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Brief Communication: Growth Velocity and Weaning $\delta^{15}N$ "Dips" During Ontogeny in *Macaca mulatta*

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KEY WORDS growth and development; lactation; nitrogen balance; stable isotopes; life history

ABSTRACT *OBJECTIVES:* A "dip" in the stable nitrogen isotope ratios ($\delta^{15}N$) of subadults in the late weaning/early post-weaning phase of growth and development has been observed. Speculatively, this is the mechanism of positive nitrogen balance operating among rapidly growing subadults. An alternate hypothesis for $\delta^{15}N$ dips is that during weaning, subadults eat lower-^{15}N foods than adults.
METHODS: This study explores the role of positive nitrogen balance in affecting $\delta^{15}N$ variation of growing subadults by comparing growth velocity with stable carbon isotope ($\delta^{13}C$) and $\delta^{15}N$ ratios of blood serum from captive rhesus macaques (*Macaca mulatta*) ($n = 14$) with controlled diets during the first 10 months of life.
RESULTS: During the first six months, $\delta^{15}N$ values are inversely correlated with growth in some of the anthropometrics (weight and sagittal circumference). Dips in some infants' $\delta^{15}N$ values below their mothers' values are observed at the end of the weaning period. However, during this time frame, $\delta^{15}N$ values of the infants are not correlated with anthropometric indices. Serum stable isotope ratios of lactating and non-lactating adult females differ significantly.
CONCLUSIONS: Growth in body mass and size explains some of the variation in infant $\delta^{15}N$ values, but are not responsible for dips in the late weaning/early post-weaning phase. It is advised that future research evaluate the extent to which growth in other body systems affects nitrogen balance and $\delta^{15}N$ dips during ontogeny, and expand on isotopic differences between lactating and non-lactating females. Am J Phys Anthropol 157:347–357, 2015.

Weaning is a risky period during ontogeny, during which an infant is exposed to a new pathogen environment, and is simultaneously withdrawn from the nutritional benefits of breast milk, including enhanced resistance to infection (Lönnerdal, 2003). In addition to risks posed in early childhood, weaning also is associated with a wide range of later-life health outcomes (Cunningham, 1995; Miller, 2014). Human beings mitigate the risks of weaning via cultural practices such as prolonged nursing, introducing select weaning foods intended to buffer the transition, and enlisting social support (Gray, 1996; McDade and Worthman, 1998; Piperata, 2009), as well as through biological adaptation, for example, through the deposition of fat reserves during gestation and early infancy (Kuzawa et al., 2007; Miller, 2014). Understanding the weaning process, and how humans culturally and physiologically mitigate the risks of weaning, are basic elements in understanding the success and biocultural diversity of our species. Weaning behaviors may be assessed using stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) from osseous or soft tissues, which is possible because $\delta^{13}C$ and $\delta^{15}N$ values are high among breastfeeding infants and decline as weaning progresses. This decline represents a drop in an infant's trophic position, as breast milk composes less of the infant's diet (Fogel et al., 1989; Fuller et al., 2006; Newsome et al., 2006; Reitsema, 2012; Romek et al., 2013).

In addition to reflecting diet, including breastfeeding, stable isotope ratios of consumer tissues reflect physiological states associated with pregnancy and lactation. Catabolism of endogenous tissue reserves as in wasting (Fuller et al., 2005; Hatch, 2012; Hobson et al., 1993) or as is the case for income breeders who draw off endogenous reserves to meet the reproductive demands (Hinde et al., 2009; Sare et al., 2005) and protein synthesis during fasting in anabolic states (Lee et al., 2012), leads to higher $\delta^{15}N$ values in an organism's tissues. Rapid growth and weight gain, as in pregnancy or during early post-natal infant growth, can lead to lower tissue $\delta^{15}N$ values in a consumer by inducing a state of positive nitrogen balance (Fuller et al., 2004). These physiological effects are independent of dietary $\delta^{15}N$ values (for a review, see Reitsema, 2013). Nitrogen balance refers to the balance between nitrogen consumed with protein, nitrogen needed and used for tissue building and maintenance, and nitrogen in excess of biological demands that is excreted as waste. The stable isotope composition (the relative abundance of the heavier isotope ^{15}N and

Abbreviations: $\delta^{13}C$, carbon isotope; $\delta^{15}N$, nitrogen isotope

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the lighter isotope ^{14}N) in these three nitrogen pools usually differs due to fractionation incurred during protein metabolism (Steele and Daniel, 1978). Stable nitrogen isotope fractionation occurs when non-essential amino acids are synthesized in vivo (transamination) and when excess protein not used for tissue building is broken down and excreted (deamination) (Gaebler et al., 1966; Macko et al., 1986). During these processes, the light isotope is usually preferentially excreted with urine, leaving tissues enriched in ^{15}N relative to both foods and to excreta (Minawaga and Wada, 1984; Schoeller, 1999; Sponheimer et al., 2003; Steele and Daniel, 1978). Physiological states that influence the degree of transamination and deamination affect the $\delta^{15}N$ signatures of tissues and excreta (Lee et al., 2012). The overall effect is measurable and usually referred to as the diet-tissue space or a "trophic effect." During rapid growth, less nitrogen is excreted, and is instead routed directly to tissues. This bypasses the fractionating steps of deamination and transamination, resulting in predicted and/or observed lower $\delta^{15}N$ values among the tissues of growing individuals (Deschner et al., 2012; Gaye-Siessegger et al., 2003; Mekota et al., 2006; Waters-Rist and Katzenberg, 2010).

A decrease in infant $\delta^{15}N$ values to adult mean values with weaning is expected as breastfeeding frequency and/or intensity declines, in keeping with the "trophic effect" seen with $\delta^{15}N$ (Fahy et al., 2014; Reitsema, 2012). However, isotopic studies of weaning also identify an unexplained "dip" in infant $\delta^{15}N$ values after weaning appears complete, to values *below* the adult population average and/or the infants' mothers' values (Beaumont et al., 2013; Eerkens et al., 2011; Henderson et al., 2014; Katzenberg et al., 1996; Richards et al., 2002; Sandberg et al., 2014; Schurr, 1997; White and Schwarcz, 1994). A provisional and plausible explanation for this dip is that during weaning, the infant also is growing rapidly, inducing a state of positive nitrogen balance. An alternate hypothesis for $\delta^{15}N$ dips is that during weaning, subadults eat lower-^{15}N foods than adults in the group. A relationship between growth and $\delta^{15}N$ values has been documented among rapidly-growing animals and in controlled settings (for a useful review, see Waters-Rist and Katzenberg, 2010), including non-human primates during weight recovery after fasting (Deschner et al., 2012). The hypothesis that growth is responsible for post-weaning $\delta^{15}N$ dips during ontogeny in anthropological studies remains speculative in the absence of complementary experimental evidence from human and/or non-human primates. Unlike $\delta^{15}N$ values, $\delta^{13}C$ values are decoupled from protein balance, and are expected to vary more with fluctuations in the lipid and carbohydrate inputs to the diet, rather than predominantly with protein inputs. This is because carbon in the blood is derived from carbohydrates, lipids, and proteins, and is subject to fractionation, primarily during respiration (Hobson et al., 1993; Hobson and Clark, 1992), and during protein metabolism, gluconeogenesis, and lactation (DeNiro and Epstein, 1977; DeNiro and Epstein, 1978; Fuller, 2003:89–90; Hare et al., 1991). Nitrogen in the blood is derived from dietary protein, and is subject to fractionation during protein metabolism (Gaebler et al., 1966; Macko et al., 1986).

If growth during ontogeny causes predictable shifts in $\delta^{15}N$ values by way of the mechanism of positive nitrogen balance, it would be possible to examine how humans and other animals mobilize endogenous versus exogenous resources to meet reproductive, growth, and developmental demands in the face of environmental challenges and at different life history transitions. This study tests the hypothesis that nitrogen balance influences infant $\delta^{15}N$ signatures during early development, by comparing growth in body mass with serum $\delta^{15}N$ values in rhesus macaques (*Macaca mulatta*) housed at the Yerkes National Primate Research Center at Emory University. In particular, whether or not the rate of body growth (relative growth gains between months, not absolute body size or body size gains in kg or cm) is associated with a dip in $\delta^{15}N$ values is explored. If growth velocity and $\delta^{15}N$ are related, it would be a step toward clarifying the role of positive nitrogen balance in $\delta^{15}N$ variation among mammals, and would provide evidence that the widely-observed dip among infants in the first year of life is brought on by a state of positive nitrogen balance as the infant's body rapidly grows. The scope of the present study is to assess relationships between growth velocity and $\delta^{15}N$ ratios. A detailed examination of the weaning process of these rhesus macaques will be described elsewhere (Reitsema et al., in prep).

MATERIALS AND METHODS

Samples of rhesus macaque blood serum that had been previously collected for another study were made available by the Yerkes National Primate Research Center at Emory University. Fourteen mother-infant dyads were studied retroactively, the infants having been born in 2011. These monkeys are provisioned with monkey chow, and supplemental foods, such as oranges, sunflower seeds, non-sugar cereal, popcorn, carrots, and other foods. Of the 14 dams, eight had been fed a standard or "normal" low fat, high fiber diet supplied by Purina® (Lab Diets 5038) and six had been fed an experimental high fat, high sugar diet supplied by Research Diet® (Diet D07091204), which was switched to the normal diet during weaning (at either one, three, or five months of infant age). Only stable isotope data from months preceding the diet switch are included from the high-calorie diet cohort for discussion in this study. This limits discussion of the high-calorie diet subjects to the two-month time point, and precludes discussion of growth velocity and stable isotope signatures during the months of the $\delta^{15}N$ "dip" from the high-calorie diet cohort. Eleven non-lactating adult females were sampled for comparison, since lactation may affect stable isotope signatures in mothers (e.g., Kurle, 2002).

Sub-samples of serum from nursing and weaning infants at ages two, five, six, seven, eight, and 10 months, and from dams at infant ages two and five months were collected from frozen serum in storage at the Yerkes National Primate Research Center, and freeze-dried at the University of Georgia Bioarchaeology and Biochemistry Laboratory (Partrick and Reitsema, 2014). 50 μl of liquid serum was found to be more than enough volume to yield the appropriate dry-weight amount for stable carbon and nitrogen isotope analysis (~450–650 μg). Serum is ~10–12% nitrogen and 41–43% carbon. After freeze-drying, samples were homogenized using an agate mortar and pestle, and 0.85–1.10 mg of powder weighed into tin capsules for analysis at the University of Georgia Center for Applied Isotope Studies. A smaller amount of 0.50–0.65 mg was weighed for non-lactating adult females

after viewing results of the infants and mothers. Freeze-dried, homogenized samples of the standard and the high-calorie monkey biscuit were also assayed. Samples were combusted using a Costech® elemental analyzer coupled to a Thermo Delta plus XL isotope ratio mass spectrometer. Stable isotope results are reported according to the equation $X = [(R_{sample}/R_{standard}) - 1] \times 1000$. The elemental analyzer measured carbon content (%C) and nitrogen content (%N) in the serum samples, values which were used to calculate carbon:nitrogen ratios (C:N) useful in assessing whether some samples may have been contaminated or altered during storage or preparation, and the lipid content in serum (c.f., Habran et al., 2010, p. 888).

Infants were weighed every month during the first year of life by the staff at the Yerkes National Primate Research Center, who made data available for this study. Infant crown-rump, crown-heel, and sagittal circumference measurements at the level of the umbilicus were also recorded at 48–72 hours, 14–17 days, and three, six, nine, and 12 months during the first year of life. Estimations of growth velocity, also referred to as growth rate in this study, were made by calculating the difference in two successive months' measurements and dividing the difference by the measurement from the collection month in question. For weight, growth rates were calculated for every month. For the other three anthropometrics, a two-month growth rate was calculated from the measurements taken at 14–17 days and three months, a six-month growth rate was calculated from the measurements taken at three and six months, and an eight-month growth rate was calculated from the measurements taken at six and nine months.

Linear regressions with SPSS® statistical software evaluate whether $\delta^{15}N$ values are influenced by growth velocity. Spearman's one-tailed correlations are used to evaluate correlations between $\delta^{15}N$ and $\delta^{13}C$ values. Mann–Whitney U tests evaluate pairwise differences between groups. Henceforth in the text, values are considered significant when $P < 0.05$.

RESULTS

For infants in the normal diet group, serum %C values range from 37.4% to 45.5%, %N values range from 9.4% to 12.6%, and C:N ratios range from 4.2 to 4.8. For infants in the high-calorie diet group, serum %C values range from 43.0% to 45.0%, %N values range from 10.8% to 11.7%, and C:N ratios range from 4.5 to 4.8. On the normal diet, mothers exhibit serum %C values from 41.1% to 44.6%, %N values from 11.5% to 12.7%, and C:N ratios from 4.0 to 4.5. Non-lactating females on the normal diet exhibit significantly higher %C values of 44.9% to 47.3% (Kruskal–Wallis, $P < 0.001$), significantly higher %N values of 11.1–12.5% (Kruskal–Wallis, $P = 0.008$), and significantly higher C:N ratios of 4.2–5.0 (Kruskal–Wallis, $P = 0.009$). On the high-calorie diet, mothers exhibit serum %C values from 42.5% to 46.1%, %N values from 10.2% to 11.4%, and C:N ratios from 4.4 to 5.3.

Mothers on the normal diet exhibit a mean $\delta^{15}N$ value of $6.9 \pm 0.3‰$ and a mean $\delta^{13}C$ value of $-19.3 \pm 0.4‰$. These values are referred to as the "maternal baseline." Non-lactating adult females on the normal diet exhibit a mean $\delta^{15}N$ value of $7.2 \pm 0.2‰$ and a mean $\delta^{13}C$ value of $-19.7 \pm 0.5‰$, which are also used as baseline values. The $\delta^{15}N$ values of mothers are significantly lower than those of non-lactating adult females (Kruskal–Wallis, $P = 0.012$), and the $\delta^{13}C$ values of mothers are significantly higher than those of non-lactating adult females (Kruskal–Wallis, $P = 0.022$). High-calorie diet mothers exhibit a mean $\delta^{15}N$ value of $9.0 \pm 0.4‰$ and a mean $\delta^{13}C$ value of $-22.0 \pm 0.3‰$, which are significantly different than normal diet mothers ($\delta^{15}N$ and $\delta^{13}C$: Kruskal–Wallis, $P < 0.001$).

Monkeys were fed one of two isotopically dissimilar supplemental biscuits: a normal biscuit, with a $\delta^{15}N$ value of $2.4 \pm 0.3‰$ and a $\delta^{13}C$ value of $-18.5 \pm 0.4‰$ (analyzed in triplicate), and a high-calorie biscuit with a $\delta^{15}N$ value of $6.1 \pm 0.2‰$ and a $\delta^{13}C$ value of $-26.5‰$. Fruits and vegetables, along with other supplemental foods offered to subjects at Yerkes, are expected to result

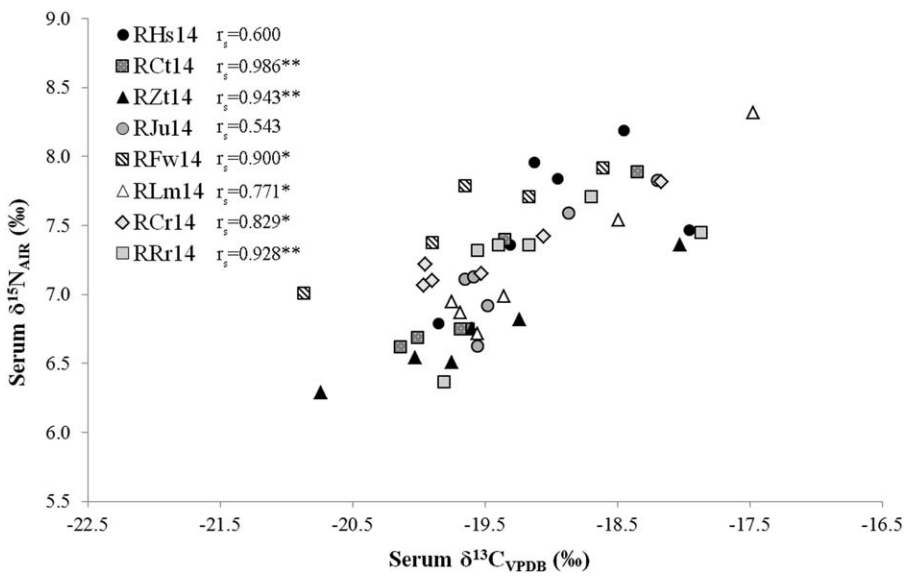

Fig. 1. Bivariate plot of infants' $\delta^{13}C$ and $\delta^{15}N$ values. All correlations are positive, and results of one-tailed Spearman's correlations are reported (correlation coefficients and their statistical significance). * $P < 0.05$; ** $P < 0.01$.

TABLE 1. Serum stable nitrogen isotope data, and growth velocity between successive sampling periods, expressed as percent gained

Diet	Infant ID	Month of sample	Infant $\delta^{15}N$ (‰)	Body weight gained (%)	Crown–rump gained (%)	Crown–heel gained (%)	Sagittal circumference gained (%)
Normal diet subjects	RHs14	2	7.5	33	23	23	28
		5	8.2	12	–	–	–
		6	7.8	10	17	17	8
		7	8.0	6	–	–	–
		8	7.4	9	5	4	13
		10	6.8	7	–	–	–
	RCt14	2	7.9	25	18	20	14
		5	7.4	9	–	–	–
		6	6.8	13	10	5	15
		7	6.6	15	–	–	–
		8	6.8	15	10	4	18
		10	6.7	17	–	–	–
	RZt14	2	7.4	29	20	12	15
		5	6.8	22	–	–	–
		6	6.5	15	14	15	27
		7	6.8	−4	–	–	–
		8	6.3	7	4	4	−1
		10	6.5	−1	–	–	–
	RJu14	2	7.8	27	17	19	19
		5	7.6	3	–	–	–
		6	7.1	6	13	11	20
		7	6.9	1	–	–	–
		8	6.6	11	17	12	15
		10	7.1	5	–	–	–
	RFw14	2	–	24	22	21	23
		5	7.9	10	–	–	–
		6	7.8	8	8	12	7
		7	7.7	6	–	–	–
		8	7.4	8	6	5	8
		10	7.0	3	–	–	–
	RLm14	2	8.3	15	17	15	5
		5	7.5	24	–	–	–
		6	7.0	13	15	15	18
		7	6.7	15	–	–	–
		8	6.9	−2	5	7	14
		10	7.0	13	–	–	–
	RCr14	2	7.8	27	15	14	16
		5	7.4	18	–	–	–
		6	7.2	13	10	10	24
		7	7.2	4	–	–	–
		8	7.1	7	10	10	4
		10	7.1	9	–	–	–
	RRr14	2	7.5	25	17	14	20
		5	7.7	16	–	–	–
		6	7.4	9	7	20	–
		7	7.4	3	–	–	–
		8	7.3	9	14	7	–
		10	6.4	7	–	–	–
High-calorie diet subjects	RA114	2	9.5	40	18	21	19
	RTk14	2	9.7	31	21	21	21
	RKk14	2	9.6	43	16	22	23
	RNp14	2	9.2	38	25	19	24
	RJs14	2	9.8	24	18	19	21
	RBv14	2	–	36	19	21	23

in a bulk non-biscuit diet value of ~3–5‰ for $\delta^{15}N$ and ~ −27‰ to −26‰ for $\delta^{13}C$ (after Reitsema, 2012). The majority of individuals reported here were fed the normal biscuit, and together with supplemental foods, the solid-food diet of these rhesus macaques is expected to have a $\delta^{15}N$ value of ~3–4‰, and a $\delta^{13}C$ value of ~ −22‰. A bivariate plot of infant $\delta^{15}N$ and $\delta^{13}C$ values shows an overall positive correlation between values, confirming the existence of two isotopically dissimilar dietary endpoints: solid foods with low-^{15}N and low-^{13}C values, and breast milk with higher $\delta^{15}N$ and $\delta^{13}C$ values (Fig. 1). When $\delta^{13}C$ and $\delta^{15}N$ values are compared for each individual, six infants exhibit significant correlations and two do not (results of Spearman's correlations reported in Fig. 1). Infants who have stronger $\delta^{13}C$ and $\delta^{15}N$ correlations are not more or less likely to exhibit a dip or to show unusually growth trajectories than infants who exhibit weaker $\delta^{13}C$ and $\delta^{15}N$ correlations or lack correlations.

Stable nitrogen isotope data and growth velocities from all infants are given in Table 1. Infant $\delta^{15}N$ values are elevated at the beginning of the collection period

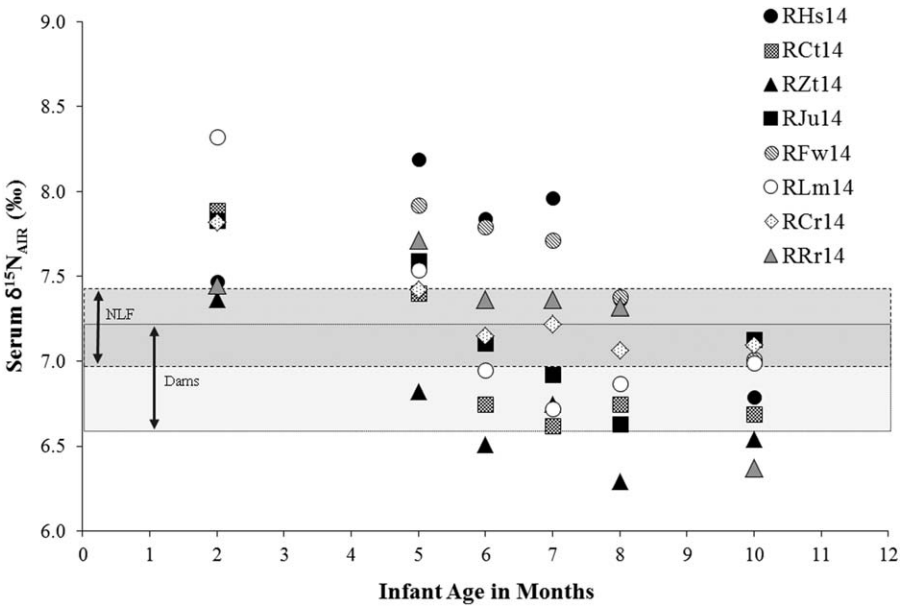

Fig. 2. Changes in infant serum $\delta^{15}N$ values are shown. Infants are plotted individually. A baseline (mean/standard deviation) of all the dams' ($n = 8$) values at infant ages 2- and 5-months is represented by the lightly shaded region ($6.9 \pm 0.3‰$). A baseline of 11 non-lactating adult females is represented by the darkly shaded region ($7.2 \pm 0.2‰$). Consistent with other studies that report a dip in infant $\delta^{15}N$ values with weaning, in later months, many infants exhibit $\delta^{15}N$ values that are below the dams' baseline value, and the dip is more apparent when non-lactating adult females are used for comparison.

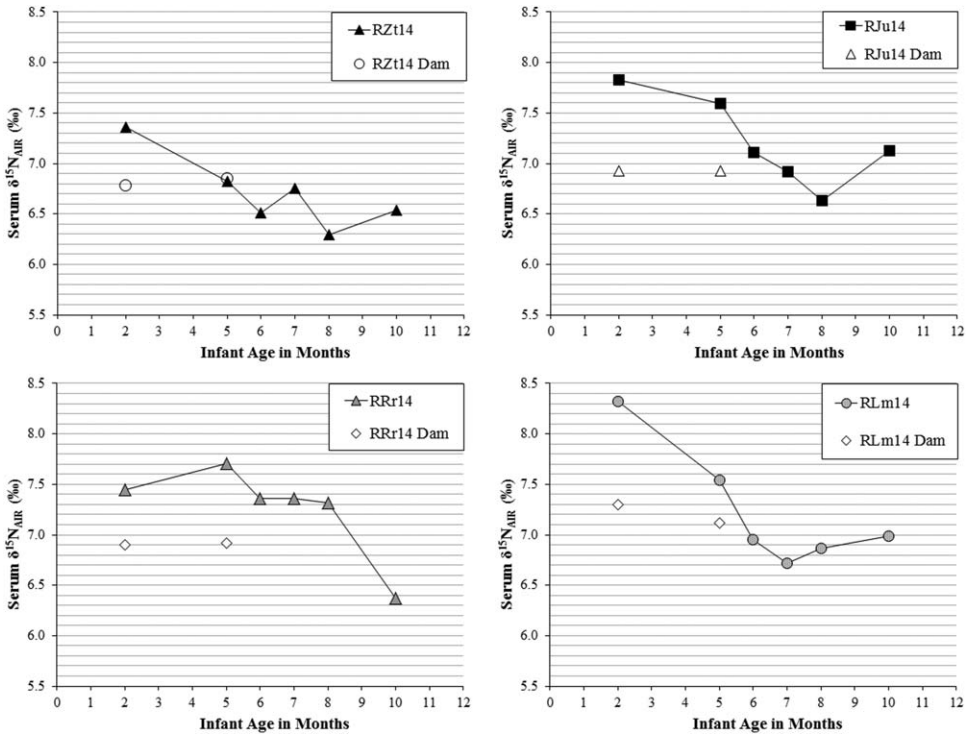

Fig. 3. Examples of "dips" in $\delta^{15}N$ values of four infants. Infant values are shown in comparison to their mothers' values. Values of dams are from samples collected when their infants were aged 2 and 5 months.

above adult females' values, given as the mean ± one standard deviation of both the dams (lightly shaded region) and the non-lactating females' (darkly shaded region) values, and subsequently decline (Fig. 2). Consistent with other studies that report a dip in infant $\delta^{15}N$ values with weaning, by eight months of infant age, several rhesus macaque infants exhibit $\delta^{15}N$ values that are below their mothers' values. Four of these individuals are displayed in direct comparison to their mothers in Figure 3. As seen in Figure 2, a dip in $\delta^{15}N$

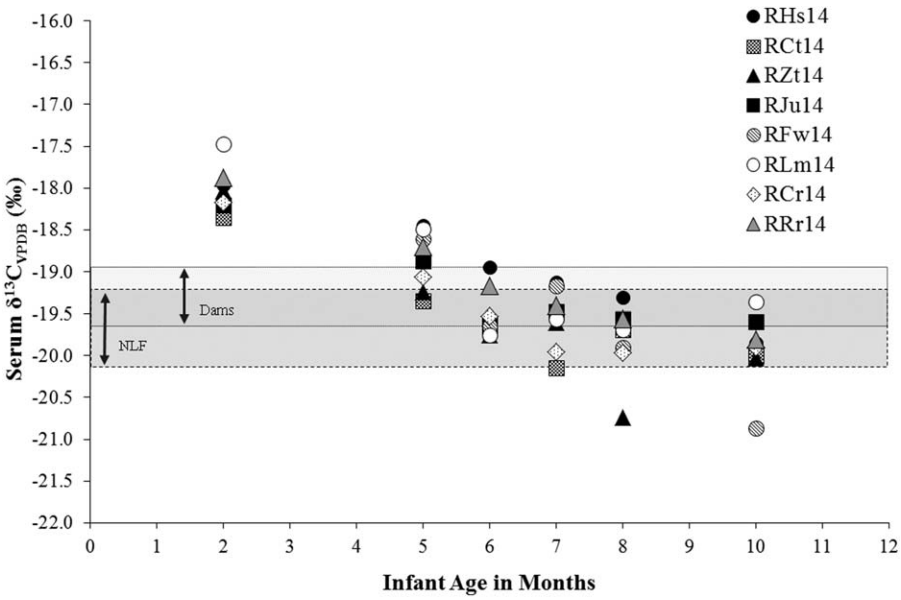

Fig. 4. Changes in infant serum $\delta^{13}C$ values are shown. Infants are plotted individually. A baseline (mean/standard deviation) of all the dams' ($n = 8$) values at infant ages 2- and 5-months is represented by the lightly shaded region ($-19.3 \pm 0.4‰$). A baseline of 11 non-lactating adult females is represented by the darkly shaded region ($-19.7 \pm 0.5‰$). In general, infant $\delta^{13}C$ values dip below the dams' values, but not below the non-lactating adult females.

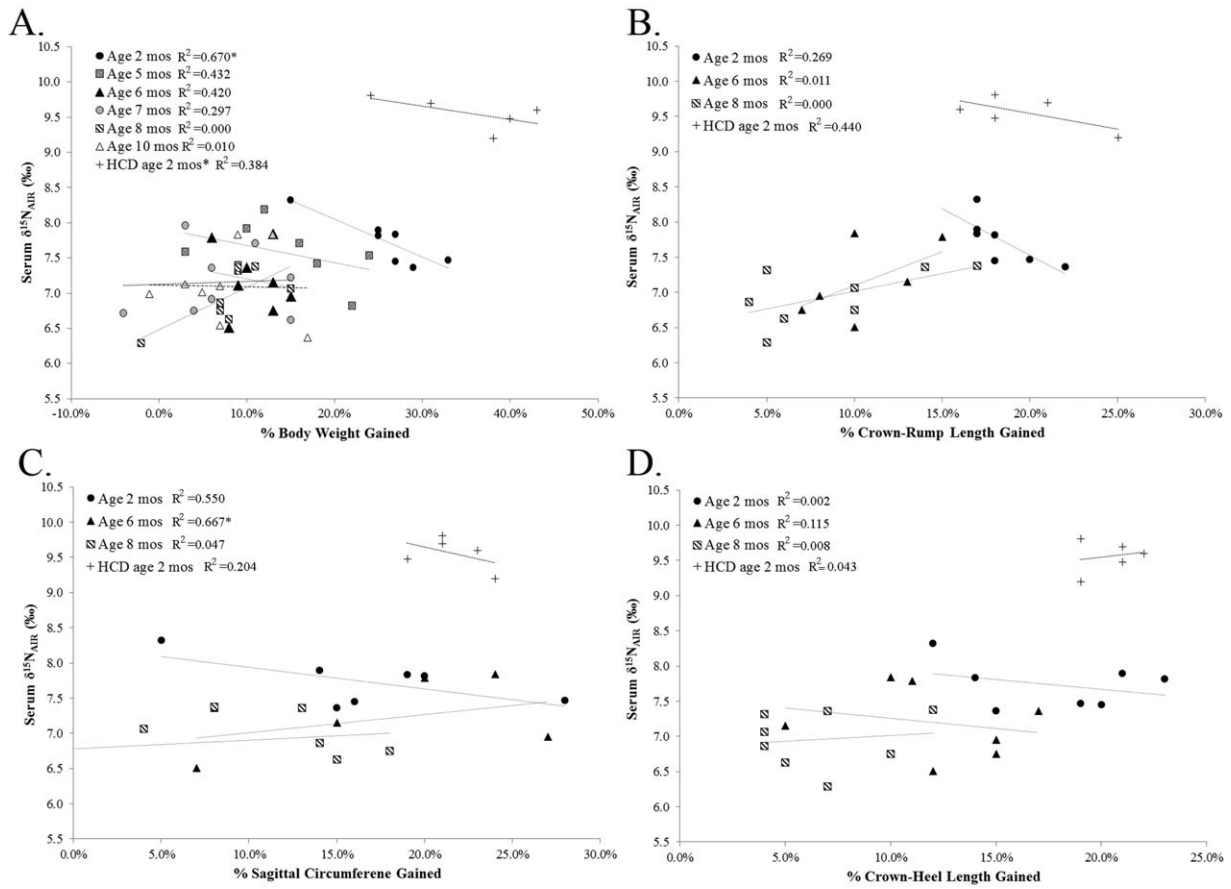

Fig. 5. Growth velocity in four anthropometric variables are shown in comparison to $\delta^{15}N$ values of infants at ages 2–10 months. high sugar diet refers to infants fed a high-calorie diet; only the 2-month samples are used from this cohort because the dams' diets changed after this time. R^2 values from linear regressions are given; $^*P < 0.05$.

TABLE 2. Results of linear regressions between serum stable nitrogen isotope ratios ($\delta^{15}N$) of infant rhesus macaques and growth velocity in four anthropometric measures at different ages (months) as described in the text

		Weight		Crown–rump		Crown–heel		Sagittal circumference	
	Age	R^2	P	R^2	P	R^2	P	R^2	P
Normal diet	2	0.672	**0.024**	0.269	0.233	0.002	0.922	0.550	0.056
	5	0.432	0.285		–		–		–
	6	0.420	0.082	0.011	0.805	0.115	0.411	0.667	**0.025**
	7	0.297	0.474		–		–		–
	8	0.000	0.967	0.000	0.986	0.008	0.834	0.047	0.642
	10	0.010	0.811		–		–		–
High-calorie diet	2	0.384	0.265	0.440	0.223	0.043	0.737	0.204	0.445

Values are considered significant at $P \leq 0.05$ (bold text).

values is most apparent when non-lactating adult females are used as the baseline for comparison, more so than when dams are used as the comparison, because the non-lactating female range is higher. Infant $\delta^{13}C$ values also decline over the sampling period. As shown in Figure 4, infant $\delta^{13}C$ values also dip below the dams' values (lightly shaded region) in later months. Infant $\delta^{13}C$ values do not appreciably dip below values of non-lactating females in later months, because the non-lactating female range is lower.

Between months two and six, rate of weight gain and infant $\delta^{15}N$ values are inversely related (linear regression R^2: two months: $R^2 = 0.676$, $P = 0.0242$; five months: $R^2 = 0.432$, $P = 0.285$; six months: $R^2 = 0.420$, $P = 0.082$; Fig. 5 and Table 2). At two months, the relationship is statistically significant. During infant ages eight and 10 months, infant growth velocity and $\delta^{15}N$ values are not correlated (seven months: $R^2 = 0.297$, $P = 0.474$; eight months: $R^2 = 0.000$, $P = 0.967$; 10 months: $R^2 = 0.010$, $P = 0.811$). Two-month-old infants whose mothers were consuming a high-calorie diet at the time of sampling also exhibit an inverse correlation ($R^2 = 0.384$; $P = 0.265$). No other time points from high-calorie individuals are considered because of the confounding effects of the mothers' diet switch during subsequent months of the sampling period.

Sagittal circumference is inversely correlated with infant $\delta^{15}N$ values during months two and six (the two earliest months for which estimations of sagittal growth velocity are available) (two months: $R^2 = 0.550$, $P = 0.056$; six months: $R^2 = 0.667$, $P = 0.025$), but there is no relationship at month eight ($R^2 = 0.047$, $P = 0.642$; Table 2). Growth velocity in crown-heel lengths and in crown-rump lengths are not correlated with infant $\delta^{15}N$ values.

Breastfeeding influences infant $\delta^{15}N$ values. In an effort to control for non-dietary $\delta^{15}N$ variation in this exploration of the effects of growth, comparisons also were made between growth velocity and $\delta^{15}N$ values among specifically those individuals/data points whose $\delta^{15}N$ values did not show signs of the inter-individually varied weaning transition. That is, month-by-month comparisons between growth and $\delta^{15}N$ values were next focused on infants whose $\delta^{15}N$ values exceeded 7.2‰, the maximum threshold of the estimated dams' baseline value, and on individuals aged 10 months, whose isotopic values indicate a complete transition off breast milk. This narrowed the comparison to all infants aged two months, all but one individual aged five months, select individuals in months six, seven, eight, and nine whose $\delta^{15}N$ values were higher than 7.2‰, and to all infants aged 10 months. When diet is partially controlled in this manner, there is no change in the significance of month-to-month relationships between rate of weight gain and $\delta^{15}N$: significant correlations in weight gain and $\delta^{15}N$ are only found at two months. There were no significant relationships between growth velocity and stable carbon isotope ratios (Fig. 6).

DISCUSSION

If $\delta^{15}N$ values vary predictably with growth by way of the mechanism of positive nitrogen balance and use of exogenous foods for tissue-building, it would be possible to examine how humans and other animals meet reproductive, growth, and developmental demands in the face of environmental challenges and at different life history transitions by utilizing stored energy versus compensatory feeding (c.f., Houston et al., 2007; Miller, 2014). This study tested the hypothesis that nitrogen balance influences infant $\delta^{15}N$ signatures during early development by comparing growth in body mass and length with serum $\delta^{15}N$ values in rhesus macaques. In particular, whether or not weight gain is associated with a dip in $\delta^{15}N$ values was explored, which would be more evidence that the widely-observed $\delta^{15}N$ dip among infants in late- or early-post-weaning is brought on by a state of positive nitrogen balance as the infant's body rapidly grows.

Results of this study lend mixed support to the hypothesis. Consistent with expectations for a state of positive nitrogen balance, faster growth rates in two variables, weight and sagittal circumference, are associated with lower infant $\delta^{15}N$ values at some time points in the first six months of life, suggesting positive nitrogen balance is operating to at least partly explain $\delta^{15}N$ values (breast milk being the major factor influencing infant $\delta^{15}N$ values at this time). These early post-natal months are indeed a time of rapid fat deposition among primates (c.f., Kuzawa et al., 2007), and the most rapid brain growth occurs during the first four months of life in *M. mulatta* (Malkova et al., 2006). This, however, is not the period of the observed $\delta^{15}N$ dip in infant values.

A dip in rhesus infant $\delta^{15}N$ values below the dams' baseline is observed among most individuals between months 6–10. During months 6–10, to which the hypothesis is chiefly addressed, more rapid growth is *not* associated with lower $\delta^{15}N$ values among rhesus infants, including weight gain, gains in body length, and gains in sagittal circumference. Some of this lack of correlation

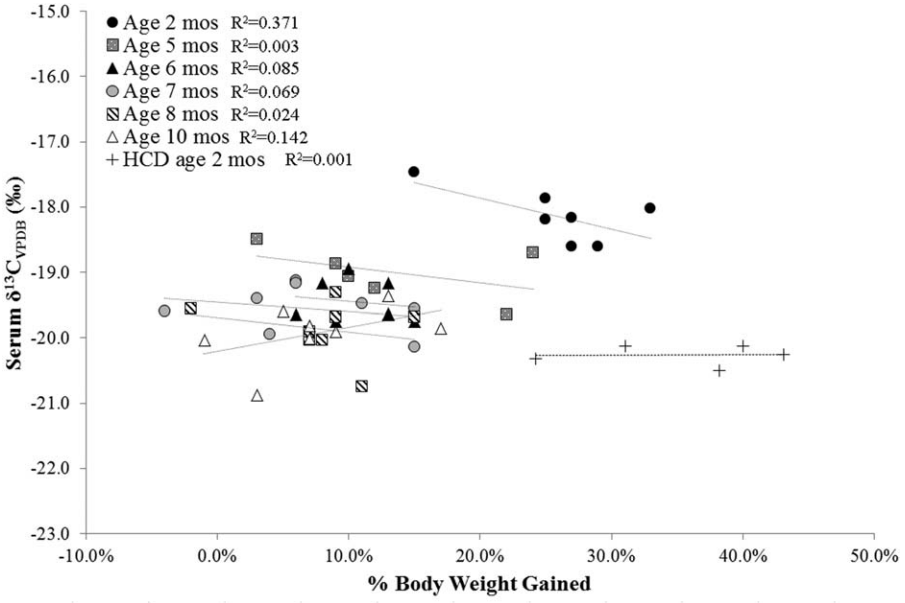

Fig. 6. Growth velocity in weight gain compared to $\delta^{13}C$ values of infants at ages 2 through 10 months. R^2 values from linear regressions are given; $^*P < 0.05$.

could be due to the fact that not one, but two potential sources of $\delta^{15}N$ variation are operating in tandem at this time: diet (the reduction of suckling) and growth. Controlling for diet by excluding individuals at time points when they appear to have weaned, and, in the case of the 10 month samples, *only* including individuals who are weaned, does not change the lack of correlations between $\delta^{15}N$ and growth during the period of the dip. This suggests growth in the examined parameters is not a source of positive nitrogen balance affecting $\delta^{15}N$ values, and that another factor is responsible for the dip in infant $\delta^{15}N$ values below maternal values at the time of weaning.

Consumption of low-^{15}N weanling foods is a possible explanation for $\delta^{15}N$ dips. It is difficult to identify which are the low-^{15}N foods in the present study—the biscuit, fruits, vegetables, and other supplemental foods are all known or expected to exhibit low $\delta^{15}N$ values on the order of 0–3‰ (Reitsema, 2012). In comparison to those of lactating females, infant $\delta^{13}C$ values also "dip" in the sample over time. Because serum $\delta^{13}C$ values so closely track the consumption of high-carbon vegetal (supplemental) foods, $\delta^{13}C$ dips would seem to corroborate the dietary explanation for $\delta^{15}N$ dips. However, when the non-lactating adult females are used for comparison to infant values (darkly shaded region of Fig. 4), the $\delta^{13}C$ dip virtually disappears, because the $\delta^{13}C$ values of non-lactating adult females are lower than those of lactating adult females (see below). Conversely, when the non-lactating adult female $\delta^{15}N$ value is used for comparison to infants, the $\delta^{15}N$ dip is exaggerated, because non-lactating adult females exhibit higher $\delta^{15}N$ values than lactating adult females. Therefore, even though $\delta^{15}N$ values are not correlated with the measured body growth parameters, it still seems possible that protein balance, and not diet, is affecting this $\delta^{15}N$ dip in late weaning – just not protein balance resulting from growth in mass, body length, and girth. Growth in other body systems is not explored in the present study, but could form the basis of future research.

Intra-group variations in diet are species- and even group-specific, whereas physiology and protein balance can be expected to show similar patterns within taxonomic groups. Few similar data including both growth and isotopic data are available for comparison from other primates, but some data already exist that indicate $\delta^{15}N$ dips and $\delta^{15}N$ correlations with growth velocity are not universal. An individual infant François' langur (*Trachypithecus francoisi*) reported by Reitsema (2012) in direct comparison to its mother exhibits neither a $\delta^{15}N$ nor a $\delta^{13}C$ dip toward the end of weaning, and a comparison between fecal $\delta^{15}N$ and growth velocity in weight measured monthly for this individual also shows no relationship ($R^2 = 0.026$; $P = 0.656$; Fig. 7). It should be noted that the most meaningful comparison of growth velocity and $\delta^{15}N$ is made when the tissue analyzed has a turnover time that matches the frequency at which growth measurements are taken. Serum, which reflects nutrients incorporated in the 1–2 weeks preceding collection (Kurle, 2002), is best accompanied by weekly growth measurements or weekly averages, whereas isotopic data from feces, which reflect the preceding 1–2 days, are best accompanied by daily measurements.

For most infants in this study, $\delta^{13}C$ and $\delta^{15}N$ are tightly correlated (Fig. 1). A correlation in $\delta^{13}C$ and $\delta^{15}N$ values is expected if individuals are consuming differing amounts of two isotopically different foods. In this case, for infants, the isotopically different foods are breast milk and the solid food diet, which consists of monkey biscuit and fruit. If $\delta^{13}C$ and $\delta^{15}N$ values were decoupled, it could indicate protein balance as a factor underlying $\delta^{15}N$ variation. For the two individuals whose $\delta^{13}C$ and $\delta^{15}N$ values are not correlated (individuals RJu14 and RHs14), $\delta^{15}N$ values do vary independently from $\delta^{13}C$ values. However, these individuals exhibit neither anomalous growth rates, nor anomalous $\delta^{13}C$ or $\delta^{15}N$ values, and they are not more or less likely to "dip" below maternal values. Because there is nothing in the growth data to explain the decoupling of $\delta^{13}C$ and $\delta^{15}N$ values for some infants, it is less

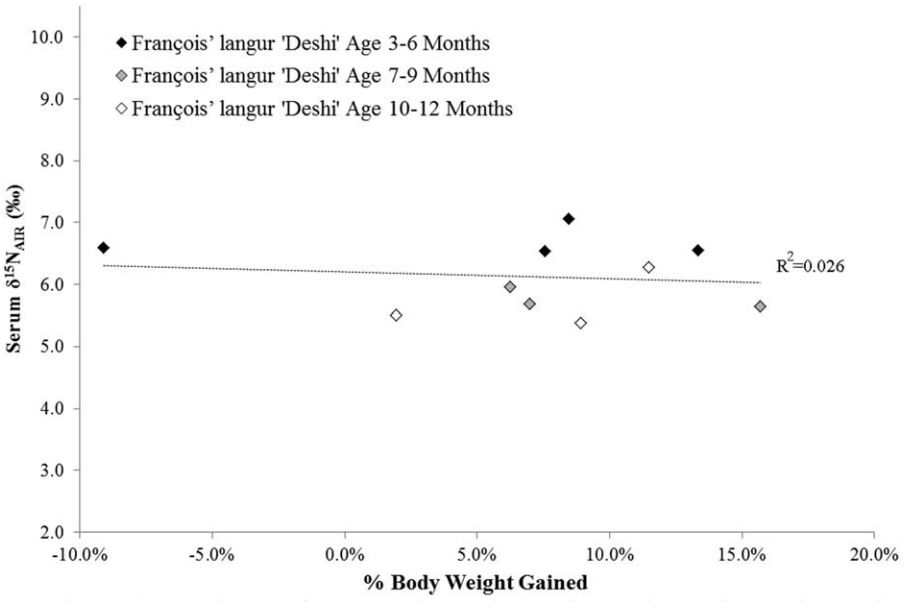

Fig. 7. Another comparison can be made between growth velocity and $\delta^{15}N$ values, drawing from previously published data from a François' langur (*Trachypithecus francoisi*) infant (Reitsema, 2012). Lower $\delta^{15}N$ values are not associated with greater growth velocity in this individual. The trendline is for all data points (all time points pooled) and the linear regression R^2 value for all data is given.

likely that the decoupling reflects shifts in protein balance, and more likely that these individuals were continuing to breastfeed while supplementing breast milk with solid foods—that is, a dietary explanation for the data. Prolonged suckling would result in occasional spikes in serum ^{15}N that were accompanied by $\delta^{13}C$ values that (tracking solid food consumption) resembled those of the rest of the group.

Although $\delta^{15}N$ dips do not track increases in body size and mass, protein balance cannot be ruled out as an explanation for the $\delta^{15}N$ data. Rapid growth in other bodily systems and not body growth *per se* may be occurring and inducing a state of positive nitrogen balance responsible for the dip. Energy and resources are not allocated homogeneously during growth and development, but are rationed for different systems at different times, according to the expectations of life-history theory (Pereira, 2002). Among primates, somatic, neural and dental development follow different growth trajectories (c.f., Batchelder et al., 2010; Dobbing and Sands, 1979; Leigh, 1992; Malkova et al., 2006). These may be growing rapidly at the time of the "dip," in pace with infant tissue $\delta^{15}N$, shifting the routing of food $\delta^{15}N$ from body growth to the growth, development and maintenance of other biological systems. Thus, it is recommended that future research in this area consider growth rates of other systems more specifically (e.g., digestive, renal, reproductive, cardiovascular, lymphatic, nervous), using standard or advanced imaging, or other proxy indicators (c.f., Batchelder et al., 2010). It is also recommended that future research take growth measurements at intervals that correspond with the turnover time of the tissue analyzed.

The isotopic values of lactating and non-lactating adult female rhesus macaques differ significantly. Lower $\delta^{15}N$ values among lactating females in the present study corroborate the report by Kurle (2002) of lactating *Callorhinus ursinus* (fur seal) exhibiting lower $\delta^{15}N$ values in comparison to pregnant and non-lactating females, despite very different lactation strategies among primates and marine mammals (c.f., Hinde and Milligan, 2011). A possible explanation for the phenomenon of lower $\delta^{15}N$ values among lactating versus non-lactating females has to do with the isotopic composition of breast milk. If breast milk is isotopically enriched in ^{15}N, its excretion should result in relatively lower $\delta^{15}N$ values of maternal tissues. This is the case among marine mammals (Kurle, 2002; Polischuk et al., 2001) but preliminary evidence suggests it is not the case in humans (Fuller, 2003), whose breast milk protein $\delta^{15}N$ values are approximately the same as tissue (fingernail) $\delta^{15}N$ values and whose bulk breast milk $\delta^{15}N$ values are actually lower than tissue values, on account of breast milk containing ^{15}N-depleted urea. Human and macaque milks are compositionally similar (Hinde and Milligan, 2011; Hinde et al., 2009). Milk stable isotope data are not currently available for rhesus macaques.

Humans and other primates utilize a mixed income/capital strategy, sustaining lactation by mobilizing body reserves and routing dietary intake to milk (c.f., Hinde et al., 2009). Among humans, breast milk $\delta^{13}C$ is lower than maternal tissue $\delta^{13}C$, indicating breast milk is synthesized in females from isotopically light carbon of tissue reserves (Fuller, 2003). The excretion of isotopically light carbon via breast milk is indicated in this study among rhesus macaques in the form of higher serum $\delta^{13}C$ values in lactating mothers compared to non-lactating females. Without more stable isotope data from breast milk, it is difficult to interpret lactating and non-lactating female stable isotope differences. Further research into the isotopic composition of breast milk across taxa will not only clarify isotopic evidence for weaning, but will also clarify the isotopic variation

among adult females in aid of bioarchaeological and modern appreciation of population-level stable isotope ratio variability.

CONCLUSION

Measurements of growth velocity (body size, length and circumference) were compared to the $\delta^{15}N$ values of blood serum from captive rhesus macaque infants to test the hypothesis that low infant $\delta^{15}N$ values of infants relative to those of the adult population are due to positive nitrogen balance. Although inverse relationships were identified between growth velocity in (1) body mass and (2) sagittal circumference at some infant ages (in months 2–6), suggesting a state of positive nitrogen balance in growing individuals, the correlations were not present during the ages at which the $\delta^{15}N$ "dip" is conspicuous. Diet is a possible explanation for observed $\delta^{15}N$ differences between subadults and adults at the time of the post-weaning $\delta^{15}N$ dip, but given the fact that infants and non-lactating adult females exhibit similar $\delta^{13}C$ values, it remains possible that growth in non-measured body systems is affecting $\delta^{15}N$ values of infants in the late- and early-post-weaning stage of development. Internal imaging and other means of assessing the maturation of organ and system function are suggested as means of further examining growth in these other systems, in the context of nitrogen balance during ontogeny.

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LITERATURE CITED

Batchelder CA, Lee CC, Martinez ML, Tarantal AF. 2010. Ontogeny of the kidney and renal developmental markers in the rhesus monkey (*Macaca mulatta*). Anat Rec 293:1971–1983.

Beaumont J, Gledhill A, Lee-Thorp J, Montgomery J. 2013. Childhood diet: a closer examination of the evidence from dental tissues using stable isotope analysis of incremental human dentine. Archaeometry 55:277–295.

Cunningham AS. 1995. Breastfeeding: adaptive behavior for child health and longevity. In: Stuart-Macadam P, and Dettwyler KA, editors. Breastfeeding: biocultural perspectives. New Work: Walter de Gruyter, Inc. p 243–264.

DeNiro MJ, Epstein S. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197:261–263.

DeNiro MJ, Epstein S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Ac 42:495–506.

Deschner T, Fuller BT, Oelze VM, Boesch C, Hublin J-J, Mundry R, Richards MP, Ortmann S, Hohmann G. 2012. Identification of energy consumption and nutritional stress by isotopic and elemental analysis of urine in bonobos (*Pan paniscus*). Rapid Commun Mass Spectrom 26:69–77.

Dobbing J, Sands J. 1979. Comparative aspects of the brain growth spurt. Early Hum Dev 3:79–83.

Eerkens JW, Berget AG, Bartelink EJ. 2011. Estimating weaning and early childhood diet from serial micro-samples of dentin collagen. J Archaeol Sci 38:3101–3111.

Fahy GE, Richards MP, Fuller BT, Deschner T, Hublin J-J, Boesch C. 2014. Stable nitrogen isotope analysis of dentine serial sections elucidate sex differences in weaning patterns of wild chimpanzees (Pan troglodytes). Am J Phys Anthropol 153:635–642.

Fogel ML, Tuross N, Owsley DW. 1989. Nitrogen isotope tracers of human lactation in modern and archaeological populations. Carnegie Inst Washington Yearbook 88:33–34.

Fuller BT. 2003. Isotopic investigation of pregnancy and breastfeeding in modern and archaeological populations. Oxford: University of Oxford. p 211.

Fuller BT, Fuller JL, Harris DA, Hedges RE. 2006. Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotope ratios. Am J Phys Anthropol 129:279–298.

Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges REM. 2004. Nitrogen balance and $\delta^{15}N$: why you're not what you eat during pregnancy. Rapid Commun Mass Spectrom 18:2889–2896.

Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges REM. 2005. Nitrogen balance and $\delta^{15}N$: why you're not what you eat during nutritional stress. Rapid Commun Mass Spectrom 19:2497–2506.

Gaebler OH, Vitti TG, Vukmirovich R. 1966. Isotope effects in metabolism of ^{14}N and ^{15}N from unlabeled dietary proteins. Can J Biochem Cell Biol 44:1249–1257.

Gaye-Siessegger J, Focken U, Abel HJ, Becker K. 2003. Feeding level and diet quality influence trophic shift of C and N isotopes in Nile tilapia (*Oreochromis niloticus L.*). Isotopes Environ Health Studies 39:125–134.

Gray SJ. 1996. Ecology of weaning among nomadic Turkana pastoralists of Kenya: maternal thinking, maternal behavior, and human adaptive strategies. Hum Biol 68:437–465.

Habran S, Debier C, Crocker DE, Houser DS, Lepoint G, Bouquegneau JM, Das K. 2010. Assessment of gestation, lactation and fasting on stable isotope ratios in northern elephant seals (Mirounga angustirostris). Mar Mamm Sci 26: 880–895.

Hare PE, Fogel ML, Stafford TW, Mitchell AD, Hoering TC. 1991. The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. J Archaeol Sci 18:277–292.

Hatch KA. 2012. The use and application of stable isotope analysis to the study of starvation, fasting, and nutritional stress in animals. In: McCue MD, editor. Comparative physiology of fasting, starvation, and food limitation. Berlin, Heidelberg: Springer. p 337–364.

Henderson RC, Lee-Thorp J, Loe L. 2014. Early life histories of the London poor using δ13C and δ15N stable isotope incremental dentine sampling. Am J Phys Anthropol 154: 585–593.

Hinde K, Milligan LA. 2011. Primate milk: Proximate mechanisms and ultimate perspectives. Evol Anthropol 20:9–23.

Hinde K, Power ML, Oftedal OT. 2009. Rhesus macaque milk: magnitude, sources, and consequences of individual variation over lactation. Am J Phys Anthropol 138:148–157.

Hobson KA, Alisauskas RT, Clark RG. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet. Condor 95:388–394.

Hobson KA, Clark RG. 1992. Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor 94:189–197.

Houston AI, Stephens PA, Boyd IL, Harding KC, McNamara JM. 2007. Capital or income breeding? A theoretical model of female reproductive strategies. Behav Ecol 18:241–250.

Katzenberg AM, Herring DA, Saunders SR. 1996. Weaning and infant mortality: evaluating the skeletal evidence. Yearbook Phys Anthropol 39:177–199.

Kurle CM. 2002. Stable-isotope ratios of blood components from captive northern fur seals (*Callorhinus ursinus*) and their diet: applications for studying the foraging ecology of wild otariids. Can J Zool 80:902–909.

Kuzawa CW, Gluckman PD, Hanson MA. 2007. Developmental perspectives on the origins of obesity. In: Fantuzzi E, and Mazzone T, editors. Adipose tissue and adipokines in health and disease. New York: Humana Press.

Lee TN, Buck CL, Barnes BM, O'Brien DM. 2012. A test of alternative models for increased tissue nitrogen isotope ratios during fasting in hibernating arctic ground squirrels. J Exp Biol 215:3354–3361.

Leigh SR. 1992. Patterns of variation in the ontogeny of primate body size dimorphism. J Hum Evol 23:27–50.

Lönnerdal B. 2003. Nutritional and physiologic significance of human milk proteins. Am J Clin Nutr 77:15375–15435.

Macko SA, Estep MLF, Engel MH, Hare PE. 1986. Kinetic fractionation of stable nitrogen isotopes during amino acid transamination. Geochim Cosmochim Ac 50:2143–2146.

Malkova L, Heuer E, Saunders RC. 2006. Longitudinal magnetic resonance imaging study of rhesus monkey brain development. Eur J Neurosci 24:3204–3212.

McDade TW, Worthman CM. 1998. The weanling's dilemma reconsidered: A biocultural analysis of breastfeeding ecology. J Dev Behav Pediatr 19:286–299.

Mekota A-M, Grupe G, Ufer S, Cuntz U. 2006. Serial analysis of stable nitrogen and carbon isotopes in hair: monitoring starvation and recovery phases of patients suffering from anorexia nervosa. Rapid Commun Mass Spectrom 20:1604–1610.

Miller EM. 2014. Chronic undernutrition and traditional weaning foods are associated with fat stores in ariaal infants of Northern Kenya. Am J Phys Anthropol 153:286–296.

Minawaga M, Wada E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between ^{15}N and animal age. Geochim Cosmochim Ac 48:1135–1140.

Newsome S, Etnier M, Aurioles-Gamboa D, -Koch P. 2006. Using carbon and nitrogen isotope values to investigate maternal strategies in northeast Pacific otariids. Marine Mammal Sci 22:556–572.

Partrick KA, Reitsema LJ. 2014. Stable carbon and nitrogen isotope data offer novel opportunities for hypothesis testing in non-human primate weaning ecology. Am J Phys Anthropol 153:204.

Pereira ME. 2002. Juvenility in animals. In: Pereira ME, and Airbanks LA, editors. Juvenile primates: life history, development and behavior. Chicago: University of Chicago Press. p 17–27.

Piperata BA. 2009. Variation in maternal strategies during lactation: the role of the biosocial context. Am J Hum Biol 21: 817–827.

Polischuk SC, Hobson KA, Ramsay MA. 2001. Use of stable-carbon and -nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. Canadian J Zool 79: 499–511.

Reitsema LJ. 2012. Introducing fecal stable isotope analysis in primate weaning studies. Am J Primatol 74:926–939.

Reitsema LJ. 2013. Beyond diet reconstruction: stable isotope applications to human physiology, health and nutrition. Am J Hum Biol 25:445–456.

Richards MP, Mays S, Fuller BT. 2002. Stable carbon and nitrogen isotope values of bone and teeth reflect weaning age at the Mediaeval Wharram Percy site, Yorkshire, UK. Am J Phys Anthropol 119:205–210.

Romek KM, Julien M, Frasquet-Darrieux M, Tea I, Antheaume I, Hankard R, Robins RJ. 2013. Human baby hair amino acid natural abundance 15N-isotope values are not related to the 15N-isotope values of amino acids in mother's breast milk protein. Amino Acids 45:1365–1372.

Sandberg PA, Sponheimer M, Lee-Thorp J, Van Gerven D. 2014. Intra-tooth stable isotope analysis of dentine: A step toward addressing selective mortality in the reconstruction of life history in the archaeological record. Am J Phys Anthropol 155:281–293.

Sare DTJ, Millar JS, and Longstaffe FJ. 2005. Nitrogen- and carbon-isotope fractionation between mothers and offspring in red-backed voles (*Clethrionomys gapperi*). Can J Zool 83:712–716.

Schoeller DA. 1999. Isotopic fractionation: why aren't we what we eat? J Archaeol Sci 26:667–673.

Schurr MR. 1997. Stable nitrogen isotopes as evidence for age of weaning at the Angel site: a comparison of isotopic and demographic measures of weaning age. J Archaeol Sci 24: 919–927.

Sponheimer M, Robinson TF, Roeder BL, Passey BH, Ayliffe LK, Cerling TE, Dearing MD, and Ehleringer JR. 2003. An experimental study of nitrogen flux in llamas: is 14N preferentially excreted? J Archaeol Sci 30:1649–1655.

Steele KW, Daniel RM. 1978. Fractionation of nitrogen isotopes by animals: a further complication to the use of variations in the natural abundance of ^{15}N for tracer studies. J Agricult Sci 90:7–9.

Waters-Rist AL, Katzenberg MA. 2010. The effect of growth on stable nitrogen isotope ratios in subadult bone collagen. Int J Osteoarchaeol 20:172–191.

White CD, Schwarcz HP. 1994. Temporal trends in stable isotopes for Nubian mummy tissues. Am J Phys Anthropol 93: 165–187.