				
Intercept	115.55	18.35	6.29	<0.0001
Race (2-1)[a]	-5.83	6.03	-0.97	0.336
Race (3-2)[b]	6.63	6.80	0.97	0.332
FFM	26.44	5.04	5.24	<0.0001

FFM= Fat free mass

Multiple linear regression models were used to determine if SMR differs by race and sex; FFM was included in the model as a covariate.

Model 1: R^2=0.28, p=<0.0001

Model 2: R^2=0.23, p<0.0001

[a] Race (African American – Caucasian)

[b] Race (Mixed/other – African American)

NOTE: Data are preliminary, additional data are forthcoming.

Table A.5b - Factors predictive of SMR at 3.5 month (N=83)

Term	Estimate	Standard Error	t Ratio	Prob >|t|
Model 1				
Intercept	186.60	39.55	4.72	<0.0001
Sex	9.63	4.97	1.94	0.05
FFM	27.13	7.80	3.48	0.0008
Model 2				
Intercept	167.24	40.48	4.13	<.0.0001
Race[a]	-6.08	13.52	-0.45	0.654
Race[b]	10.48	12.18	0.86	0.391
FFM	31.76	7.57	4.19	<0.0001
Model 3				
Intercept	171.84	40.49	4.24	<0.0001
Formula Group[c]	0.39	4.73	0.08	0.933
Sex	9.15	4.95	1.85	0.05
Formula x Sex[d]	-8.35	4.82	-1.73	0.08
FFM	30.24	8.01	3.77	0.0003

FFM= Fat free mass
Multiple linear regression models were used to determine if SMR differs by race and sex; FFM was included in the model as a covariate.
Model 1: R^2= 0.21, p=<0.0001
Model 2: R^2=0.19, p=0.0007
Model 3: R^2=0.24, p=0.0002
[a] Race (African American – Caucasian)
[b] Race (Mixed/other – African American)
[c] Enfamil= formula reference group
[d] Formula group by sex interaction
NOTE: Data are preliminary, additional data are forthcoming.

Table A.6 - List of Equations for Estimating Basal Metabolic Rate

Equation	Reference	Formula
BMR	FAO/WHO/ UNU	**BMR: Children under 3 years (kcal/d)** Male: 60.9wt (kg) -54 Female: 61.0wt (kg)-51
BMR	Schofield-wo	**BMR: Children under 3 years (kcal/d)** Male: 0.249 wt (kg) – 0.127 Female: 0.244 wt (kg) – 0.130
BMR	Schofield-wh	**BMR: Children under 3 years (kcal/d)** Male: 0.0007 wt (kg) + 6.349 ht (cm) - 2.584 Female: 0.068 wt (kg) + 4.281 ht (cm) – 1.730
BMR	Oxford-wo	**BMR: Children under 3 years (kcal/d)** Male: 0.255 wt (kg) – 0.141 Female: 0.246 wt (kg) – 0.0965
BMR	Oxford-wh	**BMR: Children under 3 years (kcal/d)** Male: 0.118 wt (kg) + 3.59 ht (cm) – 1.55 Female: 0.127 wt (kg) + 2.941 ht (cm) – 1.20

BMR – Basal Metabolic Rate FAO-Food and Agriculture Organization of the United Nations, WHO- World Health Organization, UNU- United Nations University, kcal/d- kilocalories per day, wt- weight, ht- height, wo- weight only used in equation, wh- weight and height used in equation, kg- kilograms, cm- centimeters

Table A.7a - Evaluation of equations for calculating BMR compared to measured SMR at 0.75 time point (n=102)

Predictive equations for calculating BMR in healthy children	Calculated BMR kcal/day Mean±SD *(range)*	Difference Calculated BMR-Measured SMR *kcal/d*	%SMR predicted	R^2 (RMSE)
WHO	176±25 *(120-236)*	-25	87	0.35 (22.12)
SCHwo	190±25 *(140-253)*	-11	94	0.40 (21.32)
SCHwh	172±30 *(106-293)*	-29	86	0.21(24.43)
OXwo	196±25 *(140-257)*	-5	98	0.35 (22.08)
OXwh	185±27 *(129-278)*	-16	92	0.25 (23.68)

WHO= World Health Organization, SCHwo= Schofield weight only, SCHwh= Schofield weight and height, OXwo= Oxford weight only, OXwh= Oxford weight and height, RMSE= root mean square error

Criteria for formula evaluation and model comparison included the difference of calculated BMR minus measured SMR and percentage calculated TER relative to measured SMR (group level agreement), and RMSE, Wald-statistic P value, and squared multiple coefficient of determination (R2) (individual level agreement). At the group level, the OXwo equation calculated energy requirements closest to actual SMR. At the individual level, regression of measured SMR onto the calculated BMR for each equation, the SCHwo yielded the lowest RMSE and the highest R2.

NOTE: Data are preliminary, additional data are forthcoming.

Table A.7b - Evaluation of predictive BMR equations compared to measured SMR at 3.5 time point (n=83)

Predictive equations for calculating BMR in healthy children	Calculated BMR kcal/day Mean±SD *(range)*	Difference Calculated BMR-Measured SMR *kcal/d*	%SMR predicted	R^2 (RMSE)
WHO	327±50 *(242-490)*	3	102	0.34 (38.0)
SCHwo	337±50 *(249-501)*	12	105	0.35 (37.7)
SCHwh	318±55 *(239-681)*	-6	99	0.16 (42.9)
OXwo	345±51 *(259-510)*	21	108	0.35 (37.9)
OXwh	335±49 *(255-576)*	10	104	0.27 (40.0)

WHO= World Health Organization, SCHwo= Schofield weight only, SCHwh= Schofield weight and height, OXwo= Oxford weight only, OXwh= Oxford weight and height RMSE= root mean square error

Criteria for formula evaluation and model comparison included the difference of calculated BMR minus measured SMR and percentage calculated TER relative to measured SMR (group level agreement), and RMSE, Wald-statistic P value, and squared multiple coefficient of determination (R2) (individual level agreement).

At the group level, the SCHwh equation calculated energy requirements closest to actual SMR. At the individual level, regression of measured SMR onto the calculated BMR for each equation, SCHwo and OXwo both yielded the highest R^2, but the SCHwo had the lowest RMSE.

NOTE: Data are preliminary, additional data are forthcoming.

Table A.7c - Overall evaluation of predictive equations compared to measured SMR with both 0.75 and 3.5 month time points (n=185)

Predictive equations for calculating BMR in healthy children	Calculated BMR kcal/day ±*SD (range)*	Difference Calculated BMR-Measured SMR (*kcal/d)*	%SMR predicted	R^2 (RMSE)
WHO	244±84 *(120-490)*	-12	94	0.80 (31.78)
SCHwo	256±82 *(140-500)*	-0.8	99	0.80 (31.45)
SCHwh	238±84 *(106-680)*	-19	92	0.71 (38.66)
OXwo	263±83 *(140-510)*	6	102	0.80 (31.79)
OXwh	252±83 *(129-575)*	-4	97	0.77 (34.07)

WHO= World Health Organization, SCHwo= Schofield weight only, SCHwh= Schofield weight and height, OXwo= Oxford weight only, OXwh= Oxford weight and height RMSE= root mean square error

Criteria for formula evaluation and model comparison included the difference of calculated BMR minus measured SMR and percentage calculated TER relative to measured SMR (group level agreement), and RMSE, Wald-statistic P value, and squared multiple coefficient of determination (R^2) (individual level agreement).

At the group level, the SCHwo equation calculated energy requirements closest to actual SMR. At the individual level, regression of measured SMR onto the calculated BMR for each equation, SCHwo had the highest R^2 and lowest RMSE.

NOTE: Data are preliminary, additional data are forthcoming.

Appendix B

FIGURES

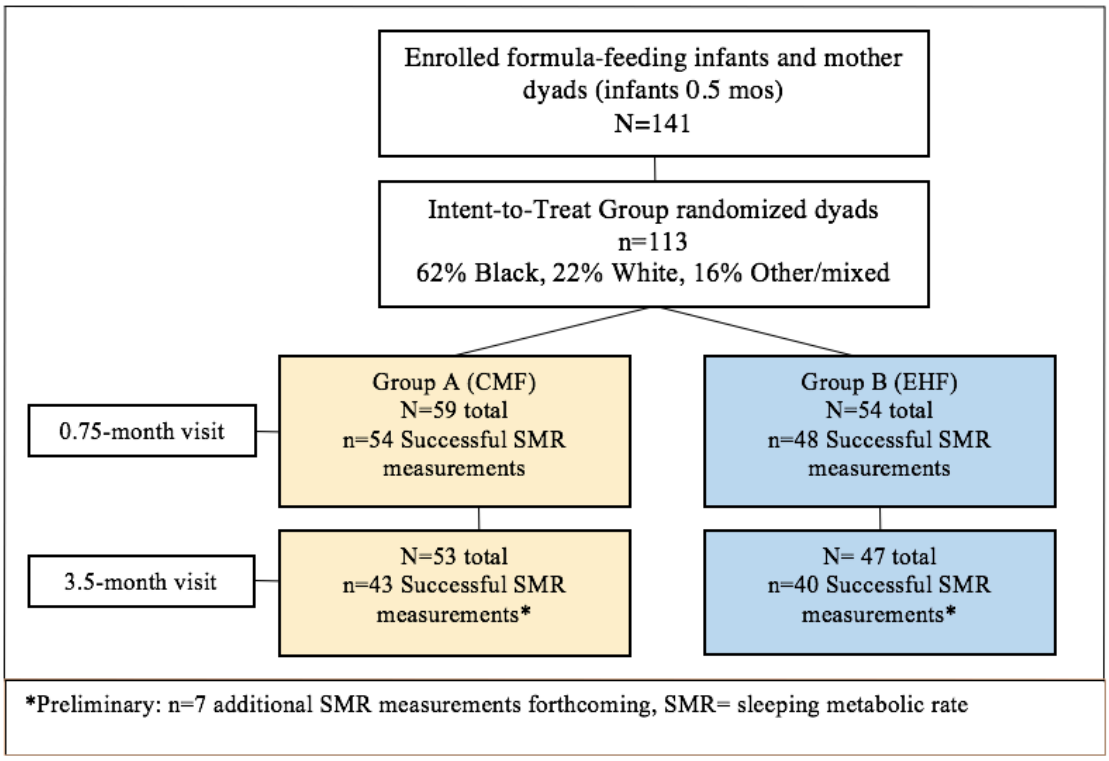

Figure B.1 - Intent to Treat Profile. Participant Flow from enrollment through 3.5-month visit

Appendix C

STUDY VISIT DOCUMENTS

A.1 Institutional Review Board Approval Letter

Approval Letter 2016

UNIVERSITY OF DELAWARE
RESEARCH OFFICE

210 Hullihen Hall
University of Delaware
Newark, Delaware 19716-1551
Ph: 302/831-2136
Fax: 302/831-2828

DATE: December 21, 2015

TO: Jillian Trabulsi, PhD
FROM: University of Delaware IRB

STUDY TITLE: [336722-7] Impact of diet composition on energy balance and satiety during infancy

SUBMISSION TYPE: Continuing Review/Progress Report

ACTION: APPROVED
APPROVAL DATE: December 21, 2015
EXPIRATION DATE: November 8, 2016
REVIEW TYPE: Administrative Review

Thank you for your submission of Continuing Review/Progress Report materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Administrative Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office.

Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Nicole Farnese-McFarlane at (302) 831-1119 or nicolefm@udel.edu. Please include your study title and reference number in all correspondence with this office.