Different Growth Patterns Persist at 24 Months of Age in Formula-Fed Infants Randomized to Consume a Meat- or Dairy-Based Complementary Diet from 5 to 12 Months of Age

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Objective To test the long-term effect on growth status at 24 months of age in formula-fed infants who were randomized to consume a meat- or dairy-based complementary diet from 5 to 12 months of age.

Study design Observational assessments, including anthropometric, dietary, and blood biomarkers, were conducted at 24 months of age, 1 year after the intervention ended.

Results The retention rate at 24 months of age was 84% for the meat group and 81% for the dairy group. Mean (±SD) protein intakes at 24 months of age were 4.1 ± 1.2 and 4.0 ± 1.1 g/kmeat (n = 27) and dairy (n = 26) groups, respectively, and comparable with the estimates of US population intake. At 24 months of age, weight-for-age z score did not differ significantly between groups and was similar to that at 12 months. Length-for-age z score remained significantly higher in the meat group compared with the dairy group, and the average length was 1.9 cm greater in the meat group. Weight-for-length z score also did not differ significantly between groups. Insulin-like growth factor 1 significantly increased from 12 to 24 months of age in both groups, but insulin-like growth factor-binding protein 3 and blood urea nitrogen did not change significantly from 12 to 24 months of age and were comparable between groups.

Conclusions The protein source-induced distinctive growth patterns observed during infancy persisted at 24 months of age, suggesting a potential long-term impact of early protein quality on growth trajectories in formula-fed infants. *(J Pediatr 2019;206:78-82).*

Trial registration ClinicalTrials.gov: NCT02142647.

Complementary foods are the first source of diet diversification in infants as they transition from sole intake of breastmilk and/or formula. Evidence-based dietary recommendations of complementary feeding are critical to informing feeding practices, which, in turn, may affect growth trajectory and later obesity. Among various dietary determinates of growth, protein has been of special interest and consensus holds that the high protein content in infant formula drives the observed accelerated weight gain in formula-fed infants.[1] Moreover, protein quantity during complementary feeding was also found to be associated with increased overweight risk and later obesity. One epidemiologic study in the United Kingdom showed that a higher proportion of energy from protein during complementary feeding was associated with higher weight and body mass index (BMI)[2]; similar results were found in other studies.[3,4] Overall, it appears that there is a positive association between protein quantity and weight status early in life and the current dietary recommendation is to limit protein intake to 15% of energy during the first 2 years of life, without clear distinction for type of protein.[5]

Emerging research suggests differential impact on growth and weight gain in infants and young children consuming protein from meat, dairy, and plant sources. In 1 observational study, dairy protein intake at 12 months of age was associated with BMI at 7 years of age, and protein from meat, plants, or cereal did not have the same effect,[6] and it was the dairy protein that drove the significant association between protein intake and later BMI.[6] Our group recently conducted a small randomized controlled trial that directly compared a high-protein, meat- vs dairy-based complementary diet in formula-fed infants from 5 to 12 months of age.[7] We found that the dairy group had a relative deceleration of linear growth (decrease of length-for-age z score [LAZ]), and the meat group had an increase in linear growth (increase of LAZ). This distinctive linear growth pattern, plus the similar increases of weight and weight-for-age z score (WAZ) for both groups, led to a significant increase of 0.76 of weight-for-length z score (WLZ)

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BMI	Body mass index
BUN	Blood urea nitrogen
CTRC	Children's Hospital Colorado Clinical and Translational Research Center
IGFBP3	Insulin-like growth factor-binding protein 3
LAZ	Length-for-age z score
WAZ	Weight-for-age z score
WLZ	Weight-for-length z score

in the dairy group from 5 to 12 months of age.[7] Here we present the follow-up assessment on growth, dietary intake, and blood biomarkers of this cohort at 24 months of age. Our primary hypothesis is that the observed growth patterns during the intervention will persist at 24 months of age.

Methods

Details of the study design and results of the intervention phase from 5 to 12 months of age were previously reported.[7] In brief, this study was a stratified, randomized controlled trial using semicontrolled feeding. Healthy 5-month old formula-fed infants were provided the same cow milk-based infant formula and consumed a complementary diet with meat or dairy as the predominant protein source from 5 to 12 months of age; total protein intake was ~ 3 g/kg/day and equal to 15% of total energy. Diet records showed that during the intervention, over 90% of protein from solid foods was from the assigned protein group and <5% was from the alternative. The primary outcome was growth, measured as longitudinal change in weight (kg), length (cm), and respective age- and sex-specific z scores. Growth indices were measured at baseline, end of intervention, and 7, 8, 9, 10, and 11 months of age during the intervention. Blood samples were collected at 5 and 12 months of age to measure selected biomarkers. The study was approved by the Colorado Multiple Institutional Review Board and registered at ClinicalTrials.gov (NCT02142647).

Follow-Up Assessment

The follow-up visit was conducted at 24 months of age, from June 2016 to August 2017. Parents or caregivers of the participants who completed the intervention were contacted by phone or email when the participant was between 23 and 24 months of age. Participants visited the Children's Hospital Colorado Clinical and Translational Research Center (CTRC) at 24 months of age and signed the consent for this visit. During this visit, length, weight, and blood samples were collected by the trained CTRC research nurses who were blinded of the group assignment; and all anthropometric measurements were performed in triplicate. Length was measured in recumbent position using an infantometer accurate to 0.1 cm (Holtain Ltc; Crosswell, Crymych, Pembs, United Kingdom). An electronic digital scale (Sartorius Corp, Bohemia, New York) was used to obtain naked weight. Parents also completed a 3-day diet record. Diet records were analyzed by the CTRC nutrition core for total calories and macronutrient contents (NDSR software; Nutrition Data System for Research, Minneapolis, Minnesota). Total protein intake was further broken down to various sources. After all the participants' 3-day food records were entered into NDSR, a CTRC dietitian exported the component/ingredient file. This file provided macro- and micronutrient data for each ingredient in the diet records, including grams of animal protein and plant protein. From this file, each participant's data were further separated into individual protein sources: liquid dairy (cow's milk), solid dairy (yogurt, cheese), meat (chicken, beef, pork, lamb), fish, egg, and plant. Diet diversity score was also calculated at 24 months

of age, based on the World Health Organization infant and young child minimum dietary diversity score.[8] Blood samples were centrifuged and serum was stored at −80°C until analyzed. The following markers were analyzed by the CTRC core laboratory: insulin-like growth factor 1 (IGF-1, chemiluminescence, DiaSorin Liaison, Cypress, California), insulin-like growth factor-binding protein 3 (IGFBP3, chemiluminescence, Siemen, Washington, DC), and blood urea nitrogen (BUN). The between assay precisions were <2.7% for IGF-1, <4.0% for IGFBP3, and <4.5% for BUN.

Statistical Analyses

Statistical analyses were performed using SAS v 9.3 (SAS Institute Inc, Cary, North Carolina). Results of growth z scores, dietary intakes, and blood biomarkers data are presented as mean ± SD. Repeated measures ANOVA (PROC GLM) were used to evaluate the main effects of time (12-24 months of age), group, and their interactions on the dependent variables between 12 and 24 months of age. The Student t test was used to compare values between groups at 24 months of age. All model assumptions were checked and P value of <.05 was considered statistically significant.

Results

Among all participants who completed the intervention (32 per group), 26 from the dairy group and 27 from the meat group completed the follow-up visit. The retention rate was similar between groups (meat: 84%; dairy: 81%). Of the 11 infants who did not complete the follow-up, 8 moved out of the state or country and 3 lost contact. Subject characteristics (birth weight, length, gestational age, and sex) did not differ between those who did not complete the study vs those that completed. Table I summarizes the characteristics of those participants who completed the 24-month visit.

Dietary Intake, Including Protein Sources

Three-day diet records were collected on 19 subjects from the meat group and 18 from the dairy group. At 24 months of age, all participants consumed whole- or low-fat (2% fat) cow's milk, except for one from the dairy group who still consumed infant

Table I. Subject characteristics*

	Meat[†] (n = 27)	Dairy[†] (n = 26)	P value
Sex[‡]	48% male	49% male	.67
Birth weight[§] (kg)	3.33 ± 0.35	3.28 ± 0.45	.65
Gestational age[§] (wk)	39 ± 1	39 ± 1	.77
Maternal BMI[§]	27 ± 7	27 ± 6	.70
Maternal height[§] (cm)	167 ± 6	166 ± 8	.22
Maternal age[§] (y)	30 ± 6	30 ± 7	.53
24 mo of age weight[§] (kg)	12.6 ± 1.0	12.4 ± 1.5	.38
24 mo of age length[§] (cm)	89.0 ± 2.3	87.1 ± 3.3	**.02**

*Mean ± SD.
†Meat: the meat-based complementary diet group; Dairy: the dairy-based complementary diet group.
‡χ² test.
§Independent Student t test.

Table II. Macronutrient distributions and caloric intakes at 24 months of age*

	Meat[†] (n = 19)	Dairy[†] (n = 14)	P value
Total calories (kcal/d)[‡]	1350 ± 565	1150 ± 370	.21
Total calories[‡] (kcal/kg/d)	106 ± 48	97 ± 27	.29
Carbohydrate[‡] (g/kg/d)	13.3 ± 5.1	12.4 ± 3.9	.55
Carbohydrate[‡](%energy)	50 ± 8	53 ± 6	.32
Fat[‡] (g/kg/d)	3.8 ± 1.2	3.4 ± 1.1	.23
Fat[‡] (%energy)	35 ± 7	32 ± 5	.12
Protein[‡] (g/kg/d)	4.1 ± 1.2	4.0 ± 1.1	.45
Protein[‡] (%energy)	16 ± 3	16 ± 3	.52

*Mean ±SD.
†Meat: the meat-based complementary diet group; Dairy: the dairy-based complementary diet group.
‡Independent Student t test.

formula. Total protein intake was 4.06 ± 1.21 and 4.00 ± 1.10 g/kg/day for meat and dairy groups, respectively. Macronutrient distributions and total caloric intakes are presented in **Table II** and showed no difference between groups in macronutrient or caloric intakes. Total protein intakes were further categorized to various sources (**Figure 1**). Overall, there was no difference between groups for any source of protein consumed, except for egg protein, which was higher in the meat group (0.24 ± 0.27 g/kg/day) than in the dairy group (0.10 ± 0.15 g/kg/day) (P = .04). No difference of reported diet diversity was found between meat and dairy. The average scores were 4.74 ± 1.07 and 4.17 ± 1.01 for meat and dairy groups, respectively (P = .11).

Anthropometric Measurements at 24 Months of Age

Growth z scores (WAZ, LAZ, and WLZ) of the intervention and the follow-up are summarized in **Figure 2**. Data from the intervention (5 and 12 months of age) include only participants who completed the follow-up assessment at 24 months of age, and all the z scores were considered well within normal
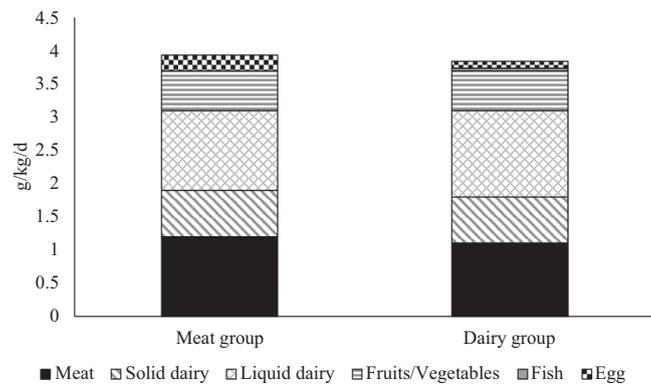
Figure 1. Protein intake at 24 months of age from different sources between groups. Meat n = 19; dairy n = 18; By independent Student t test between groups, only fish intake was different between groups and was higher in the meat group (P = .04).
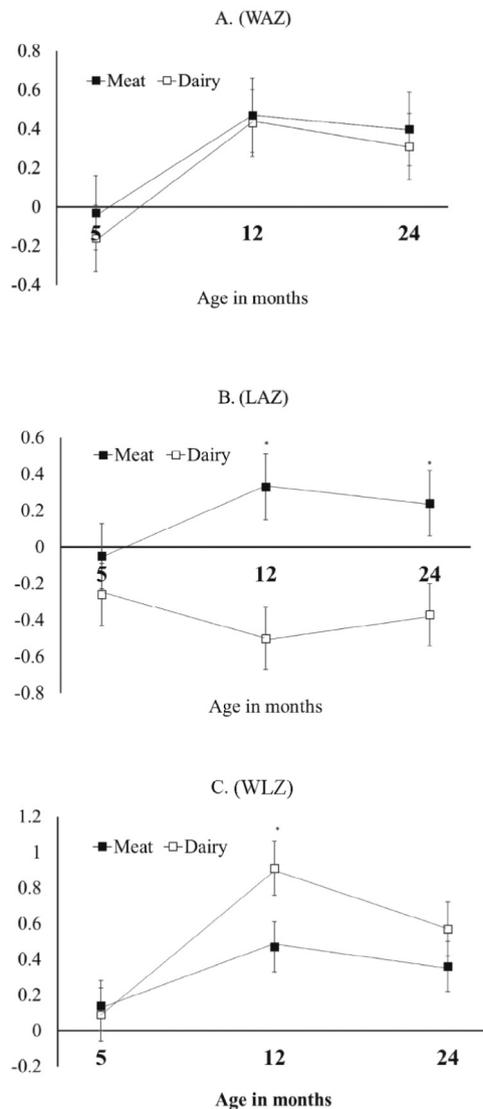
Figure 2. Growth z score at 5, 12, and 24 months of age for participants who completed the 24- month assessment. Repeated measure ANOVA compared time (12 and 24 months of age), group (meat and dairy), and their interactions. Independent Student t test was used to compare between groups at 24 months of age. **A,** No significant differences for WAZ between groups at 24 months of age (P = .65) or over time (effect of time P = .42). **B,** Significant difference of LAZ at 24 months of age (P = .01) but no change from 12 to 24 months of age for either groups (effect of time P = .44). **C,** No significant difference between groups of WLZ at 24 months of age (P = .40) or change over time (effect of time P = .15).

range. WAZ did not differ significantly between groups at 24 months of age (P = .65) or change significantly from 12 to 24 months of age (effect of time P = .42). At 24 months of age, LAZ was still significantly higher in the meat group (0.19 ± 0.62), compared with the dairy group (−0.37 ± 0.88, P = .01). Although the change in LAZ did not statistically differ from 12 to 24 months of age for either diet group (meat: P = .33; dairy: P = .23), the dairy group had a slight increase of LAZ

Table III. IGF-1, IGFBP3, and BUN concentrations at 24 months of age in meat and dairy groups*

	IGF-1 (ng/mL)	IGFBP3 (ng/mL)	BUN (mg/L)
Normal range	29-186 (male) 19-147 (female)	1269-4104 (male) 1198-4182 (female)	7-26
Meat[†] (n = 18)	106 ± 31	2784 ± 498	15 ± 3
Dairy[†] (n = 16)	97 ± 29	2505 ± 462	14 ± 3
P value between groups	.44	.13	.49

*Mean ±SD.
†Meat: the meat-based complementary protein group; Dairy: the dairy-based complementary protein group.

(+0.13 ± 0.12). This increase of LAZ, although not significant, produced WLZ values at 24 months of age that no longer differed between groups (P = .40, **Figure 2**). Weight (kg) and length (cm) between groups at 24 months of age are presented in **Table I** and consistent with the growth z scores. The average length was 1.9 cm higher in the meat group compared with the dairy group.

Blood Biomarkers

Table III shows IGF-1, IGFBP3, and BUN concentrations by group. At 24 months of age, there were no significant differences in the IGF-1, IGFBP3, or BUN values between the meat and dairy groups. IGF-1 significantly increased from 12 to 24 months of age in both groups (effect of time P = .002) but no significant change was observed for IGFBP3 or BUN. Each of the average biomarker values remained within the normal range for this age.

Discussion

Protein intake from complementary foods during infancy has important implications for growth. Our randomized controlled trial of formula-fed infants showed greater length gain in those who consumed meat compared with dairy from 5 to 12 months of life.[7] This follow-up study at 24 months of age further demonstrated that the growth pattern observed during infancy persisted at 24 months of age, 1 year after the end of the intervention. Although LAZ did not differ between 12 and 24 months of age for either diet group, the slight (nonsignificant) increase of LAZ in the dairy group made WLZ no longer different between groups at 24 months of age (**Figure 2**, C). LAZ was still significantly higher in the meat group compared with dairy at 24 months of age and the length difference between groups was comparable at 12 months of age (1.8 cm)[7] and 24 months of age (1.9 cm). These findings were consistent with our hypothesis that the protein-induced growth patterns at 12 months of age would persist after the intervention ended and further underscored the importance of early dietary patterns, especially protein quality, on long-term growth. Several studies have also shown that diet-induced weight gain during infancy has a prolonged impact on long-term growth and health.[9-11]

One potential mechanism of protein intake and accelerated weight gain is the "early protein hypothesis," which proposes that high protein intake increases circulating IGF-1 concentration, which enhances growth and weight gain.[12] However, IGF-1 had a similar increase from 12 to 24 months of age in both groups in the present study and cannot explain the persistent differential linear growth patterns between groups. If it was indeed the effect of protein source consumed, dietary intakes may provide some insights. During the intervention, participants did not consume the assigned alternative protein source. For example, infants from the dairy group consumed a minimum amount of meat (2%) from solid foods and vice versa.[7] After the intervention ended at 12 months of age, consumption of protein from meat or dairy sources were no longer restricted, and this resulted in a relative increase of meat consumption in infants from the dairy group. The 3-day diet records at 24 months of age showed that the infants consumed protein from various sources including both meat and dairy, and there were no significant differences between groups (**Figure 1**). Specifically, both groups consumed ~1 g/kg/day of protein from meat. This increase in meat intake in the dairy group after the intervention could potentially explain the improved linear growth in the dairy group at 24 months of age.

Research, especially randomized controlled trials, is quite limited for the association between protein intakes on infant growth during complementary feeding. A previous cohort of breastfed infants from Denver by our research team showed that consuming a meat-based complementary diet (2.7 g /kg/day) increased LAZ by an average of 0.27 and a low protein, cereal-based diet (1 g/kg/day) was associated with a decline in LAZ by an average 0.33 over 3 months (6-9 months of age).[13] Another study that randomized breastfed infants to consume a low-meat (19% energy) vs high-meat (21% energy) diet from 4 to 10 months of age found no difference in length or weight gain.[14] Similar intervention trials also did not find differences between high- vs low-meat consumptions on growth in infants.[15,16] These studies, however, had iron status as the primary outcome, not growth,[14-16] and the differences in protein quantity between groups were usually small. Thus, more high-quality research during the complementary feeding period is needed to elucidate the role of protein quality with growth as the primary outcome.

Participants at 24 months of age consumed a quantity of protein similar to the national average in the US. Data from the 2016 National Health and Nutrition Examination Survey (NHANES) showed that infants aged 12-23 months consumed an average of 4.1 g/kg/day of protein,[17] and the participants in our study consumed ~4 g/kg/day at 24 months of age. In addition, the most recent findings from the Feeding Infants and Toddlers Study[18] also reported that the median total protein intake for 12-23.9 months of age was 46 ± 0.4 g/day and the 75th percentile was 54 g/day, and in our study it was 49 ± 2 g/day at 24 months of age. Thus, overall, the amount of protein consumed at 24 months of age was comparable between diet groups and similar to national average for the US, suggesting that protein quantity at 24 months of age may not contribute to the persistent growth patterns observed from 12

to 24 months of age. In terms of carbohydrate, fat, and energy, infants from the present study also had comparable intakes to the reported values in the Feeding Infants and Toddlers Study.[18]

One of the strengths of the study was that the research nurses at Children's Hospital Colorado, who were blinded to the group assignment, conducted the anthropometric measurements for the 24-month visits. In addition, the protein source breakdown from the 3-day diet record was helpful to demonstrate protein intakes from meat, dairy, and other common sources for this age group, although the dietary data was not from the full 24-month sample. One limitation was that we did not include a reference breastfed group for comparison with our participants who were formula-fed during infancy. Further, 6 participants from the dairy group and 5 participants from the meat group were lost to follow-up, which could create potential confounding, although subject characteristics at baseline did not differ between the participants who completed the study and those who did not. Finally, LAZ at 24 months of age in both the meat and dairy groups might reflect the possibility of a regression to the mean[19]; and whether this trend will continue or not needs further research with longer follow-up time.

In summary, this follow-up observation to a randomized controlled trial suggests that consumption of meat-based complementary diet by formula-fed infants in the early complementary feeding period promotes linear growth, which persists at 2 years of life. Moreover, reintroducing meat to participants who consumed only dairy as the main protein source during infancy appeared to ameliorate the earlier decline of LAZ. However, the sample size was relatively small, and it is unclear whether this greater linear growth in the meat group would have any health benefits in well-nourished infants and toddlers. To our knowledge, this is a novel example of how diet in the first year of life can impact short- and possibly longer term growth patterns. The current Dietary Guidelines for Americans has very limited guidance for infants and children from birth to 24 months of age because of limited evidence available. Findings from this study reinforce the need for more high-quality research on dietary patterns and nutrient intakes during the first 2 years of life in relation to the quality of growth. ∎

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