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Cohort profile for the Nurture Observational Study examining associations of multiple caregivers on infant growth in the Southeastern USA

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ABSTRACT

Purpose: Childcare has been associated with obesity in children in cross-sectional and longitudinal studies, although some observed no association. Few studies have focused on care during infancy, a period when children may be especially vulnerable.

Participants: The Nurture Study is an observational birth cohort designed to assess longitudinal associations of childcare and the presence of multiple caregivers on infant adiposity and weight trajectories throughout the first year of life. We examine as potential mediators feeding, physical activity, sleep and stress. We completed recruitment in 2015. Of the 860 women who enrolled during pregnancy, 799 delivered a single live infant who met our inclusion criteria. Of those, 666 mothers (77.4%) agreed to participate in the study for themselves and their infants.

Findings to date: Among the 666 women in the study, 472 (71%) identified as black, 127 (19%) as white, 7 (1%) as Asian or Asian American, 6 (1%) as Native American and 49 (7%) as other race or more than one race; 43 (7%) identified as Hispanic/Latina. Just under half (48%) had a high school diploma or less, 61% had household incomes <$20 000/year and 59% were married or living with a partner. The mean (SD) infant gestational age was 41.28 weeks (2.29) and birth weight for gestational age z-score was −0.31 (0.93). Just under half (49%) of infants were females, 69% received some human milk and 40% were exclusively breast fed at hospital discharge. Data collection began in 2013, is currently underway, and is scheduled to conclude in late 2016.

Future plans: Results will help assess the magnitude of associations between childcare in infancy and subsequent obesity. Findings will also inform intervention and policy efforts to improve childcare environments and help prevent obesity in young children.

Strengths and limitations of this study

• Previous cohort studies have not included sufficient representation of participants from racial or ethnic minority groups. Our cohort consists of predominately black women and infants living in the Southeastern USA.

• Results of this study will provide new information on childcare, caregivers and obesity, and may help determine associations in instances where the relationships among these variables have been unclear. Findings will also inform intervention and policy efforts to improve childcare settings and help prevent obesity in very young children.

• Our cohort includes a relatively low-income population of predominately black women. The demographic composition of our sample includes a higher representation of black women than the local population, and results from the study may not be directly generalisable to other populations. It may also limit our ability to compare findings with previous studies examining a similar research question in predominately higher income populations of women.

• A number of women withdrew from the study or could not be reached just after delivery. Consequently, bias from loss to follow-up may be an issue.

• Available funding also limited our ability to collect saliva to measure maternal and infant cortisol on the entire sample. While information on the subsample will be helpful in describing the role that stress plays in the pathway between childcare and obesity, having this information on the larger sample would be ideal.

INTRODUCTION

Childcare attendance has been associated with obesity and weight gain in children in cross-sectional and longitudinal studies in Canada, China, Denmark, the UK, the Netherlands and the USA, although some observed no association. Most of these studies found that less formal types of care, including care by relatives and care in day-care homes, were most often associated with obesity. A study in Japan and another in the USA identified grandparents...
specifically as the informal care providers most associated with obesity in children.\textsuperscript{5,13}

The association between childcare and obesity may depend, in part, on the age of the children in care. Prior studies of infants, including our own work, have linked childcare in the first year of life with obesity.\textsuperscript{1,2,4,5,9} Recently, we examined childcare attendance in infancy and weight outcomes at 12 months in a cohort of Danish children and found that childcare was associated with a higher body mass index z-score and increased risk of obesity at 12 months.\textsuperscript{1} Additionally, in a sample of US children, we found that any type of childcare outside of the home in the first 6 months of life was associated with greater adiposity at 12 months, and this relationship was still evident at 3 years.\textsuperscript{2}

Childcare may promote the development of obesity through the provision of less healthy foods and beverages, shorter durations of breastfeeding and fewer opportunities for children to be physically active. Studies in the USA and the Netherlands found that meals and snacks served in out-of-home childcare often lacked fruits, vegetables and whole grains and included excessive fats and sugars.\textsuperscript{14–18} A study in the USA showed that non-maternal care was associated with decreased odds of continued breastfeeding.\textsuperscript{19} Studies in Canada, Australia, Scotland and the USA demonstrated that children were mostly inactive and engaged in insufficient physical activity in childcare.\textsuperscript{20–25} Media use and screen time can also be excessive in childcare—especially in the less formal types of care.\textsuperscript{26,27}

Childcare may also affect obesity risk through additional pathways such as inadequate sleep, chronic stress and psychological and emotional distress.\textsuperscript{28,29} Short sleep duration has been associated with childhood obesity in numerous studies.\textsuperscript{29–34} Napping during the day while children are in childcare may delay sleep onset at night\textsuperscript{35,36} and decrease the duration and quality of night-time sleep.\textsuperscript{37–39} Childcare outside of the home has been associated with greater stress in children in a number of prior studies,\textsuperscript{40–42} and many previous studies have shown a potential link between elevated cortisol levels and obesity.\textsuperscript{39,40}

For the ongoing Nurture Study, we will assess the relationship between childcare and obesity and examine specific factors that may influence this relationship, especially the role of multiple caregivers, in a sample of children from birth to 12 months of age. Assessing the impact of childcare requires us to examine all childcare providers—adults other than parents—caring for infants. Our central hypothesis is that energy intake, energy expenditure, stress and sleep, all influence the development of obesity, and that these behaviours can be modified to prevent excessive weight gain in children (figure 1). Results of this study will provide new information on childcare, caregivers and obesity, and may help determine associations in instances where the relationships among these variables have been unclear. Findings will also inform intervention and policy efforts to improve the childcare system and help prevent obesity in very young children.

**COHORT DESCRIPTION**

**Study purpose and setting**

The Nurture Study is an observational birth cohort designed to assess longitudinal associations of early childcare and multiple caregivers on infant adiposity and weight trajectories throughout the first year of life. We are specifically interested in the potential mediators of feeding, physical activity, sleep and stress. The Nurture Study takes place in the Southeastern USA. We recruited and enrolled women in mid-pregnancy to late-pregnancy from a private prenatal clinic and the local county health department prenatal clinic in Durham, North Carolina, USA.

**Study population**

To minimise attrition, pregnant women were required to be 20–36 weeks gestation, be pregnant with a singleton with no known congenital abnormalities, be at least 18 years of age, speak and read English, intend to keep the baby and plan to stay within the area until at least 12 months post partum. Shortly after delivery, women were required to reconfirm their interest in participating in the study through a second consent process. At that time, we excluded infants who were born prior to 28 weeks gestation, had congenital abnormalities that could affect growth and development, were in the

![Figure 1](nurture_conceptual_model.png)
hospital for 3 or more weeks after birth or were not able to take food by mouth at the time of hospital discharge.

We began recruitment in 2013 and recruited our final participant in early 2015. We enrolled approximately one out of every two eligible women we approached in the prenatal clinics. Of the 860 women who enrolled during pregnancy, 799 delivered a single live infant who met our inclusion criteria. Of those, 42 withdrew from the study and 91 were lost to follow-up (unable to reach) to consent after birth, leaving a final sample size of 666 mother/infant dyads (77.4%). Using data collected from all women at enrolment in pregnancy, we compared the 666 women included in the sample with the 133 who withdrew or were excluded. Fewer women included in the sample were Hispanic/Latina (6.5% vs 12.0%, p=0.04) and fewer were pregnant with their first child (37.0 vs 49.1, p=0.04). Women included were somewhat older (27.1 vs 25.9 years, p=0.02) and more included women had a partner (59.5% vs 50.0%, p=0.04). The included women were comparable with respect to other covariates such as prepregnancy BMI, race, education or household income.

When infants were 6 months of age, we invited, with the permission of the mother, fathers and other partners, resident (living with mother and infant) and non-resident (living elsewhere), to participate in the study. We asked all 666 women if they would like us to contact the father of the baby. Of those 666, 385 women reported that the father was involved in the baby’s life and gave us permission to contact him. The remaining 281 women did not want us to contact the father of the baby about the study. Of the 385 mothers who approved and provided contact information, 271 fathers enrolled in the study. We also invited other non-parental caregivers to participate, defined as any adults who cared for infants 3 or more hours per week on a regular basis. Non-parental caregivers included family members, friends, neighbours, nannies, babysitters and childcare providers, regardless of payment for this care. We enrolled a total of 33 formal and 223 informal non-parental caregivers into the study, with an average of 1.5 per infant.

We obtained written informed consent from each woman at recruitment into the study during pregnancy and then confirmed participation of mother and infant shortly after delivery. Fathers and other caregivers provided consent to participate in the study through completion of the questionnaire.

Data collection and measures

Overview

We conducted home visits when infants were 3, 6, 9 and 12 months of age (figure 2). Each visit lasted ~1.5 hours, although the visits at 6 and 12 months could take up to 3 hours due to additional assessments. Women received automated interactive voice response (IVR) telephone calls in months 1, 2, 4, 5, 7, 8, 10 and 11 to assess a limited set of behaviours, including care-giving arrangements, infant feeding practices, infant motor milestone achievement and infant sleep (figure 2). Data collection began in 2013, is currently underway, and is scheduled to conclude in late 2016.

Exposures

Our primary exposures were infant childcare and care-giving arrangements, assessed longitudinally throughout the first year of life using questions from the Early Childhood Longitudinal Survey—Birth Cohort. We asked mothers to provide detailed information about any non-parental caregivers (ie, family members, friends, neighbours, nannies, babysitters and childcare providers, regardless of payment) who interacted with and cared for their infants for 3 or more hours per week on a regular basis. We assessed the type and location of care, the hours per week in each type of care and the age when care started and stopped, by month. We asked detailed questions at each home visit when infants were 3, 6, 9 and 12 months. We also asked a more abbreviated set of questions during the IVR telephone calls at months 1, 2, 4, 5, 7, 8, 10 and 11.

Outcomes

The main outcome was infant adiposity measured via weight, length and skinfold thickness at each home visit. We were also interested in infant weight trajectories over the course of the 12 months. Trained data collectors measured infant recumbent length to the nearest one-eighth inch using a ShorrBoard Portable Length Board and weight using a Seca Infant Scale to the nearest 0.1 pound. We had study scales professionally calibrated annually. We monitored the scales with calibration weights every 2 weeks to check for drift. If the weighed value deviated from the expected value more than ±0.091 kg of the calibration weight, we had the scale professionally calibrated. Infants were weighed and measured in light clothing without shoes. We calculated age-specific and sex-specific weight-for-length z-scores using WHO reference standards. We measured the abdomen, subcapular and triceps skinfold thicknesses to the nearest 0.2 mm using standard techniques. We used the sum of subcapular and triceps (SS+TS) skinfold thickness as a proxy for overall fatness, and the ratio of subcapular to triceps (SS:TS) skinfolds thickness as a proxy for centrally deposited fat. We conducted all assessments in triplicate and used an average of the three measurements. Body composition measures like skinfolds and weight-for-length are important anthropometric measures because they can be easily implemented in large-scale studies using portable equipment. However, these body composition measures vary in their relation to body fat mass and adiposity. Despite these limitations, the WHO includes child growth standards for skinfolds and weight-for-length values and highlights these body composition measures as international tools for growth and nutritional assessment.
Potential mediators: feeding, physical activity, sleep and stress

Feeding: Mothers reported infant feeding method as formula only, mixed feeding (some formula and some human milk) or exclusive breastfeeding and the introduction of solid foods to infants via questionnaire at each home visit and IVR call. We also asked mothers to document all foods and beverages provided to infants via questionnaire at each home visit using questions from the Infant Feeding Practices Study II and the Feeding Infants and Toddlers Study. Women completed the Infant Feeding Style Questionnaire (IFSQ) at each home visit, and fathers and caregivers completed the IFSQ at 6 and 12 months only. The IFSQ is a 63-item questionnaire that assesses infant feeding beliefs and behaviours. The IFSQ was developed by Thompson et al. and was tested for reliability and validity in a sample of predominately low-income black women living in North Carolina. We measured food security status at enrolment in pregnancy and again at 3, 6, 9 and 12 months using the US Household Food Security Survey Module: Six-Item Short Form. Women received a food security score ranging from 0 to 6, which we dichotomised into high or marginal food security (0–1) and low food security (2–6) consistent with scoring protocol and recent literature.

Physical activity: Infants wore an ActiSleep+ device (Actigraph, Pensacola, Florida, USA) continuously on the left ankle for 4 full days (96 hours) continuously over 2 week days and 2 weekend days using a 30 Hz sampling rate. The ActiSleep+ is water resistant and uses a three-axis Medication Event Monitoring System (MEMS) accelerometer. Many researchers, including ourselves, have used accelerometers to assess physical activity in young children, but only a few have used these devices with infants. We recognise that infant movement may be generated and controlled by outside forces (e.g., the mother may be carrying the infant, causing movement, but not energy expenditure in the baby); therefore, although we believe we can contribute valuable insights to physical activity through this physical activity assessment, we approach with caution. These data will provide information on infant activity to the extent possible, as the accelerometer does not specify whether an infant is engaging in movement themselves or an adult is moving the infant. The accelerometer will still, however, yield important data on infant inactivity.

Additionally, to assess infant fine and gross motor development, we conducted the Bayley Scales of Infant and Toddler Development: Third Edition during each home visit at 3, 6, 9 and 12 months. The Bayley Scales of Infant and Toddler Development assesses large muscle coordination, movement and balance, is tailored to the age of the infant and includes item sets corresponding to our ages for assessment. Obesity in infancy may delay achievement of gross motor milestones, and infants who attain motor milestones at later ages may be heavier or less physically active later in childhood. We also assessed maternal report of time infants spent in...
restrictive devices (eg, car seats, strollers) via questions that the study team developed, as existing scales or assessment instruments were not available. Mothers and fathers completed the Rothbart Infant Behaviour Questionnaire, Revised Very Short Form when infants were 6 and 12 months to assess parents’ perceptions of infant behaviour and temperament. We also asked mothers to report infant screen time using questions from the Children’s Media Use in America at each home visit at 3, 6, 9 and 12 months.

Sleep: In addition to providing information about physical activity, the ActiSleep+ device worn by infants for 4 continuous days and nights also provides information on sleep during that time. These accelerometer data will yield average daily sleep duration, night-time sleep, night wakings, daytime sleep and nap frequency and duration. Using accelerometers to assess sleep in children is consistent with a handful of other paediatric studies. However, given that actigraphy has not been used extensively to measure sleep in infants and has some potential limitations, we added additional maternal report of infant sleep duration. Mothers reported their infants’ average daily sleep duration at each home visit and IVR call. Mothers completed the Children’s Sleep Wake Scale and the Brief Infant Sleep Questionnaire at each home visit.

Stress: We measured salivary cortisol as a proxy for stress in a subsample of 25 mothers and infants. Assessing cortisol in saliva is a widely accepted technique in children and adults, and researchers report a strong positive correlation between salivary and serum cortisol. Assessment via saliva has the advantage of eliminating stress introduced through blood draw and enabling mothers to collect the samples themselves for their infants at home in a more natural setting. Mothers collected four saliva samples from themselves and their infants over the course of 3 days (2 week days and 1 weekend day), documenting the time of day each sample was collected, and any potential problems with the collection. Mothers collected samples on waking, 30 min after waking, at 17:30 or when she retrieved her infant from childcare and at bedtime. Mothers collected infant samples by absorbing saliva from the back side of the mouth or under the tongue for 90 s until the swab was saturated using a SalivaBio Children’s Swab (Salimetrics, State College, Pennsylvania, USA). We asked mothers to wait until after the first morning collection to feed infants, and to rinse and wipe their mouths prior to the second morning collection.

Mothers collected their own saliva samples by placing the SalivaBio swab under their tongues for 90 s. We asked mothers to avoid lotions, makeup, toothpaste, foods and beverages prior to the collection, and to refrain from cigarette smoking and drinking alcohol. We also requested that mothers wait at least 60 min after eating or consuming caffeine before collecting the 17:30 or bedtime sample. We provided mothers with compliance boxes that recorded the time and date whenever the lid was opened. These compliance boxes functioned like a MEMSCap, but were larger and designed to hold the supplies needed for multiple days of saliva collection. Mothers stored the samples in home freezers until a study team member returned to retrieve and transfer them to a laboratory to be stored in a −80°C freezer until being shipped in batches to the Salimetrics (Salimetrics, State College, Pennsylvania, USA) laboratory for analysis. This protocol is consistent with other studies examining changes in cortisol throughout the day for children in childcare.

Other measures
We recorded medical history, pregnancy and birth outcomes via electronic medical record review, interviews and questionnaires. Infant variables of interest included race, sex and birth weight for gestational age as a continuous z-score. We collected demographic information from mothers via interviews and questionnaires at recruitment, at birth and during each home visit and IVR call. Maternal variables of interest included race, ethnicity, age, parity, education and prepregnancy BMI. We weighed mothers to the nearest 0.1 kg at each home visit using a Tanita BWB-800 Scale. We measured height at the first home visit only, to the nearest 0.1 cm using a Seca stadiometer. At enrolment and at each home visit we asked mothers to report household income, and whether they participated in the Special Supplemental Nutrition Program for Women, Infants and Children (WIC) programme during the past 3 months. Mothers completed the Healthy Home Survey to describe the home environment as it relates to feeding and physical activity at 6 months only. We included a number of additional measures to assess stress within the home. We measured household chaos using the Confusion, Hubbub and Order Scale (CHAOS) at each home visit. Mothers also completed the Edinburgh Postnatal Depression Scale and the Perceived Stress Scale. We referred mothers to a clinician with expertise in postpartum depression if needed based on questionnaire responses. Fathers completed the Perceived Stress Scale at 6 and 12 months only. Fathers and caregivers provided demographic information (eg, race, ethnicity and age) at 6 and 12 months. Fathers also completed multiple assessments on engagement and involvement in infant caretaking at 6 and 12 months.

Analysis and sample size calculation
Serial measurements collected over the course of the first year of life will allow us to address the primary aims of the study, namely to characterise temporal trends in these variables, as well as the temporal role played by the potential mediators. We will use Bayesian linear mixed models to analyse these data. Given careful choice of the prior distribution, Bayesian methods can overcome the unreliability of likelihood-based variance components estimators. As most of the measurements will be collected in a serial manner, we will fit models with serially correlated, within-subject innovations; we will assume a first-order
autoregressive (AR1) structure, but will verify robustness and goodness of fit to alternative specifications, including independence. We will investigate transformations of the adiposity measures to normality as necessary to facilitate normal theory analyses and will consider complementary summaries of the primary exposure variables, such as age-at-onset of non-parental childcare. In particular, we will fit longitudinal mixed-effects models with subject-specific outcomes measured at 3, 6, 9, 12 months. Variables that are measured monthly through IVR calls will be averaged to create a quarterly exposure value. We will model our outcome measures of weight-for-length z-score and skinfold thicknesses separately, but will experiment with a joint model for the two outcomes. We will include important potential covariates like birth weight for gestational age z-score and breastfeeding duration in all models to help address selection bias.

To estimate power and sample size, we assumed a two-sided, level 0.05 test, and estimated the power to detect a 0.09 increase in the 1-year WFL z-score for each 10-hour/week increment spent in childcare to be 0.990 via simple linear regression modelling. We have based these estimates on our previous study of infants in childcare.\(^2\) Given that frequent, longitudinal data collection will result in less recall error and an improved ability to characterise within-subject and between-subject variability (due to the finer scale of measurement), we anticipated even greater power to detect group differences. For purposes of these calculations, we assumed that WFL z-score is linear in time/week (TPW) (in fractions of 10-hour increments) with baseline (TPW=0, i.e., children not in childcare) level (y-intercept) =0.27, slope =0.09 and residual SD=1.01. Therefore, 666 (77.4\%) of the 860 infants will yield power >90\% to detect differences in WFL z-score if we are able to retain at least 85\% through 12 months.

**FINDINGS TO DATE**

Among the 666 women included in the study, 472 (71\%) identified as black, 127 (19\%) as white, 7 (1\%) as Asian or Asian American, 6 (1\%) as Native American and 49 (7\%) identified as Hispanic/Latina (Table 1). Just under half of women (48\%) had a high school diploma or less, 61\% had household incomes <$20 000/year and 59\% were married or living with a partner. Of the 666 women, 161 (24\%) were food insecure in pregnancy. The mean (SD) gestational age was 41.28 weeks (2.29) and infant birth weight for gestational age z-score was $-0.31$ (0.93). Just under half (49\%) of infants were females, 69\% received some human milk and 40\% were exclusively breastfed at hospital discharge.

**STRENGTHS AND LIMITATIONS**

When the Nurture Study is completed, we expect to describe the relations between childcare in the first year of life, caregivers and infant adiposity. If we find that infants in childcare or with multiple caregivers are heavier than those cared for by a parent, we also hope to identify and quantify the modifiable risk factors that mediate the relationship, and assess whether these factors differ by type and location of care, or age of care onset. Previous cohort studies have either not included sufficient representation of children from racial or ethnic minority groups, or have not asked detailed

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<th>Table 1 Characteristics of mothers and infants participating in the Nurture Study (n=666)</th>
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<td><strong>Characteristics</strong></td>
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<td>Mothers’ characteristics</td>
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<td>Exclusive breastfeeding at discharge</td>
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WIC, Special Supplemental Nutrition Program for Women, Infants and Children.
questions about childcare and care-giving arrangements. Additionally, we used objective measures where possible (eg, sleep and physical activity assessment) rather than relying on maternal report, to help minimise social desirability bias and recall bias. Findings from this study may have important public health and childcare implications, as they will inform policy and intervention efforts to improve this setting where the majority of US infants spend time.

Our study has some limitations. Our cohort includes a relatively low-income population of predominately black women. The demographic composition of our sample includes a higher representation of black women than the local population, and results from the study may not be directly generalisable to other populations. We may also be limited in our ability to compare findings to previous studies examining a similar research question in predominately higher income populations of women. But, our population is a priority, given the high prevalence of obesity. Additionally, 6% of infants did not meet inclusion criteria and 16.6% of women withdrew from the study or could not be reached just after delivery. Consequently, bias may influence our results. Funding also limited our ability to collect saliva to measure maternal and infant cortisol on the entire sample. While information on the subsample may be helpful in describing the role that stress plays in the pathway between early childcare and obesity, having this information on a larger sample or on the entire sample would be ideal. However, we did not conduct a power analysis to determine whether we will be able to answer our intended research question. In fact, given the small sample size, we likely have insufficient power to analyse the cortisol data to answer the research question. All analyses we conduct will be exploratory in nature and presented with the noted limitation of sample size. Additionally, data from the cortisol assessment can provide information about the feasibility of conducting this measure in a future study. The cortisol assessment was complicated by the fact that mothers had to collect their own saliva samples and that of their infants at three different scheduled time points throughout the day over 4 days. This is a difficult task and makes compliance challenging. Feedback from mothers in the Nurture Study who collected saliva samples will help improve and refine this assessment for future studies. Finally, our sleep and physical activity assessments are based, in part, on actigraphy. While this is a highly novel approach, there are limitations and challenges associated with accelerometer data in infants.62

Obesity rates have increased globally over the past decade, even in young children.87–89 Rates of obesity are even greater in high-income countries, where childcare outside of the home is more common.90–91 Obesity in early childhood has been linked to chronic health conditions such as diabetes mellitus and cardiovascular disease in adulthood.92–93 Even in infancy, excessive weight gain is associated with later obesity.94–96 Frequent assessments at four time points throughout infancy may help pinpoint when children begin to gain weight excessively and help identify risk factors associated with obesity. Infancy appears to be a window of opportunity for the prevention of obesity and the Nurture Study will provide important information about childcare, caregivers and weight gain throughout the first year of life.

COLLABORATION

When the proposed study is complete, we will have data on infant care-giving arrangements and childcare outside of the home, infant weight and adiposity over the first year of life, and information on the potential mediators of feeding, physical activity, sleep and stress (subsample only). Data will be available in late 2017 and may be available to other researchers interested in these variables.


