The Effects of Feeding Style on Subcutaneous Adipose Tissue Deposition within the First Year of Life

Meriah Schoen

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ABSTRACT
The Effects of Feeding Style on Subcutaneous Adipose Tissue Deposition within the First Year of Life

**Background:** Fat distribution, rather than total body fat, has been identified as a significant risk factor for chronic disease. Patterning of subcutaneous fat, in particular, may play a pervasive role in shaping the metabolic milieu that is critical for disease development. Several studies have shown that early-life nutrition may influence later body composition. The effect of breastfeeding and formula feeding on early patterns of subcutaneous fat deposition, however, are uncertain.

**Objective:** At a time when early infant growth is emerging as a predictor for later chronic disease, it is the aim of the present analysis to investigate whether feeding style (breastfeeding versus formula feeding) modifies subcutaneous fat growth rates and trajectories in the first year of life with a focus on the historical iterations of WHO infant feeding recommendations (0 to 4 months, 4 to 6 months, and 6 to 12 months of age).

**Methods:** This is an ex post-facto design that utilizes data collected as part of a longitudinal growth study in the first year of life. Subcutaneous fat mass was anthropometrically assessed weekly by skinfold thickness (triceps, calf, subscapular, suprailiac, midaxillary, and abdominal) in 21 infants. Feeding data were collected through daily parental records and are entered here as a categorical variable (predominantly breast fed and predominantly formula fed). Multi-level mixed effects models for repeated measures were used (STATA 14) adjusting for age, sex, weight, birthweight, and number of feeding episodes per day. Statistical significance was accepted at $p<0.05$ and trends were represented between $p=0.05$ and $p=0.10$.

**Results:** Infants experienced fat accretion only during the first four months, and this was limited to peripheral skinfolds. Thereafter, subcutaneous skinfolds followed a trend of declining rates. Breastfed and formula fed infants, however, demonstrated different patterns of subcutaneous fat deposition in both the sum of skinfolds and in each skinfold site. During the first four months, formula fed infants experienced greater rates for the subscapular, abdominal, suprailiac, trunk, quadriceps, sum of skinfolds ($p<0.05$), and triceps ($p=0.066$) skinfolds. Between four and six months of age, formula fed infants followed more negative slopes than breastfed infants in all but the lower limb and suprailiac skinfolds. Beyond six months, formula fed infants had more negative slopes in the suprailiac ($p<0.01$) and midaxillary ($p=0.10$) skinfolds, while breastfed infants had more negative slopes in the calf ($p<0.05$), abdominal ($p=0.062$), and limb skinfolds ($p=0.092$). These time-constrained slope comparisons actually reflect different overall deposition trajectories by feeding style. By comparison with growth rate in the first four months of life, formula fed infants experience a more dramatic decline in rate in the subsequent four to six-month interval in the trunk skinfolds ($p<0.01$) than their breastfed peers. Beyond six months, breastfed infants subsequently demonstrate a more dramatic decline in rate in the trunk skinfolds ($p<0.01$).

**Conclusion:** Weekly skinfold assessments of seven subcutaneous sites have identified that feeding style predicts differences in deposition patterns in the first year of life. Breastfed infants demonstrated both slower rates of accretion and decline by comparison with their formula fed peers. This analysis further suggests that the first four months may be a critical period for subcutaneous fat deposition. Feeding specific effects were identified for truncal deposition and utilization, which suggests that future studies may benefit from depot-specific inquiries.
The Effects of Feeding Style on Subcutaneous Adipose Tissue Deposition within the First Year of Life

by

Meriah Schoen

A Thesis

Presented in Partial Fulfillment of Requirements for the Degree of Master of Science in Health Sciences

The Byrdine F. Lewis School of Nursing and Health Professions Department of Nutrition
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# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADP</td>
<td>air displacement plethysmography</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>DXA</td>
<td>dual-energy X-ray absorptiometry</td>
</tr>
<tr>
<td>IMCL</td>
<td>fat deposition in the soleus muscle</td>
</tr>
<tr>
<td>LR</td>
<td>likelihood ratio test</td>
</tr>
<tr>
<td>MONW</td>
<td>metabolically obese normal-weight</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>NCDs</td>
<td>non-communicable diseases</td>
</tr>
<tr>
<td>NEFA</td>
<td>non-esterified fatty acids</td>
</tr>
<tr>
<td>PBF</td>
<td>predominantly breastfed</td>
</tr>
<tr>
<td>PFF</td>
<td>predominantly formula-fed</td>
</tr>
<tr>
<td>STR</td>
<td>ratio of subcutaneous to triceps skinfold</td>
</tr>
<tr>
<td>TBK</td>
<td>total body potassium</td>
</tr>
<tr>
<td>TOBEC</td>
<td>total body electrical conductivity</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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CHAPTER I: INTRODUCTION

Statement of the problem

Numerous epidemiological reports have suggested that obesity is the predominant risk factor for a range of chronic diseases, including type II diabetes mellitus, cardiovascular disease, and cancer.\textsuperscript{1,2} Yet, there has not been a consistent association between obesity and the metabolic abnormalities that characterize these conditions.\textsuperscript{3,4} In part, this reflects the fact that obesity is often defined in relation to body mass index (BMI), a weight phenotype that is less predictive than previously assumed, and attention has turned to patterns of adipose tissue deposition as a more sensitive indicator of lifespan health\textsuperscript{5}. Evidence for this derives from samples of both infants and adults, for which the amount and distribution of adipose tissue have not only been discordant with weight status, but also prognostic for later disease.\textsuperscript{6,7} As deposition patterns in infancy have been found to track into adulthood, and particularly among children with excess adiposity,\textsuperscript{8} early developmental influences may be critical for fat patterning and long-term health outcomes.

Feeding practices in infancy represent one important route whereby adiposity may be programmed. This is the fundamental premise of The Developmental Origins of Health and Disease Model, which posits that early nutritional experiences can alter organ structure and essential homeostatic mechanisms during critical periods of development.\textsuperscript{9} Among humans, data to date has identified that early feeding choices produce long-term differences in body composition.\textsuperscript{10} From 15 predominantly longitudinal studies, a recent meta-analysis has demonstrated that there is a switch from higher adiposity among breastfed infants at three to four months of age to greater adiposity among formula-fed infants by 12 months of age.\textsuperscript{10} Imai and colleagues\textsuperscript{11} extended these findings in a longitudinal analysis that has demonstrated both faster growth during infancy and greater BMI at age six among infants who were formula-fed and
provided complementary food prior to six months of age. Beyond feeding style, the duration of breastfeeding has also been deemed a significant influence for body composition. From the West Australian Pregnancy Cohort Study, a 20-year longitudinal analysis, it has been shown that children breastfed for four months or less have the greatest probability of exceeding the 95th percentile for BMI from one to eight years of age as compared to children breastfed for greater than 12 months. Additionally, the cessation of exclusive breastfeeding before six months has been associated with an increased prevalence of overweight or obesity at 20 years of age.

Collectively, the beneficial outcomes associated with breastfeeding have been attributed to the unique and individualized composition of breastmilk, which can change with the time of day and over the course of a feed. Beyond the essential macro- and micronutrients, breast milk also contains a plethora of biologically active components (immune-related compounds, cytokines, hormones, growth factors, enzymes, essential fatty acids, etc.) that may function as key players in both the long- and short-term health benefits associated with breastfeeding. Infant formula, which does not share the same dynamic composition as breast milk, has been associated with an increased risk for common childhood infections (ear infections and diarrhea) and several chronic diseases (type 2 diabetes, asthma, childhood obesity). While it is possible, that changes in formula composition, and particularly the protein content (amount, source, and hydrolyzation), may attenuate some of these effects, the current WHO infant feeding recommendations align with the greater body of evidence that supports positive breastfeeding-associated health effects. As exclusivity and duration of breastfeeding have been deemed significant, the WHO recommendation now promotes exclusive breastfeeding through the first six months of life, in addition to continued breastfeeding for the first two years of life.
In spite of the WHO recommendation, several studies have failed to find any significant long-term differences in weight and/or body composition from distinctions in feeding style\textsuperscript{18-20} or duration of exclusive breastfeeding.\textsuperscript{21} This lack of consistency may derive from a diversity of data acquisition and measurement approaches.\textsuperscript{22} More importantly, however, these studies all predominantly use BMI and weight as proxies for adiposity, which provide misleading information about body composition, and particularly body fat content.\textsuperscript{23} As there is a now a large evidence base that supports an association between adiposity and health,\textsuperscript{5} it is of critical importance to determine if early feeding choices directly modulate adipose tissue morphology.

Adipose tissue morphology, or the general tissue phenotype, is determined by two features: cell size and cell number. While adipose tissue development begins around the 14\textsuperscript{th}-28\textsuperscript{th} week of gestation,\textsuperscript{24} the augmentation of these cellular features occurs primarily in the postnatal period and depends on two distinct growth mechanisms: hypertrophy and hyperplasia, an increase in cell size and cell number, respectively.\textsuperscript{25} These processes are reported to occur independently of weight status.\textsuperscript{26} Over time, these growth patterns produce distinct adipose tissue phenotypes and metabolic profiles. Increased hypertrophy leads to adipose tissue depots composed of few but large cells, while hyperplasia manifests as many but small fat cells.\textsuperscript{27} Due to significant association with insulin resistance, type 2 diabetes, dyslipidemia, and hypertension, a deposition pattern characterized by increased hypertrophy relative to hyperplasia is considered ‘risky’.\textsuperscript{28-30} An increase in hyperplasia, in contrast, is believed to result in a more benign metabolic profile with fewer long-term metabolic consequences.\textsuperscript{28-30}

The contribution of adipocyte cell number and size to the overall growth of a fat depot varies among individuals, by site, and with age.\textsuperscript{31,32} When assessing regional differences in these features, it has been found that cell number is a more significant contributor to overall fat mass in
the visceral adipose region; whereas, both cell number and cell size are equal contributors to mass in the subcutaneous adipose region.\textsuperscript{33} Within the subcutaneous depot, the relationship between cell size and number also differs by sub-depot. This has been postulated to be the product of different preadipocyte populations, which have innate variability in proliferative potential, adipogenesis, and gene expression.\textsuperscript{34} These differences ultimately translate into distinct growth mechanisms. From studies that have assessed the effects of overfeeding on the size and cellularity of healthy adult adipocytes,\textsuperscript{34} the truncal depots have been found to expand predominantly through hypertrophy, while the peripheral depots have been observed to expand through hyperplasia. Due to the tendency for truncal depots to develop a smaller number of larger cells, deposition within these subcutaneous depots is considered more ‘risky’ in terms of health sequelae.\textsuperscript{26}

Despite the growing understanding of how adipose tissue mass and distribution produce variable metabolic profiles,\textsuperscript{35} the majority of research in this area has focused on adult samples. There remain few prospective longitudinal studies investigating how these growth processes unfold in infants and children. Among those that have, differences in the populations studied and methodology used have made it unclear whether fat depot expansion early in development occurs independently through cell size and number, or by concomitant increases in both variables.\textsuperscript{25,31,36,37} The most compelling evidence comes from a study comparing adipocyte developmental trajectories in both longitudinal and cross-sectional cohorts from 4 months to 24 years of age, which suggests that adipocyte number and size have distinct growth trajectories beginning in the first year of life.\textsuperscript{31} Moreover, it is reported that expansion by cell number, rather than cell size, is the more prominent contributor to early growth.\textsuperscript{31} As cell number is an important contributor to fat mass,\textsuperscript{38} and studies suggest that weight loss among both children and
adults is associated solely with reductions in cell size,\textsuperscript{27,39} it is important to determine whether feeding practices can modulate early adipose tissue growth, and specifically increases in cell number.

While evidence for this question is largely absent in human cohorts, animal studies have demonstrated that early nutritional influences can be important modulators of adipocyte growth patterns. Among rats, changes in caloric intake during the suckling period have been found to alter both cell size and cell number in the epididymal fat pad as early as five weeks of age.\textsuperscript{40} More specifically, cell size and cell number have been found to augment by a greater rate and magnitude in rats with an increased caloric intake relative to rats with a restricted intake.

Synonymous to the deposition trajectories described by \textsuperscript{31} in both the longitudinal and cross-sectional human cohorts, initial growth in the rat epididymal adipocytes has also been found to expand primarily by increases in cell number.\textsuperscript{40} Rat studies assessing effects of early intake levels on growth beyond the first 20 weeks of life, suggest that these differences in adiposity remain relatively consistent after weaning, even when intake restrictions are no longer present.\textsuperscript{41} This may represent long-term effects on appetite regulation, and emphasizes the essential role of early feeding strategies for altering behavioral and physiological factors that contribute to adiposity.

While respecting species distinctions, the findings from animal models suggest that early nutritional experiences in humans may be similarly critical for shaping adipose tissue morphology. While early investigations of infant adiposity began nearly five decades ago with studies that employed a cellular lens,\textsuperscript{25,31,36} more recent investigations regarding feeding effects on adiposity have adopted an approach more focused on overall body composition.\textsuperscript{10} A fundamental factor underlying this shift has been the advent of several advanced measurement
technologies (i.e. DXA, MRI, ADP, CT), which have illuminated differences in fat versus fat-free mass, in addition to regional adipose tissue distinctions (visceral versus subcutaneous fat). While these techniques provide greater measurement precision and more specific information on body composition than weight or BMI alone, they have decreased site specificity. This is of critical importance, as adipose tissue distribution is determinative of the mechanism by which adipose tissue expands.\textsuperscript{26,34} For this reason, clarifying the metabolic risks associated with adiposity requires a more site-specific approach, such as that provided by classic anthropometry for the subcutaneous region. Given subcutaneous fat has been found to compose 70-80\% of total body fat in infants,\textsuperscript{42} this is an appropriate investigative approach for the inquiry at hand. This methodology further represents the best technique for a non-invasive longitudinal design, which is necessary for accurately exploring the associations between feeding style and patterns of subcutaneous fat deposition over the first year of life.

\textit{Project Aims}

The primary aim of this study is to investigate whether subcutaneous adipose tissue deposition patterns are modified by feeding style within the first year of life. Over the past 20 years, the World Health Organization (WHO) recommendations for exclusive breastfeeding have changed from prescribing adherence for the first four to six months of life\textsuperscript{43} to a stricter exclusive practice for the first six months of life, followed by continued breastfeeding for the first two years as foods are added.\textsuperscript{17} With the WHO’s extended duration of exclusive breastfeeding aiming to promote “optimal growth, development, and health,” it is of interest to assess whether there are developmental differences in adiposity among infants who have met this recommendation. The present analysis will investigate subcutaneous fat deposition patterns during the following time frames: 0 to 4 months, 4 to 6 months, and from 6 to 12 months of age. The term ‘deposition
patterns’ rather than ‘growth’ of subcutaneous adipose tissue will be employed because the mechanisms by which growth of adipose tissue occurs (hypertrophy or hyperplasia) remain unknown in the depots and time frame that will be investigated.\textsuperscript{31,32}

Secondary Aims

(1) In view of the evidence associating different metabolic consequences with truncal versus peripheral patterns of subcutaneous fat deposition,\textsuperscript{44} a secondary aim of this study is to assess whether differences in fat deposition patterns in the abdominal and femoral depots by feeding style are evident; specifically, if predominant formula feeding is associated with increased tissue deposition in so-called high risk depots.

(2) Based on the current WHO infant feeding recommendation, an additional aim is to investigate whether feeding style modifies deposition patterns for subcutaneous adipose tissue depots among infants who continue breastfeeding beyond the first six months of life; specifically, whether breastfeeding is associated with more gradual rates of subcutaneous fat deposition, and thus confers a potential protective effect on the development of adiposity.

Hypotheses

(1) The null hypothesis employed here is that feeding style has no statistically significant effects (p<0.05) on subcutaneous adipose tissue deposition rates for each of the depots examined (triceps, quadriceps, calf, subscapular, suprailliac, mid-axial, and abdominal) for each of the three time frames (0 to 4 months, 4 to 6 months, and 6 to 12 months of age).

(2) The null hypothesis employed here is that feeding style has no statistically significant effects (p<0.05) on deposition rates of subcutaneous adipose tissue depots associated with
risk for the development of chronic disease (abdominal skinfold) by comparison with non-risky depots (femoral skinfold) during each of the three time frames (0 to 4 months, 4 to 6 months, and 6 to 12 months of age).

(3) The null hypothesis employed here is that feeding style has no statistically significant effects (p<0.05) on subcutaneous adipose tissue deposition patterns for each of the depots beyond the first 6 months of life. This hypothesis tests feeding style categories as composed of continued breastfeeding beyond the first six months of life and formula-fed or weaned by six months of age.
CHAPTER II: REVIEW OF THE LITERATURE

How infant feeding strategies affect early growth patterns is a question that has been considered for decades. A plethora of studies have explored differences in weight and length accretion among breast and formula-fed infants,\textsuperscript{45-48} and can be summarized as showing variable results. These diverse findings have been attributed to problems of confounding, reverse causality, and selection biases, all of which obscure biology.\textsuperscript{49} It is well known, moreover, that weight and other proxies of adiposity do not accurately capture body fat content.\textsuperscript{23} To better understand how nutrition modulates early patterns of adipose tissue deposition, numerous studies and meta-analyses have also investigated body composition differences among breastfed and formula-fed infants over the first year of life with methods ranging from anthropometry to a wide variety of in-vivo measurement techniques.\textsuperscript{10,20,50-53} These studies have also employed a variety of outcome measures, the majority of which have been total body fat or percentage body fat. While these studies have not assessed the direct effect of diet on adipose tissue cellularity, a reported positive association between cell size, cell number, and percent body fat\textsuperscript{31} provides insight into how these studies may translate at the cellular level. These studies do not, however, describe how feeding practices affect the distribution of adipose tissue deposition. As it is critical to assess both the amount and distribution of adipose tissue, literature describing site-specific differences in adiposity is more limited.

\textit{Effect of feeding strategy on subcutaneous skinfolds}

One of the earliest investigations of feeding strategy on fat deposition patterns in infants was conducted by Oakley\textsuperscript{54} In this analysis, three feeding groups were utilized to compare skinfold thickness: fully breastfed infants, fully formula-fed infants, and mixed formula- and cereal-fed infants over the first six weeks of life. Infants were measured twice (within 24-hours
of birth and at five to seven weeks of age), and subcutaneous fat deposition was assessed through an average of the triceps and subscapular skinfolds. Despite a similar weight gain between the groups, breast-fed infants demonstrated a greater increase in subcutaneous fat over the six weeks by comparison with the formula-fed infants (p < 0.001); and further, infants fed formula plus cereal demonstrated an increase in subcutaneous fat that was intermediate between that of the formula-fed and breastfed infants. This study hypothesized that differences in fatty acid composition between breast milk and cow’s milk formula was the predominant factor driving differences in the quantity and composition of subcutaneous fat.

In a sample of healthy singleton infants who were breastfed, bottle-fed, or concomitantly breast and formula-fed, D’Souza and Black\textsuperscript{51} similarly assessed skinfold thickness prospectively over the first seven weeks of life. In alignment with Oakley\textsuperscript{54}, an average of the triceps and subscapular skinfold measurements was assessed 24 hours after birth and at five to seven weeks of age. Analogous to the findings of Oakley\textsuperscript{54}, a significantly greater increase in skinfold thickness was found among the entirely breastfed infants compared to their peers (breast and formula-fed infants and solely formula fed) (p<0.01). This effect, however, was found only among boys; female infants demonstrated no significant differences in skinfold thickness over the seven weeks. D’Souza and Black\textsuperscript{51} postulated that the greater growth observed among breast fed infants may represent greater overall consumption of milk. This interpretation, however, is problematic as it negates many studies that suggest formula fed infants consume more energy, protein, and micronutrients than breastfed infants.\textsuperscript{55-57}

While these studies used comparable methodologies and report similar conclusions, it is important to note that neither study discusses the possibility of reverse causality in their findings, wherein robust breast-feeders are maintained on breastmilk while those with poorer perceived fat
deposition are changed to formula.\textsuperscript{\ref{58}} This was first broadly considered in the 1980s and subsequently became a concern for the clarity of any study aiming to draw these simple comparisons.\textsuperscript{\ref{59}}

**Effect of feeding strategy on total body fat and/or percentage body fat**

An investigation by Shepherd et al.\textsuperscript{\ref{60}} assessed body composition in 82 healthy exclusively formula-fed and exclusively breastfed infants over the first three months of life. Fat mass was measured not only by anthropometry (triceps skinfolds), but also by total body potassium (TBK) (utilized in a four-compartment model) on day 10, day 28 ± 3, day 42 ± 4, and day 90 ± 5. Feeding information was provided by maternal diaries. Using t-tests, no significant differences in mean skinfold size between feeding groups was found across the study interval. Nevertheless, there was a significantly greater gain in total body fat as measured by TBK among male formula fed infants relative to the breastfed infants between days 10 and 90. This pattern of increased fat accretion among formula fed boys, however, was only mirrored in triceps skinfold thickness from day 10 to 28. This upward trend in fat deposition was attributed to greater intakes among formula fed infants, yet no specific calorie data were presented.

Utilizing TOBEC and sum of skinfold thickness (from triceps, subscapular, quadriceps skinfolds), de Bruin et al.\textsuperscript{\ref{52}} assessed body composition measures and nutrient intakes at 1, 2, 4, 8 and 12 months of age in a cohort of full-term infants exclusively breastfed or formula-fed for at least four months. Intakes were estimated by weighing infants (breastfed group) or bottles (formula-fed group) prior to and after feedings, and were corrected for regurgitation after feedings. Intake of non-milk foods and fluids were recorded by infants’ mothers. Analysis by multiple linear regression revealed that formula-fed infants had higher macronutrient and gross energy intakes during the exclusive feeding period, with statistically significant differences at
two and four months. A comparison of both TOBEC measures and sum of skinfolds by feeding group at each age point demonstrated a greater fat gain among formula fed infants (only girls) from one to four months of age. Differences in total body fat by feeding groups, however, were only found at four and eight months of age, with formula-fed girls displaying greater total body fat at these time points.

Among infants that were either exclusively breastfed or exclusively formula-fed until four months of age (thereafter feeding was at the discretion of infants’ parents), Butte et al.\textsuperscript{50} compared fat mass and fat-free mass at 0.5, 3, 6, 9, 12, 18, and 24 months of age. Fat mass data specifically was attained using four different body composition methods: a multicomponent body composition model (with data derived from total body water (TBW) and total body potassium (TBK)), anthropometry (mean skinfold thickness), total body electrical conductivity (TOBEC), and dual x-ray absorptiometry (DXA). Feeding patterns were quantified via histories taken at each three- and six-month study interval, in addition to three-day weighted intake records (quantified at 3, 6, 12, and 24 months), which clarified macronutrient intakes. From the weighted records, breastfed infants were found to have significantly lower intakes of metabolizable energy, protein, fat, and carbohydrates at 3 and 6 months of age (p=0.001), but there was no correlation found between intakes and fat mass or percentage fat mass at three, six or 12 months of age. The body composition analysis revealed no significant differences in skinfold thickness (triceps, subscapular, flank, quadriceps and sum of skinfolds) or DXA measurements at any age assessed. From the multicomponent model, fat mass and percentage fat mass were significantly higher in infants at three months of age and six months of age among boys. These findings were mirrored and extended by those of the TOBEC, which found a greater percentage fat mass at nine months of age among boys. As nutrient intake was only related to infant weight gain and not
fat mass or percentage fat mass, Butte et al.\textsuperscript{50} suggested that other bioactive factors in breastmilk may be responsible for the noted body composition differences.

Buyken et al.\textsuperscript{61} assessed the effect of breastfeeding on percentage body fat at 6 months of age and the rate of fat accrual from six months to seven years of age among 434 healthy infants. Skinfold measurements were taken three times in the first year, two times in the second year, and annually thereafter at the biceps, triceps, subscapular, and suprailiac sites. Using these measurements and an equation from Duerenberg et al.\textsuperscript{62}, percentage body fat was calculated. Prospective data on the duration of breastfeeding was attained through maternal interview at each study visit and weighed 3-day dietary records were kept during the first year of life. Based on this information, infants were classified as breastfed for a long duration (fully breastfed $>$ 17 weeks), shortly breastfed (fully breastfed for greater than two weeks and up to a maximum of 17 weeks), and never breastfed (not, partially, or fully breastfed for up to two weeks). Using a linear mixed effects regression model, infants who were breastfed for a longer duration were found to have a decreased percentage body fat at six months of age. Additionally, a longer duration of breastfeeding only affected the rate of body fat accrual in a subgroup of male infants with overweight mothers.

A recent meta-analysis by Gale et al.\textsuperscript{10} assessed differences in fat mass, fat-free mass, and percentage fat mass among healthy term infants that were either breastfed or formula-fed from 0-12 months of age. Data were derived from 15 studies that used either longitudinal or cross-sectional designs, in addition to a wide range of techniques used to measure body composition (TBK, isotope dilution, TOBEC, DXA, MRI, ADP, and multicomponent models). Pooled mean differences between breastfed and formula-fed infants revealed a significantly lower fat mass and percentage fat mass among formula-fed infants at four and six months of age. No significant
differences in fat mass or percentage fat mass between the feeding groups were detected at 12 months of age. Formula fed infants, however, displayed a trend toward higher fat mass than their breastfed counterparts. Subgroup analyses, which assessed patterns using only a single type of body composition measurement technique revealed differences of the same magnitude and direction as the larger analysis. The higher fat mass found among breastfed infants was postulated as reflecting a yet to be clarified mechanistically, evolutionary mechanism selected to support the infant during a precarious developmental period.

These investigations represent the bulk of studies that have assessed the effect of early feeding strategies on total or percentage body fat. While it is beneficial that these studies utilized longitudinal designs, comparing the results of these studies is challenging due to a range of other methodological inconsistencies and differences in study design.

**Methodological Considerations**

Specific methodological concerns include variability in the definition of feeding groups, differences in the measurement time-frame and frequency, differences in sample size, variability in infant formula composition, differences in outcome variables, uncontrolled confounders, and the use of different growth chart references.\textsuperscript{47,50} Additionally, not all studies have employed a longitudinal design, which is necessary when trying to capture a developmental process that is both complex and non-linear.\textsuperscript{31} Due to the immense heterogeneity among these variables, both the magnitude and direction by which feeding strategy affects weight and body composition is unclear. This is in addition to the problems associated with confounding, bias and reverse causality as discussed by Kramer et al.\textsuperscript{49,59} From an analytic viewpoint, there are further issues in these earlier studies. Several studies use simple comparisons of mean sizes with no confirmation that the data are normally distributed. It has become clear that growth in the early
months reflects adjustments based on size at birth and, therefore, regressions incorporating size at birth are fundamental tools for assessing early growth patterns. None of these studies considered these elements.

More importantly for the aims of the present study, these studies did not consider fat a variegated depot. In studies that have utilized subcutaneous skinfold measurements, data from different sites was aggregated or utilized within a formula to attain total body fat or body fat percentage. This mirrors the reported data from more recent in-vivo techniques (DXA, MRI, computerized tomography, ADP, isotope dilution, TOBEC, and TBK) that similarly only captured fat as a single depot. As it is now clear that fat is not a homogenous tissue, and the distribution of fat may be more a significant risk factor than total body fat, these outcome variables may not be specific enough to determine whether feeding strategy can program long-term differences in metabolic health. Furthermore, as deposition in certain subcutaneous depots may be more ‘risky’ than others, this remains an important gap in the literature. Only three studies have employed site-specific approaches.

**Effect of feeding strategy on specific subcutaneous sites**

The first of the three studies was a prospective longitudinal study by Ferris et al. assessed the relationship between feeding method and subcutaneous fat deposition patterns among 92 female infants. Feeding groups were defined as infants fed breast milk alone, infants fed breast milk with food supplements (formula or milk solids >50kcal/day), infants fed formula alone, and infants fed formula with food supplements (solid food >50kcal/day). Measurements of the triceps, subscapular, suprailiac, and chest skinfolds were obtained monthly for six months. No significant differences were reported among the feeding groups; however, formula-fed infants that started solids before two months of age did demonstrate the greatest mean increase in
skinfolds. Yet, by five months of age, the skinfolds of formula-fed infants ultimately re-aligned with the other feeding groups.

A second study by Bergmann et al.\textsuperscript{67} followed a cohort of 480 infants to assess differences by feeding strategy in skinfold thickness and the prevalence of adiposity from six months to six years of age. Nutritional data and skinfold measurements at the triceps and subscapular sites were collected at 3, 6, 12, 18 months, and annually thereafter. Infants were categorized as bottle fed (never breastfed or breastfed greater than two months) or breastfed (breastfed greater than three months), and feeding effects on skinfold growth patterns were assessed separately for each site. In the triceps skinfold, a significant difference in the growth pattern between feeding groups was identified at 6 months and from 48 months on; whereas, for the subscapular skinfold, statistically significant differences were found at 24, 48, and 72 months of age.

Another prospective cohort was carried out by Durmuş et al.\textsuperscript{53} assessed the association of breastfeeding (never; ever), breastfeeding duration (never; < 4 months, > 4 months), and breastfeeding exclusivity (never; non-exclusive until 4 months; and exclusive until 4 months) with peripheral, central, and total subcutaneous fat deposition among 779 children in the first two years of life. Information about breastfeeding was acquired from delivery reports and postal questionnaires at the ages of two, six, and 12 months after birth. Skinfold thickness was measured at the ages of one and one half, six, and 24 months and outcome variables were calculated as the sum of skinfold sites. Total subcutaneous fat was calculated from the sum of the biceps, triceps, suprailliac, and subscapular skinfolds. Central subcutaneous fat was calculated as the sum of the suprailliac and subscapular skinfolds, and peripheral subcutaneous fat was calculated as the sum of the biceps and triceps skinfolds. A linear regression model with 10
covariates, which aimed to account for pre- and post-natal maternal characteristics, was utilized for statistical analysis. Based on feeding strategy alone, infants that were never breastfed were found to have greater peripheral fat mass at 6 months of age. When comparing duration, shorter breastfeeding was associated with greater peripheral and total subcutaneous fat mass at 6 months of age, with no significant differences found at 24 months of age. A comparison between infants breastfed exclusively for four months and those who were never and non-exclusively breastfed identified higher peripheral and total subcutaneous fat mass at six months and higher central fat mass at 24 months.

While these studies have employed longitudinal designs, it is unclear if sampling frequency is sufficient to capture developmental differences between subcutaneous depots. As early feeding strategy is a modifiable factor, it is a priority to assess whether feeding mode may be affecting the developmental trajectory for subcutaneous fat, in addition to the specific deposition patterns through which feeding may lead to later metabolic consequences.
CHAPTER III: METHODS

Subjects and Research Design

This is an ex post-facto design that utilizing data collected for a longitudinal growth study in the first year of life. Sixty-eight twenty-one clinically normal, American infants (12 females and 9 males; 19 Caucasian, 1 Hispanic) were studied between the ages of one day and 21 months (Table 1). The present sample is composed of middle class individuals and is biased towards those willing to document their infants’ daily behaviors. Participants were recruited through birthing classes and subject referral. All infants were measured weekly for four to 18 months after parental informed, written consent of a Human Subjects’ Committee protocol approved by the University of Pennsylvania Committee on Human Subjects. Parents were asked to note each day whether their infant was breastfeeding and/or formula-feeding, and to record whether their infant experienced any illness (vomiting, diarrhea, fever, rash, congestion, or other medically diagnosed condition). The focus of this analysis will be the feeding data and the anthropometric measures, which will be analyzed up to 12 months of age.

Anthropometry Measurement Protocol

Home visits were made weekly. During each of these visits, seven limb and trunk skinfolds (triceps, quadriceps, calf, subscapular, suprailliac, midaxillary, and abdominal) were measured by the same observer (M.L.) with Holtain skinfold calipers according to standard techniques. As this was a time-intensive longitudinal study, skinfold measurements provided the most economical, noninvasive technique. While methods such as air displacement plethysmography (ADP), magnetic resonance imaging (MRI), and dual-energy x-ray
absorptiometry (DXA) may allow for greater measurement precision, these techniques often require access to specialized facilities and are not feasible for weekly measurements. Moreover, Yajnik et al. have demonstrated that skinfold measurements can function as an effective approach for assessing regional body subcutaneous fat distribution among infants.

A pilot study established the intra-observer technical error of measurement for all skinfolds as <0.2 mm based on 52 infants each measured two times with a 3-s compression time. This protocol was identified as the tolerance limit for multiple skinfold measures in a serial study of infants with measurement errors in line with previously published studies.

Statistical Analysis

This study aims to investigate feeding style effects on infant subcutaneous fat depot deposition rates and trajectories. Deposition rates were assessed as the slopes of size for age across the three age-based feeding intervals (0 to 4 months, 4 to 6 months, 6 to 12 months) and deposition trajectories were assessed as changes in slopes between these intervals, or the changes at 120 and 180 days from the immediately preceding interval. The residuals for each anthropometric variable were tested for normality (Shapiro-Francia) and Box-Cox-identified power transformations were investigated as needed using the gladder and ladder tests. Transformations were undertaken as appropriate. Infant feeding style was categorized as predominantly breastfed (PBF) and predominantly formula-fed (PFF) (Table 1). These feeding groups align with the WHO’s infant feeding categories, which specify breastmilk or formula as the predominant source of infant nourishment, while allowing for other liquids in the infant’s diet. For project aims one and two, predominant breastfeeding was defined as predominant breastfeeding for the first four months of life or until study termination, and predominant formula feeding was defined as formula feeding for the first four months of life or until study
termination, respectively. For project aim three, predominantly breastfed was defined as breastfed beyond the first six months of life, and predominantly formula-fed was defined as formula-fed or weaned at six months of age.

*Aim 1* is to clarify if feeding styles have homogeneous effects on the deposition rate of each depot and the deposition trajectory for each depot across time. The null hypothesis that feeding styles have no significant effects on subcutaneous fat expansion rates and trajectories was assessed by piecewise regression across three time intervals (<4 months, >4 to ≤6 months, and >6 months) generated by the *mkspline* procedure, with the *marginal* option. This analysis was implemented in a two-way multilevel mixed model that permitted random intercepts and slopes at the feeding style group level, and random intercepts at the sex and individual levels (*xtmixed with individual nested in sex*). This approach accommodates variability in repeated measurement intervals (each measurement was not exactly at seven day intervals), and number of measurements per individual; inter-sex and inter-individual growth rates (deposition rates) and random error components among individuals not otherwise captured in the regression model. The feeding style effects on growth rates and patterns were specifically investigated as an interaction term between slopes and feeding styles. The random slopes and intercepts model allowed for randomness in slopes from the unbalanced occasions, and the individual subject’s variability in fat deposition rate. The appropriateness of the random intercept and random slope model was assessed via the likelihood ratio (LR) test. For all skinfolds, a LR test with a p-value of <0.05 determined that it was appropriate to reject the null hypothesis that the intercept was the same across all skinfolds, as the regression model assumes. Furthermore, a LR test with a p-value of <0.05 allowed the random intercept model to be rejected in favor of a random coefficient model. For all analyses, statistical significance was estimated after adjusting for the
nested-level error structure (individuals within groups) with maximum likelihood estimation. Birth weight, weight, and the number of feeding episodes per day were investigated as covariates due to the well-established associations between these factors and adiposity.\textsuperscript{73-75} Statistical significance was accepted if $p<0.05$. Significance greater than $p=0.05$ and less than $p=0.10$ was considered a trend.

\textit{Aim 2:} The null hypothesis that ‘risky’ depots have identical growth rates and growth trajectories according to feeding style was investigated in a mixed-effects regression as above. In this analysis, skinfolds were grouped by ‘high risk’ depots (truncal) vs ‘low risk’ depots (peripheral) and interaction terms for ‘risk’ and growth rate by feeding group were included in the analysis. The truncal depot represented the sum of the abdominal, subscapular, suprailiac, and midaxillary skinfolds; whereas the peripheral depot represented the sum of the quadriceps, triceps, and calf skinfolds. Statistical significance was estimated as above.

\textit{Aim 3} investigated whether feeding style is associated with post-six month differences in expansion rates and trajectories and was tested by the null hypothesis that feeding style has no significant moderation effects on the slopes of each measured variable across the third segment of time, as estimated in the regression described for Aim 1 with the amended feeding style variable.

Short-comings of study design and execution that may influence study outcomes include several methodological aspects. These include, but are not limited to: (1) insufficient power due to a small sample size may result in a lack of resolution to identify feeding style differences, and 2) the choice of the investigative time frames, selected with an interest from a nutritional perspective, may not align with biological growth trajectories.
Table 1. Infant feeding groups by aim. Predominantly breastfed (PBF) describes infants whose predominant source of nourishment was breastmilk for four months or greater. Predominantly formula fed (PFF) represents infants whose predominant source of nourishment was always formula or infants who were breastfed for fewer than four months (Day of life 120).

<table>
<thead>
<tr>
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<th>Study Duration (days of life)</th>
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<th>Total Formula Feeding Duration (Days)</th>
<th>Feeding Group: Aims 1 and 2</th>
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<td>PBF</td>
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CHAPTER IV: RESULTS

Data Exploration and Model Considerations

Prior to assessing differences by feeding style in growth rates and trajectories, growth patterns were explored using raw anthropometric data. Fractional polynomial regression 
\( \text{fracpoly regress} \) and fractional polynomial plots \( \text{fracplots} \)\textsuperscript{72} assessed the empirical best-fit models for the growth patterns of each feeding group. These procedures identified significant differences in the growth curve forms between breast and formula-fed infants (Figure 1). Upon inspection of the polynomial fit for each age interval (0 to 4 months, 4 to 6 months, 6 to 12 months), a first power fit (linear regression) was not a significantly poorer fit than higher power models, and linear piecewise regressions were chosen as the analytic approach for comparing the quantitative differences in growth rates and trajectories.

Figure 1: Growth Curve for Sum of Skinfolds Over the First Year of Life
Effect of Feeding Style on the Growth Rates and Trajectories of Subcutaneous Fat Depots

Multilevel mixed-effects modeling identified significantly different patterns of subcutaneous fat deposition between predominantly breastfed and predominantly formula-fed infants in both the sum of skinfolds and in each of the seven skinfold sites. Fat accretion was exhibited only during the first four months of life, and this was limited to the peripheral skinfolds (Figure 2). Thereafter, all subcutaneous skinfolds followed a trend of declining rates.

Figure 2  Fixed-effects results of peripheral skinfold growth rate over the first year of life among predominantly breastfed and predominantly formula-fed infants. Graphs represent the three age intervals in which the data was analyzed: 0 to 4 months, 4 to 6 months, 6 to 12 months.
**Growth Rates**

During the first four months of life, formula-fed infants experienced greater rates of deposition relative to breastfed infants for the trunk, peripheral, and sum of skinfolds ($p<0.01$) (Figures 2-4). Regarding specific sites, formula-fed infants demonstrated greater rates of deposition for the subscapular, abdominal, suprailiac, and quadriceps skinfolds ($p<0.05$). An increasing trend in growth rate was also demonstrated in the triceps ($p=0.07$) skinfolds among formula-fed infants. No statistically significant differences by feeding style were identified in the growth rates of the calf ($p=0.27$) and midaxillary skinfolds ($p=0.36$) during this interval.

Between four and six months of age, formula-fed infants followed more negative slopes for the trunk and sum of skinfolds ($p<0.01$) (Figures 3 and 4), with no overall significant differences noted for the peripheral depot ($p=0.61$) (Figure 2) (Table 2). Relative to breastfed infants, formula-fed infants demonstrated site specific slope decreases in the abdominal, midaxillary, triceps, and subscapular skinfolds ($p<0.05$). The growth rates of the quadriceps ($p=0.14$), calf ($p=0.86$), and suprailiac ($p=0.39$) skinfolds showed no statistically significant differences between breast and formula-fed infants (Table 3).

Beyond six months, five of the 21 infants continued to be breastfed. No overall differences by feeding style were identified in the deposition rates for the trunk ($p=0.47$) and sum of skinfolds ($p=0.96$) (their slopes were not statistically different) (Figures 3 and 4) (Table 2). Formula-fed infants did, however, demonstrate a more negative trend in the peripheral skinfolds than breastfed infants ($p=0.09$). Site-specific trends were not as clear as those in earlier time frames. Formula-fed infants had more negative slopes in the suprailiac ($p<0.01$) and midaxillary ($p=0.10$) skinfolds, while breastfed infants had more negative slopes in the calf ($p<0.05$) and abdominal ($p=0.06$) skinfolds. No significant differences by feeding style were identified in the
deposition rates of the quadriceps (p=0.57), triceps (0.14), and subscapular (p=0.26) skinfolds (Table 3).

Figure 3  Fixed-effects results of trunk skinfold growth rate over the first year of life among predominantly breastfed and predominantly formula-fed infants. Graphs represent the three age intervals in which the data was analyzed: 0 to 4 months, 4 to 6 months, 6 to 12 months.
Figure 4  Fixed-effects results for sum of skinfolds growth rate over the first year of life among predominantly breastfed and predominantly formula-fed infants. Graphs represent the three age intervals in which the data was analyzed: 0 to 4 months, 4 to 6 months, 6 to 12 months.

Table 2  Growth rates in predominantly breastfed infants relative to predominantly formula-fed infants for each composite subcutaneous site

<table>
<thead>
<tr>
<th>Composite Skinfold</th>
<th>Intercept</th>
<th>0 to 4 Months</th>
<th></th>
<th>4 to 6 Months</th>
<th></th>
<th>6 to 12 Months</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Coefficient (SE)</td>
<td>p</td>
<td>Coefficient (SE)</td>
<td>p</td>
<td>Coefficient (SE)</td>
<td>p</td>
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<tr>
<td>Trunk</td>
<td>-0.056 (0.013)</td>
<td>&lt;0.01</td>
<td>0.081 (0.016)</td>
<td>&lt;0.01</td>
<td>0.008 (0.011)</td>
<td>0.47</td>
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<tr>
<td>Peripheral</td>
<td>-2.59 (0.919)</td>
<td>&lt;0.01</td>
<td>-0.593 (1.16)</td>
<td>0.61</td>
<td>-1.31 (0.777)</td>
<td>0.09</td>
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<tr>
<td>Sum</td>
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<td>&lt;0.01</td>
<td>0.087 (0.025)</td>
<td>&lt;0.01</td>
<td>0.001 (0.015)</td>
<td>0.96</td>
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</table>

The coefficient describes the difference in growth rates between predominantly breastfed and predominantly formula-fed infants for each composite subcutaneous skinfold site, while accounting for baseline differences in size.
Table 3  Growth Rates in predominantly breastfed infants relative to predominantly formula-fed infants for each subcutaneous site

<table>
<thead>
<tr>
<th>Skinfold</th>
<th>Intercept</th>
<th>0 to 4 Months</th>
<th>p</th>
<th>4 to 6 Months</th>
<th>p</th>
<th>6 to 12 Months</th>
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</thead>
<tbody>
<tr>
<td>Abdominal</td>
<td>3.38</td>
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<td>0.02</td>
<td>0.034 (0.006)</td>
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<td>-0.007 (0.004)</td>
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<td>Midaxillary</td>
<td>3.89</td>
<td>-0.003 (0.004)</td>
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<td>Quadriceps</td>
<td>5.36</td>
<td>-0.018 (0.007)</td>
<td>&lt;0.01</td>
<td>-0.013 (0.008)</td>
<td>0.14</td>
<td>-0.003 (0.005)</td>
<td>0.57</td>
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<tr>
<td>Calf</td>
<td>2.26</td>
<td>-0.001 (0.001)</td>
<td>0.27</td>
<td>0.0001 (0.001)</td>
<td>0.86</td>
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<td>Triceps</td>
<td>1.66</td>
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<td>Suprailiac</td>
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<td>0.001 (0.001)</td>
<td>0.39</td>
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<tr>
<td>Subscapular</td>
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<td>0.003 (0.001)</td>
<td>&lt;0.01</td>
<td>0.0004 (0.000)</td>
<td>0.26</td>
</tr>
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</table>

The coefficient describes the difference in growth rates between predominantly breastfed and predominantly formula-fed infants for each subcutaneous skinfold site, while accounting for baseline differences in size.

Growth Trajectories

The growth trajectories describe the overall patterns of deposition across time. In the present analysis, they estimate the inflection points, or the changes in growth rates (slopes) between sequential intervals: a comparison of slopes prior to 120 days and slopes from 120 to 180 days; and a comparison of slopes following 180 days to slopes covering 120 to 180 days. Formula-fed infants, whose slopes had exceeded that of the breastfed infants in the first four months, demonstrated a more significant decline in slope during the subsequent four to six-month interval for the trunk and sum of skinfolds (p<0.01) (Table 4), as well the individual sites (abdominal, midaxillary, triceps, and subscapular) (p<0.05) relative to breastfed infants, whose attenuation in slope was less negative. No significant rate changes were identified in either the
peripheral composite variable (p=0.23) or the related specific sites (quadriceps, p=0.681 and calf, p=0.464) (Table 5).

Formula-fed infants, already on a steeper negative slope than breastfed infants from 120 days, do not further experience change at 180 days. Breastfed infants exhibit a later onset in slope diminution, which is evident at 180 days when their significant slope change occurs. Relative to the four to six-month interval, breastfed infants demonstrated a more dramatic decline in deposition rate for the trunk and sum of skinfolds (p<0.01), and the specific truncal sites (abdominal and subscapular skinfolds, p<0.01, for both) (Table 4 and 5). No significant differences in rate change at day of life 180 were noted for the midaxillary (p=0.16), quadriceps (p=0.36), calf (p=0.20), triceps (p=0.36), and suprailiac (p=0.38) skinfolds among all infants (Table 4).

Table 4  Growth trajectories in predominantly breastfed infants relative to predominantly formula-fed infants for each composite subcutaneous site

<table>
<thead>
<tr>
<th>Composite Skinfold</th>
<th>Intercept</th>
<th>Change in Slope at DOL 120</th>
<th>Change in Slope at DOL 180</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Coefficient (SE) p</td>
<td>Coefficient (SE) p</td>
</tr>
<tr>
<td>Trunk</td>
<td>18.4</td>
<td>0.140 (0.023) &lt;0.01</td>
<td>-0.073 (0.019) &lt;0.01</td>
</tr>
<tr>
<td>Peripheral</td>
<td>-38.1</td>
<td>2.00 (1.66) 0.23</td>
<td>-0.716 (1.39) 0.61</td>
</tr>
<tr>
<td>Sum</td>
<td>20.23</td>
<td>0.167 (0.036) &lt;0.01</td>
<td>-0.086 (0.030) &lt;0.01</td>
</tr>
</tbody>
</table>

The coefficient describes the difference in growth trajectory (change in slope between sequential intervals: prior to and following 120 days, prior to and following 180 days) between predominantly breastfed and predominantly formula-fed infants for each composite subcutaneous skinfold site, while accounting for baseline differences in size.
Table 5  Growth trajectories in predominantly breastfed infants relative to predominantly formula-fed infants for each subcutaneous skinfold site

<table>
<thead>
<tr>
<th>Skinfold</th>
<th>Intercept</th>
<th>Change in Slope at DOL 120</th>
<th>Change in Slope at DOL 180</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Coefficient (SE)</td>
<td>p</td>
</tr>
<tr>
<td>Abdominal</td>
<td>3.38</td>
<td>0.045 (0.008)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Midaxillary</td>
<td>3.89</td>
<td>0.017 (0.007)</td>
<td>0.02</td>
</tr>
<tr>
<td>Quadtriceps</td>
<td>5.36</td>
<td>0.005 (0.013)</td>
<td>0.68</td>
</tr>
<tr>
<td>Calf</td>
<td>2.26</td>
<td>0.001 (0.002)</td>
<td>0.46</td>
</tr>
<tr>
<td>Triceps</td>
<td>1.66</td>
<td>0.003 (0.001)</td>
<td>0.02</td>
</tr>
<tr>
<td>Suprailiac</td>
<td>1.96</td>
<td>0.005 (0.002)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Subscapular</td>
<td>1.81</td>
<td>0.006 (0.001)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The coefficient describes the difference in growth trajectory (change in slope between sequential intervals: prior to and following 120 days, prior to and following 180 days) between predominantly breastfed and predominantly formula-fed infants for each composite subcutaneous skinfold site, while accounting for baseline differences in size.

**Effect of Feeding Style on the growth rates and trajectories of subcutaneous fat depots beyond six months of life**

Continued breastfeeding beyond six months of age was associated with a significant decline in growth rates for the trunk and sum of skinfolds (p<0.01), such that they achieved similar (not statistically significantly different) slopes to the formula-fed infants in the six to 12-month interval. Relative to formula-fed or weaned infants, more significant negative slopes within the six to 12-month interval were noted only for the calf (p<0.05) and the abdominal (p=0.06) skinfold sites among continued breast feeders. It should be noted this comparison involved infants who were breastfed after six months and all others. This confounds the strict comparison of prolonged breastfeeding to infants who were previously breastfed and weaned at six months.
CHAPTER V: DISCUSSION AND CONCLUSIONS

Summary of Findings

The present study adds a unique perspective on site-specific patterns of fat deposition during the first four months of life by comparison with earlier reports. Previous research has documented important distinctions between growth trajectories of length, weight, and weight for length between breast and formula-fed infants. While these studies suggest a divergence in growth patterns by feeding group after two to three months of age, the present study adds details on distinctive patterns of fat deposition originating in the earliest months. Of the three investigative time frames (0 to 4 months, 4 to 6 months, and 6 to 12 months), the first four months were the only period in which infants in the present study experienced subcutaneous fat accretion. Thereafter, subcutaneous skinfolds followed a trend of declining rates. A synonymous growth pattern has been previously reported in the literature, with the finding of marked increases in skinfold thickness, body fat mass, and body fat mass as a percentage of body weight among infants in the first three months of life, followed by a sharp decline in relative fat mass for the remainder of the first year. The decline in adiposity described by Enzi et al. and identified in the present study, has been attributed to several changes that occur later in infancy, including increased energy expenditure related to greater levels of physical activity, increased protein consumption, and/or to more efficient appetite regulation. The present study adds to these findings by identifying that breastfed infants demonstrate both slower rates of subcutaneous fat accretion and decline by comparison with their formula-fed peers.

Beyond different patterns of overall subcutaneous fat deposition, this study identified different growth patterns among breastfed and formula-fed infants in each subcutaneous site.
This study is novel in its consideration of subcutaneous fat as a variegated depot, and provides the first documentation of differences in fat deposition patterns by feeding style in seven different skinfold sites. Utilizing this unique methodological approach, this study identified site-specific feeding effects that were not apparent when all skinfold measurements were combined as single variable (sum of skinfolds). As the effects of feeding on body composition have been assessed to date predominantly using total body fat or body fat percentage as outcome variables, site-specific differences in subcutaneous fat development may have been overlooked. Only one prior longitudinal study has assessed variability in growth patterns for different subcutaneous depots among breast and bottle-fed (non-breast milk) from the age of six months to six years. While these skinfolds were utilized as proxies for total body adiposity, Bergmann et al.\textsuperscript{67} reported distinct patterns of growth between the two subcutaneous depots. Specifically, in the triceps skinfold a significant difference in the growth pattern between feeding groups was identified at 6 months and from 48 months on; whereas, in the subscapular skinfold, statistically significant differences were found at 24, 48, and 72 months of age. Additionally, skinfold thickness was reported to be relatively stable among the breastfed group, while the bottle-fed group demonstrated a more than three-fold increase in subscapular skinfold thickness starting at 12 months of age.\textsuperscript{67}

In accordance with the observations of Bergmann et al.\textsuperscript{67}, breastfed infants in the present sample similarly demonstrated a significantly steadier decline in subcutaneous fat deposition in the subscapular skinfold, in addition to other subcutaneous sites (abdominal, midaxillary, triceps, subscapular, trunk, and sum of skinfolds) starting from four months of age. While the present study did not investigate growth patterns beyond the first year of life, it is possible that formula-fed infants could follow a similar increase in subscapular deposition as described by Bergmann
et al. beyond 12 months of age. The present sample demonstrated no significant differences in growth rate between feeding styles in the subscapular, triceps, quadriceps, trunk, and sum of skinfolds from six to 12 months of age; however, the inclusion of both formula-fed and weaned (breastfed prior to six months) infants into a single feeding group during this interval may have produced a growth pattern that deviated from that of purely formula-fed infants. Notable findings during the six to 12-month interval included greater declines in deposition among formula-fed infants in the suprailiac and midaxillary skinfolds, and greater declines in deposition in the calf, abdominal, and limb skinfolds among breastfed infants. Synonymous to the findings of Bergmann et al. the rate of deposition in the triceps skinfold demonstrated no significant difference between feeding groups during this interval.

**Illuminating Feeding Style Differences with Growth Trajectories versus Size**

Several previous studies that have utilized subcutaneous skinfolds to examine total adiposity patterns within the first year of life have reported no significant differences in deposition patterns by feeding strategy alone. Significant methodological differences, including varied measurement intervals and frequency, different feeding group definitions, and diverse calculation methods for the primary outcome variable (total subcutaneous fat, sum of skinfolds, total body fat, or body fat percentage) distinguish these reports.

Beyond divergent data and analytic choices, the unique findings of this study reflect the fact that it investigates the modulation of subcutaneous skinfold growth patterns by feeding strategy, rather than merely differences in skinfold size over time. The present study’s examination of growth rate changes between age intervals, in addition to time-constrained or interval-specific rate comparisons, has illuminated that breast and formula-fed infants follow significantly different overall deposition trajectories. This finding contrasts with the only other
study\textsuperscript{61} that has analyzed changes in fat accrual rate by feeding style. Beyond differences in the analytic time frame (0 to 6 months in the present study versus 6 months to 7 years) and statistical model, the findings of the present study may differ from that of Buyken et al.\textsuperscript{61} due to the choice of outcome variable. Buyken et al.\textsuperscript{61} used percent body fat (calculated from the biceps, triceps, suprailiac, and subscapular skinfolds); whereas, the present study identified growth trajectories for the sum of skinfolds, in addition to each subcutaneous site. By using a site-specific approach, the present analysis could demonstrate that feeding style influenced growth trajectories specifically within the truncal subcutaneous depots, clarifying that previous methodological approaches with a focus on total or percentage body fat may not be sensitive enough to capture these critical depot differences. This same limitation characterizes many recent analyses of body composition that utilize more precise measurement tools (DXA, MRI, ADP) but fail to capture fat as more than a single depot.\textsuperscript{10}

**Identifying ‘Risky’ Deposition Patterns**

A unique finding of the present study is the documentation that distinct patterns of truncal deposition begin in the first year of life associated with infant feeding. By comparison with breastfed infants, formula-fed infants demonstrated a pattern of more rapid accrual within the truncal depot. This contrasts with deposition patterns in the peripheral depot, where no significant differences by feeding style were found. As deposition within the truncal depot has been considered more ‘risky’ due to increased adipocyte hypertrophy,\textsuperscript{26,34} it is significant that formula consumption stimulates an earlier and more rapid expansion in this region. If significant differences in hyperplastic growth are not be present within the first year of life, as was demonstrated in the trajectories published by Knittle et al.\textsuperscript{31}, it is possible that the increased...
hypertrophy experienced by the formula fed infants in the truncal depots may necessitate an earlier recruitment of additional adipocytes and a greater overall expansion of fat mass.

A plethora of factors associated with feeding style may be related to the more rapid accrual of subcutaneous fat in the truncal depot compared to peripheral sites. These may reflect differences in dietary composition, the timing of feeds, and/or physiological responses that distinguish these two styles. In the absence of more specific data, these details cannot be illuminated in the present study. Based on previous research it can be hypothesized that mechanisms underlying the observed truncal deposition patterns may include increased expression and activity levels of lipoprotein lipase. Relative to the peripheral depots, greater levels of lipoprotein lipase mRNA and enzyme activity have been found in the truncal depots, and predominantly in the abdominal depot.\textsuperscript{77} As lipoprotein lipase activity is present in preadipocytes and may be the key regulator of fat accretion during the early postnatal period,\textsuperscript{78} it is possible that the greater release of insulin that follows bottle-feeding could increase expression of truncal lipoprotein lipase and subsequently lead to an earlier and more rapid filling of fat cells.\textsuperscript{79,80}

\textit{The First Four Months as an Important Nutritional Target}

With the goal of clarifying whether an extended duration of exclusive breastfeeding modifies subcutaneous fat deposition patterns, the historical iterations of the WHO’s Infant Feeding Recommendation guided the investigative times frames (0 to 4 months, 4 to 6 months, and 6 to 12 months) utilized in this study. Contrary to this study’s hypothesis, the magnitude of fat deposition did not continue to rise across the first year of life, and prolonged breastfeeding was not associated with a more gradual increase in fat deposition. Instead, this analytic approach identified the first four months as the only period during which both feeding groups
demonstrated notable fat accretion. Beyond the first 120 days of life, both feeding groups followed an overall trend of declining deposition rates.

As there are few studies that describe the developmental trajectories of adipose tissue in the early postnatal period at this time,\textsuperscript{81,82} this is a valuable finding with important public health implications. The present study suggests that patterns of subcutaneous fat accretion may be influenced within the first 120 days by feeding style, with potential risks for later development. Rather than the present single emphasis on the benefits of prolonged exclusive breastfeeding, this study emphasizes the relevance of shifting promotional efforts to encourage greater breastfeeding initiation rates. In the United States, current breastfeeding rates fall below the national goals as outlined by Healthy People 2020.\textsuperscript{83} Relative to the goal of 81.9\% for breastfeeding initiation, a survey in 2010 identified that only 76.5\% of US mothers have initiated breastfeeding.\textsuperscript{84} There is a strong evidence-based argument that efforts need to be augmented to develop strategies that make breastfeeding a more feasible and acceptable feeding choice for a wider range of the population.

\textit{Appetite Modulation as a Mechanism for Altered Deposition Patterns}

Among the potential underlying mechanisms, the development of more sensitive appetite regulatory mechanisms may be an important driving factor for the more stable deposition patterns and slower rates of accretion observed among breastfed infants. As early as three months of age, significant differences in protein and energy intake have been identified among breast and formula-fed infants, with formula-fed infants found to have a 66-70\% higher intake of protein and 15-20\% higher intake of energy within the first six months of life.\textsuperscript{85} While mode of delivery (breast versus bottle) has been proposed as a causal mechanism for these appetitive distinctions, Bartok \textsuperscript{86} found no statistically significant differences between infants fed
breastmilk by bottle or breast in both fat mass and percentage fat mass at any age in the first four months of life. Although this finding does not nullify delivery mode as an important appetite modulator, it emphasizes the relevance of further exploring how the compositional differences of breastmilk and formula may result in differential effects on growth. Additionally, it suggests that delivery mode may not be a predominant confounder in this analysis.

**Trajectories beyond four months as a Reflection of Energy Balance**

From four months of age, formula-fed infants embark on a steeper decline in subcutaneous fat deposition than breastfed infants that continues throughout the first year of life. While this was an unexpected observation for which the present data provides no simple explanation on its own, it is important to note that these statistical findings reflect the different biological trajectory of the two feeding groups. Rather than implying a decline in “growth,” the results reflect the significantly greater deposition rates experienced among the formula feeders during the early months which leads to a peak and relative decline pattern. This is contrary to the more modest accretion and gradual attenuation exemplified by breastfed infants.

To interpret these patterns, it may be useful to consider concepts of energy balance. It is possible that the more passive style of formula feeding, which is characterized by higher levels of maternal control, may result in greater surpluses of energy intake than the more active, infant-driven style associated with breastfeeding. Furthermore, the more consistent flavor profile that is associated with formula consumption may affect infants’ sensitivity to the energy composition of feeds. This may alter infants’ ability to later regulate consumption patterns throughout the day, leading to greater fluctuations of within-day energy balance. While young children have been shown to maintain stable patterns of energy intake across several days, despite the
consumption of high- or low-energy foods, infants who are unable to adjust their intake to match energy differences may experience increases in fat deposition over time.\textsuperscript{89}

\textbf{Strengths and Limitations}

The predominant strengths of the present investigation are the sampling protocol and the analytic methods. Repeated weekly skinfold measurements from seven different subcutaneous sites allowed for both time- and site-specificity in the documented growth patterns, and greater investigation of intra-individual differences. Additionally, the use of a multi-level mixed model that accommodated for random slopes and intercepts, while controlling for weight, birthweight, and number feeding episodes per day, provided a very powerful analytic tool for analyzing this dynamic developmental process.

Despite the unique nature of this sample’s time-intensive data, the relatively small sample size may have made some feeding style effects unresolvable, while over exaggerating others. Moreover, this sample only encompassed clinically normal infants that were predominantly Caucasian and middle class. Hence, these results may not be generalizable to infants with clinical conditions, or those of different ethnic backgrounds, and/or living in poor environmental conditions.

Beyond sample-specific limitations, there are two critical confounding factors in this analysis. One involves the comparison of infants who were breastfed after six months to all other infants in the sample. Due to the small sample size, three infant groups could not be created to address this study’s aim regarding continued breastfeeding. The use of merely two feeding groups confounds the comparison of infants who were breastfed beyond six months to those that were previously breastfed and weaned at six months. The second confounding factor is the possibility that infants who were initially breastfed may have been switched to formula if they
were not displaying healthy growth. Thus, the formula-fed group may include infants who were not growing well due to reasons that are unrelated to feeding style.90

Another important consideration regarding the present results is that due to sample size, sex differences may not have been well-captured. Sex is a well-documented modifier of fat and/or body composition.91 While sex differences were controlled in the analytic model as a nested effect, future investigations would benefit from analyzing the growth trajectories independently by sex. Finally, it is important to note that the reported differences in skinfold deposition or decline between feeding groups may have been statistically significant but may not represent meaningful biological differences. As the risk associated with adipose tissue distribution and cellular mechanisms of expansion ultimately depend on an individual’s overall fat morphology, it unclear whether statistically significant differences in growth patterns align with purported population-level schemas for risk.

Conclusion

Weekly skinfold assessments of seven subcutaneous sites have identified that feeding style predicts differences in deposition patterns in the first year of life. Breastfed infants demonstrated slower rates of both accretion and decline by comparison with their formula-fed peers. Moreover, feeding-specific effects were particularly apparent in the truncal depots. Distinctions in truncal deposition and utilization identified a pattern of ‘risky’ growth that has not been previously documented within the first year of life. As this finding was possible due to this study’s unique analytic approach, future studies may benefit from more time- and depot-specific inquiries. This analysis further suggests that the first four months may be a critical period for subcutaneous fat deposition. Nutrition interventions that promote greater breastfeeding initiation rates may have specific, adipose tissue-mediated long-term benefits for the health of the
population due to modifications occurring during the critical period of adipose tissue deposition in early infancy.
REFERENCES


