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Original Research Article

Infant Growth and the Thymus: Data from Two South American Native Societies

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The thymus plays an important role in the development of the immune system, yet little is known about the patterns and sources of variation in postnatal thymic development. The aim of this study is to contribute cross-cultural data on thymus size in infants from two South American native populations, the Tsimane of Bolivia and the Pumé of Venezuela. Thymic ultrasonography was performed and standard anthropometric measures collected from 86 Tsimane and Pumé infants. Patterns of infant growth and thymus size were compared between the two populations and the relationship between nutritional status and thymus size was assessed. Despite nearly identical anthropometric trajectories, Tsimane infants had larger thymuses than Pumé infants at all ages. Population, infant age, and infant mid-upper arm circumference were significant predictors of thymus area in the Tsimane and Pumé infants. This finding reveals a cross-cultural difference in thymus size that is not driven by nutritional status. We suggest that future studies focus on isolating prenatal and postnatal environmental factors underlying cross-cultural variation in thymic development. Am. J. Hum. Biol. 24:768–775, 2012.

The thymus, a lymphoepithelial organ, is the main site of maturation of T-lymphocytes that orchestrate cell-mediated immune function (Abbas and Lichtman, 2011; Murphy et al., 2008). Thymus size correlates strongly with body size at birth (Aaby et al., 2002; Iscan et al., 2000; Yekeler et al., 2004) and grows postnatally, attaining maximum size at 4–6 months (Hasselbalch et al., 1999b; Yekeler et al., 2004). Thymic involution is the process by which its functional tissue shrinks and its cellular composition changes. This process begins in late infancy (Bertho et al., 1997; Miller, 2002; Yekeler et al., 2004) and continues across the life course (George and Ritter, 1996; Shanley et al., 2009; Steinmann et al., 1985). Thymus size generally corresponds to its functional capacity (Chevalier, 1994; Cohen-Stuart et al., 1998; Jeppesen et al., 2004; Ngom et al., 2004) and predicts early childhood survivorship (Aaby et al., 2002; Garly et al., 2008). Population differences in thymus size have been documented (Aaby et al., 2002, Park et al., 2008), though methodological issues inhibit broad cross-cultural comparisons (Varga et al., 2011). Postnatal environmental conditions inevitably play a role in shaping thymic developmental variation (Zeyrek et al., 2008). However, the patterns and sources of this variation remain poorly understood.

To explore the nature of cross-cultural variability in thymic development, we conducted a cross-sectional study of the infant thymus in two South American native populations, the Tsimane of Bolivia and the Pumé of Venezuela. The aim of this study is to provide empirical data on thymus size for infants who experience nutritionally and epidemiologically challenging postnatal environmental conditions. Specifically, we compare anthropometric indices and thymus size between the Tsimane and Pumé infants.

VARIATION IN THYMIC SIZE

Many ultrasonographic studies have attempted to establish standards for thymic size in infancy (Hasselbalch et al., 1999b; Kizilcan et al., 1995; Varga et al., 2011; Yekeler at al., 2004). These studies tend to rely on healthy, well-nourished infants inhabiting sanitary, urban environments (Hasselbalch et al., 1999b; Kizilcan et al., 1995; Yekeler et al., 2004). These settings are not representative of the postnatal experience in many contemporary societies. Studies of infants from less-developed countries provide data that illuminate the need to better understand its developmental influences (Aaby et al., 2002; Chevalier et al., 2002; Collinson et al., 2003).

The sensitivity of the thymus to suboptimal postnatal conditions suggests that the energetic costs of thymic tissue maintenance are substantial (McDade, 2003). The thymus is susceptible to acute involution upon prenatal and postnatal malnutrition (Chandra, 1992; Cromi et al., 2009; Savino et al., 2002), antigenic assault (Savino et al., 2007), stress hormones (Savino and Dardenne, 2000), maternal smoking (Zeyrek et al., 2008) and prenatal exposure to environmental toxins (Moore et al., 2009; Park et al., 2008). In hyperseasonal environments, both birth and measurement in the hungry season are associated with smaller thymic size (Aaby et al., 2002; Collinson et al., 2003).

TRADE-OFFS AND THYMIC DEVELOPMENT

We employ a life history theoretical perspective in this study of postnatal thymus development. Under this theoretical framework, fitness is maximized when limited energetic resources are allocated optimally to competing life history functions (Charnov, 2004; Gadgil and Bossert,

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1970; Hill and Hurtado, 1996; Kaplan et al., 2000; Kramer and Ellison., 2010; Smith and Fretwell, 1974). Immune function is an important component of survival effort and entails physiological trade-offs with growth and reproduction (Lochmiller and Deerenberg, 2000; Muehlenbein et al., 2010; Norris and Evans, 2000; Sheldon and Verhulst, 1996). During infancy, trade-offs are pronounced between the demands of immunologic maturation and rapid brain and body growth (McDade, 2005). For example, infection in infancy impairs growth (Bogin, 1999; Martorell, 1980), which suggests that resources are reallocated from growth to immunologic activation (McDade et al., 2008; Panter-Brick et al., 2000). Given the profound nature of these trade-offs, it is not surprising that infants are quite vulnerable to mortality by infectious disease in resource-scarce environments (McDade, 2005; Stoll, 2006).

Postnatal thymic development requires considerable energetic investment (Steinmann, 1986). If adaptive responses to energetic trade-offs affect thymus size, then the thymus may develop according to environmental cues of lifetime nutritional availability and disease exposure (George and Ritter, 1996; Moore et al., 2009). In pathogenically protected, nutritionally adequate environments, rapid postnatal thymic growth and prolonged maintenance of a large thymus may be the norm (Benn, 2001). In contrast, less investment in thymic maintenance might be expected in more epidemiologically and nutritionally challenging environments. Despite the apparent contradiction between a small thymus and high demand for immune responsiveness, early involution and T-lymphocyte peripheralization may suggest that rapid immunological maturation is needed to survive in such challenging environments (McDade, 2003).

By studying the thymus in South American native infants, we provide a lens onto the conditions and environmental pressures shaping thymic development throughout much of human history. The Tsimane and Pumé do not have access to modern sanitation; have high rates of infectious disease and infant mortality (Godoy et al., 2010; Gurven et al., 2007; Kramer and Greaves, 2007). Our objectives are threefold: *First*, we provide empirical data from these unique study populations to contribute to cross-cultural understanding of thymic development. *Second*, we compare infant anthropometrics and thymus size between them. We expected that Pumé infants would have smaller thymuses than Tsimane infants because the Pumé have less access to resources and medical care. *Third*, we evaluate anthropometric predictors of thymic size. We expected that infant body size, a marker of nutritional status, would correlate positively with thymus size.

METHODS

Study populations

The Tsimane of Bolivia. Based on previous census data (INE, 2003) and population growth rates, roughly 13,000 Tsimane forager-horticulturalists currently inhabit the Maniqui River system in northeastern Bolivia. Data were collected in 2006 in the Beni Biosphere Reserve, a nationally protected area encompassing 1,350 km^2 (Miranda, 1995). The communities of Cedral, Chacal, Monte Rosa, and Puerto Triunfo are located 32–42 km downstream from the town of San Borja. They can be reached by dugout canoe or road in a day or less. Each community had a school and one had a trained health promoter.

Tsimane blend traditional subsistence strategies (horticulture, fishing, hunting, and gathering) with wage labor and market exchange. Traditional diets are supplemented by market foods such as refined sugar, salt, and canned fish. Marriages are mostly monogamous and stable (Winking et al., 2009). Total Tsimane fertility rate (average number of children expected to be born to a woman across her reproductive lifespan) is 8.5 (Kaplan et al., 2010). The average age of first marriage for women is 16 years (Rucas et al., 2006) and average age of first birth is 18.6 years (Walker et al., 2006). Following birth, mothers and neonates spend a week resting in a mosquito net. Young infants are worn close to mothers in slings or rocked in hammocks while mothers engage in household tasks. Infants are breastfed exclusively in the first months of life and weaning usually occurs by 2 years of age.

The Tsimane infant mortality rate (IMR) was 12.6% from 1990 to 2002 (Gurven et al., 2007). Drinking water is obtained from rivers and most households maintain hand-dug latrines. Domestic pigs, dogs, and chickens roam the villages and their feces litter pathways between houses. Tsimane children exhibit elevated C-reactive protein levels, which indicates a high infectious disease burden (McDade et al., 2005). Parasitic infections (Blackwell et al., 2011; Tanner et al., 2009), anemia (Lindsay et al., 2003), and growth stunting (Foster et al., 2005; Godoy et al., 2010) are common in Tsimane children. Tsimane seek medical care in the nearby town of San Borja, either at the main hospital or at Horeb, a New Tribes Mission health clinic, which provides intermittent access to medicines. Vaccinations are available free at the hospital to mothers and small children. A team from the hospital occasionally enters villages to vaccinate children and provide health care, and since 2001, the Tsimane Health and Life History Project's (THLHP) medical team has made visits to Tsimane villages.

The Pumé of Venezuela. Approximately 8000 Pumé (INE, 2001) inhabit the low, flat savannas (*llanos*) of Apure, Venezuela. The llanos have a hyperseasonal climate pattern of drought from November to April followed by heavy rain and flooding from May through October. Most Pumé (~7200) live along the Capanaparo, Cinaruco, and Riecito rivers in small villages and towns. They have a mixed subsistence base of fish, manioc horticulture, animal husbandry, wild foods and engage in occasional wage labor. The Pumé who live in the interior between these major rivers (~800) subsist mainly on wild resources (Kramer and Greaves, 2011). The Pumé distinguish these differences in community type as the river Pumé and savanna Pumé, respectively (Greaves, 2006). This study was conducted in 2007 in two remote savanna Pumé villages and three small river communities situated within a 400 km^2 area. None of these villages has a school, health clinic, store, electricity, well water, or can be reached by permanent road.

The savanna Pumé are mobile foragers who move camps throughout the year in response to seasonal variation in subsistence and water availability. Dry season subsistence is centered on fish and feral mangos. When the llanos flood during the wet season, subsistence shifts to hunting, wild tubers and small inputs of cultivated manioc. Food scarcity occurs annually during the wet season. In contrast, river Pumé began to reside in permanent villages along major rivers around 50 years ago. Like the savanna Pumé, they have a mixed subsistence base of

fishing, hunting, and wild foods. However, river Pumé are more reliant on garden returns (manioc and other crops), and animal husbandry provides additional caloric input. River Pumé men engage in compensated labor at small-scale ranches more frequently than do savanna men (Kramer and Greaves, 2007). River Pumé therefore experience less seasonal variation in food availability.

Despite differences in economy and food availability, river and savanna Pumé communities maintain many similarities. Both the river and savanna Pumé are essentially monolingual. Few river Pumé (3%) and no savanna Pumé ever attended school (Kramer and Greaves, 2007). They also inhabit a similar reproductive environment. Serial monogamy is the most common marriage pattern (Kramer and Greaves, 2011). Fertility is high (cohort fertility rate for women aged >40 = 7.41 for savanna and 7.75 for river women) (Kramer and Greaves, 2007). Most girls marry by 15 years and average age at first birth is 15.5 years (Kramer et al., 2009; Kramer 2008). There is no formal post-partum isolation of mothers and newborns. Pumé infants are held, cared for and occasionally nursed by others from the day they are born. During the first months of life, infants are exclusively breastfed. They are generally weaned by 3 years old.

The Venezuelan llanos present an epidemiologically challenging environment for both the river and the savanna Pumé. In addition to malaria, endemic diseases include Chagas, tuberculosis, amoebiasis, intestinal parasites, yellow fever, measles, and respiratory diseases (Barreto and Rivas, 2007; Lizarralde and Seijas, 1991). In river communities, IMR is 13.2%, much higher than the Venezuelan national average (1.96%) and comparable to the Tsimane rate (Kramer and Greaves, 2007; PRB, 1996). In savanna communities, IMR is 34.6% (Kramer and Greaves, 2007). Health-care workers rarely reach the study communities; of children under the age of 3 years (n = 43) observed in 2007, only 7% had been vaccinated.

Data collection

Data on infant length, weight, mid-upper-arm circumference, and thymus size were collected in a cross-section of 86 infants (Tsimane n = 57, Pumé n = 29). All children aged 0–2 years were considered eligible to take part in the study. Participation was based on consent from the child's parents. The research protocols, including informed consent, were approved by the University of New Mexico Institutional Review Board, the University of New Mexico Health Sciences Center Human Research Review, and the Committee and Committees on Research Involving Human Subjects at Stony Brook University.

Aging children. Tsimane parents were asked infant birthdates, which were cross-checked with data maintained by the schoolteacher in Cedral and with THLHP demographic data (Gurven et al., 2007). The Pumé do not keep written vital records, nor have an absolute means for retrospectively aging older children and adults. Parents can accurately report the ages of young children by moon or season counts up to 4 years. All Pumé children included in this study were born proximately to the 2005–2007 field seasons and their mothers were interviewed within at most several months of their birth (Kramer and Greaves, 2007).

Anthropometry. Infant recumbent length was measured using a baby board and maternal standing height was measured using a Seca Portable Stadiometer. Infant mid-upper arm circumference (MUAC) was measured on the left upper arm at the point halfway between the tip of the shoulder and the elbow. Mothers and infants were weighed using a digital scale to the closest tenth of a kilogram. Children who could not stand on their own were weighed by subtracting mother's weight while holding the child. Children were weighed naked and without shoes. If mothers were pregnant, non-pregnant weights were obtained from existing Tsimane and Pumé anthropometric datasets (if available) from 1 year before or after data collection. Scales were routinely calibrated against known weights and repeated measures were taken to minimize measurement error.

Thymus measures. Thymus ultrasonography was performed using a solar-powered High Technology, Inc. PU-2200 ultrasound with a 7.5 MHz linear pediatric probe. Measures were taken by a single observer (AV). Due to limitations of solar power and the less developed nature of the right thymus lobe, only the left lobe was scanned. To maintain consistency with previously published data from South American children, a planimetric measure was taken of the thymus area of the lobe (sagittal plane) (Chevalier, 1997; Chevalier et al., 1994). Depth of the lobe (left anterior–posterior dimension) also was measured. Body temperature was taken under the arm to control for confounding effects of fever. Attempts were made to minimize crying in infants, which causes the thymus to appear smaller.

Data analysis. Z-scores for age-corrected height/length (HAZ), weight (WAZ), and arm circumference (MAZ) were calculated using WHO Anthro software. General linear models (GLMs) were used to compare anthropometry and thymus size between Tsimane and Pumé infants, and to test for predictors of thymus size. Analyses were conducted using PASW Statistics 18.

RESULTS

Descriptive statistics for the Tsimane and Pumé mothers and infants are provided in Table 1. Differences in maternal age were not significant (t = 0.18, p =0.86). Pumé mothers were lighter than Tsimane mothers (t = –1.86, p = 0.03), but there were more Tsimane women (4/57) for whom non-pregnant weights were unavailable. When these were excluded (along with a Pumé woman who had just given birth) there were not significant differences in maternal weight (n = 81, t = –1.02, p = 0.16). The Tsimane and Pumé infants fell below slightly below the WHO mean for height and weight (mean HAZ= –1.2, mean WAZ= –0.8, n = 86) and adhered to the WHO mean for mid-upper arm circumference (mean MAZ = 0.0, n =

TABLE 1. Sample characteristics

	Pumé	Tsimane
Total sample size	29	57
Mean age infant in months (range)	8.5 (0.2–24)	11.28 (0.7–23.5)
Total girls (boys)	15 (14)	28 (29)
Mean age mother (years)	26.6	26.3
Mean weight mother (kg)	50.8	54.1
Mean weight infant (kg)	7.7	7.7
Mean length/height infant (cm)	66.7	68.5
Mean arm circ. infant (cm)	13.6	14.4
Mean thymic depth (mm)	10	13
Mean thymic area (mm^2)	257	321

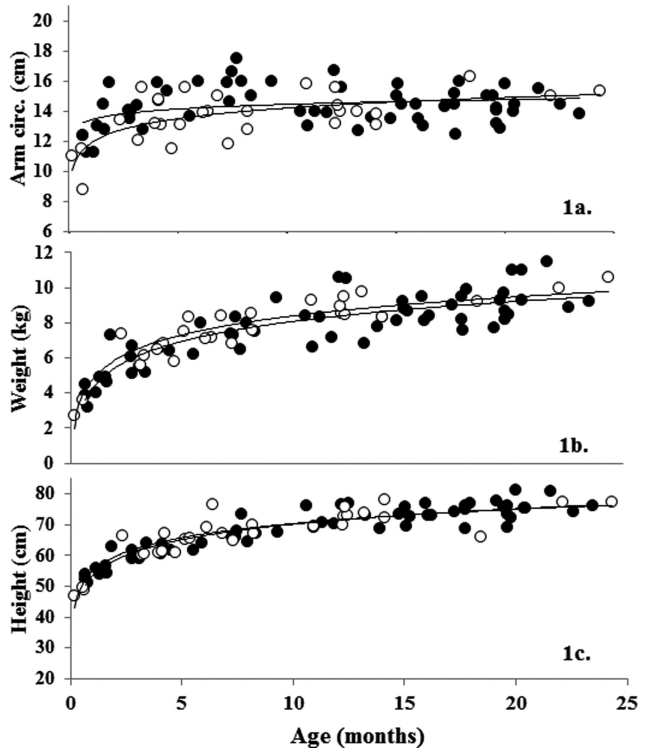

Fig. 1. Arm circumference (1a), weight (1b), and length/height (1c) by age in Tsimane (closed circles) and Pumé (open circles) infants.

56 infants aged 6–24 months), a measure commonly used in nutritional screening of older infants (Roy, 2000; Velzeboer et al., 1983). Tsimane and Pumé anthropometric trajectories in infancy were nearly identical (see below). Tsimane mean thymus area (321 mm^2, SD = 96) corresponds to values published for 37 malnourished Bolivian schoolchildren aged 6–55 months who had undergone 9 weeks of immuno-nutritional rehabilitation (their mean thymus area was 348.7 ± 21.1 mm^2, Chevalier et al., 1994). Pumé mean thymus area was much smaller (257 mm^2, SD = 99) and variance was high in both groups.

Cross-cultural differences

Height (n = 86, p = 0.32) and arm circumference (n = 86, p = 0.10) did not differ statistically between Tsimane and Pumé infants (Fig. 1). Pumé infants were slightly heavier but the difference was not statistically significant (n = 84, p =0.07, Fig. 1b). Thymus area decreased linearly with age in the Tsimane (r = –0.60, p = <0.01) and Pumé (r = –0.64, p = <0.01) infants (Table 2, Fig. 2a), as did thymus depth (Tsimane r = –0.41, p = 0.01 and Pumé r = –0.65, p = <0.01, Table 2, Fig. 2b). Thymus area and depth were smaller in the Pumé than in the Tsimane at all ages (Fig. 2a, b; Table 2, Model 1). However, because the savanna and river Pumé have different subsistence strategies, and infants experience different mortality risks, it was difficult to interpret these findings. To evaluate whether river/savanna Pumé differences in thymus size were confounding the Tsimane/Pumé differences, thymus dimensions between the Tsimane infants and river Pumé (n = 22) infants only were compared (Table 2, Model 2). The resulting model was very similar to the original including all 29 Pumé infants.

TABLE 2. Differences in thymus area and depth in Tsimane and Pumé

Model 1. Tsimane (n = 57) and River/ Savanna Pumé infants (n = 29)	Thymus area			Thymus depth		
Parameter	*B*	St. error	Sig	*B*	St. error	Sig
Intercept	419.04	17.05	<.001	16.09	0.69	<.001
Group (Pumé = 1; Tsimane = 0)	−88.33	17.69	<.001	−4.20	0.71	<.001
Infant age (months)	−8.67	1.22	<.001	−0.25	0.05	<.001

Model 2. Tsimane (n = 57) and River Pumé infants only (n = 22)	Thymus area			Thymus depth		
Parameter	*B*	St. error	Sig	*B*	St. error	Sig
Intercept	416.59	16.97	<.001	16.10	0.72	<.001
Group (Pumé = 1; Tsimane = 0)	−87.63	18.71	<.001	−4.09	0.79	<.001
Infant age (months)	−8.45	1.23	<.001	−0.25	0.05	<.001

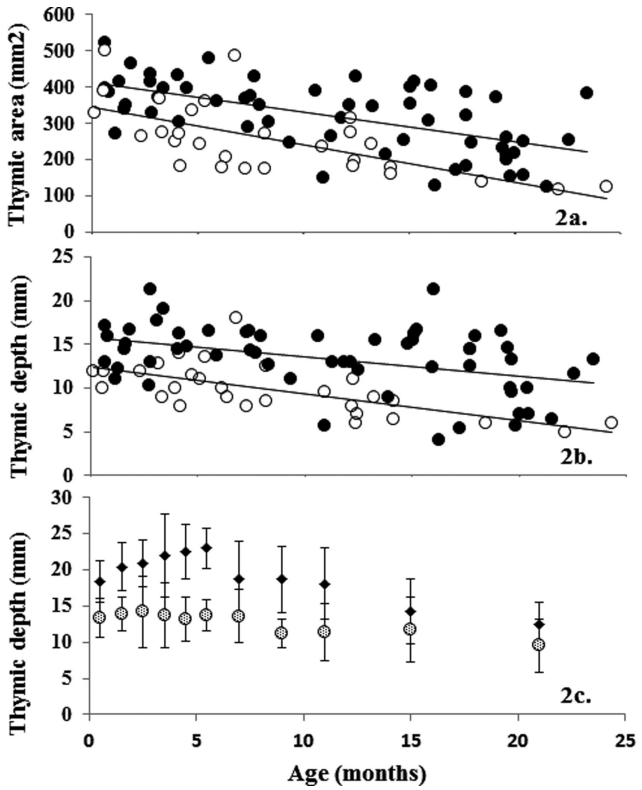

Fig. 2. Scatterplots of thymic area (2a) and depth (2b) in Tsimane (closed circles) and Pume (open circles) infants. In 2c, thymic depth values from 151 Turkish infants (diamonds) are compared to combined Tsimane/Pume means (dotted circles). Values are reported as means per age interval (see text) with standard deviations. Turkish means are from Yekeler et al., 2004.

To contextualize our findings, Tsimane and Pumé thymus depth values were combined and averaged to form a South American native infant cohort. Their thymus depth

TABLE 3. MAZ predicts thymus area in (n = 56) infants aged 6–24 months

Parameter	*B*	Std. error	Sig
Intercept	388.12	35.13	<0.001
Age	−6.59	2.16	0.002
WHO MAZ	25.79	9.70	0.008
Group (Pumé = 1; Tsimane = 0)	−86.15	23.06	<0.001

means were then plotted alongside published mean values from 151 Turkish infants (Yekeler et al., 2004) in Figure 2c. The Turkish infants were sampled monthly from 0 to 6 months, every 2 months from 6 to 12 months, once from 12 to 18 months and once from 18 to 24 months. Turkish and South American native thymus depth means are plotted with standard deviations at the midpoint of each of these designated time periods. Plot 2c does not control for population differences in body size not inter-observer variation in thymic ultrasonography. It is therefore intended only to qualitatively evaluate differences in thymic depth between the two infant populations.

The Turkish infants had larger thymic depth at all ages than the South American native infants. Population-level differences in thymic depth increased across early infancy and were most pronounced at 5 and 6 months of age. In the Turkish cohort, thymus depth increased postnatally, peaked at 6 months, and then gradually declined over the second year. In contrast no pronounced growth peak was observed in the South American native cohort. Turkish and South American native thymic depth values were not markedly different in the second year of life. Only at the two, five, six, and nine month designated points were their standard deviations non-overlapping.

Predictors of thymus size

Anthropometric and maternal variables that might affect thymus size were then evaluated. To assess the explanatory value of body size on thymus size, thymus area and depth were regressed on the age-controlled HAZ, WAZ, and MAZ. Given the complex relationships between age, body size, and thymus size, we included age in these models to avoid interpretation problems associated with colinearity (e.g., age strongly correlates *positively* with body size and *negatively* with thymus size. WAZ and HAZ were not significant predictors of thymus area ($p = 0.467$ and $p = 0.922$) or depth ($p = 0.563$ and $p = 0.727$) after controlling for group (Tsimane or Pumé) and infant age.

Arm circumference is commonly used in nutritional screening of older infants (Roy, 2000; Velzeboer et al., 1983) and is a predictor of mortality in children $\geq$ 6 months of age (Zemel et al., 1997). Analyses using MAZ were therefore restricted to ($n = 56$) infants aged 6–24 months. MAZ was a significant predictor of thymus area ($\beta = 31.24$, $p = <0.004$) but not its depth ($\beta = 0.77$, $p = 0.111$) after controlling for age. After controlling for both population and age, MAZ was still a highly significant predictor of thymus area (Table 3). This result was consistent in both the Tsimane infants ($n = 39$, $\beta = 26.18$, $p = 0.044$) and the Pumé infants ($n = 17$, $\beta = 41.72$, $p = 0.010$).

Maternal weight and age were evaluated for effects on infant thymus size because maternal condition influences infant health outcomes (Kirksey et al., 1994; Medhin et al., 2010). All analyses controlled for infant age. Maternal age was not a predictor of thymus area or depth ($p = 0.147$, $p = 0.358$, respectively). Maternal weight was not a significant predictor of thymus area or depth in the Tsimane infants ($n = 57$, $p = 0.539$ and $p = 0.628$, respectively) but approached significance in small sample of Pumé infants ($n = 29$, $\beta = 3.38$, $p = 0.075$ and $\beta = 0.095$, $p = 0.081$, respectively). Potential confounding variables such as birth season (wet/dry), infant sex, and presence of fever also were evaluated for effects on thymus size. None of these proved to be a significant predictor of thymus dimensions (data not shown).

DISCUSSION

Although the Tsimane and Pumé infants had similar growth trajectories, the Pumé had smaller thymuses than the Tsimane at all ages. This finding indicates that population variation in thymus size does not reflect a simple phenotypic correlation with body size. Intervening factors in the prenatal and postnatal environment most likely contribute to cross-cultural variation in thymic size between these two South American native populations. Maternal condition and related prenatal factors are likely to underlie differences in thymus size at birth. Pathogen exposure and neuroendocrine factors most likely influence thymic developmental trajectories in postnatal life as immune defenses mature.

Prenatal factors such as maternal smoking (Zeyrek et al., 2008), environmental toxin exposure (Moore et al., 2009; Park et al., 2008), and intrauterine growth restriction (IUGR) (Cromi et al., 2009) have been associated with a small thymus at birth. Unfortunately it is not possible to control for these with the data available. Maternal smoking is rare in the Tsimane and unlikely to influence newborn thymus size. In contrast, ritualized tobacco smoking is practiced by Pumé men and women, although the magnitude of maternal smoking is not quantified. The extent of environmental toxin exposure experienced by the two populations is also unknown. The Tsimane villages are located downstream on the Maniqui River from San Borja and the river is likely to be contaminated (Gurven et al., 2007), while the Pumé reside in a remote region far from any major urban centers or agricultural enterprises.

Maternal condition is associated with newborn size (Kirksey et al., 1994; Wells, 2010) and thymus size at birth correlates strongly with birth weight (Aaby et al., 2002; Iscan et al., 2000). It is therefore likely that unmeasured variation in maternal condition contributes to differences in thymus size in newborns. Both populations of mothers measured were similar in weight after controlling for pregnancy status, however, Pumé mothers experience profound seasonal fluctuations in nutritional status (Kramer, 2008) that are not observed in Tsimane mothers. In addition to pronounced nutritional fluctuations, Pumé mothers experience greater epidemiologic stress and less access to health care than do Tsimane mothers. This combination of adverse prenatal factors is likely to negatively impact fetal thymic development and contribute to smaller thymus size in Pumé newborns.

Differences in thymus size between the Tsimane and Pumé persisted across infancy and the rate of change was similar in the two populations. Postnatal thymic development is influenced by the environment; pathogen exposure (McDade, 2003), neuroendocrine factors (Borghetti

et al., 2009), and seasonal influences all affect thymic development (Aaby et al., 2002; Collinson et al., 2003). Unfortunately biomarkers of infection, immune function, and endocrine function were not being collected from Tsimane infants at the time of this study. Comparable medical studies among the Pumé are currently problematic following the *Darkness in El Dorado* scandal (Borofsky, 2005; Gregor and Gross, 2004; Tierney, 2000). Birth season was not a predictor of thymus size in the Tsimane or Pumé, but there might be a confounding effect of measurement season since all Pumé infants were measured at the same time (end of the dry season, a period of relative abundance) and all Tsimane infants were measured at the same time (in the wet season, when gastrointestinal infections increase).

While environmental factors clearly contribute to population differences in thymus size, we cannot rule out a genetic component. As we cannot distinguish between genetic and environmental effects with the data currently available, future studies may be able to address this question. This can be achieved by comparing infant thymic development in relatively genetically homogenous populations such as the Pumé, with distinctive subsistence economies in river and savanna ranges (Kramer and Greaves, 2007), or the Tsimane, who experience variable infant mortality by region depending on access to healthcare facilities (Gurven et al., 2007). Such comparisons were not feasible given our small sample size in the Pumé and the environmentally homogenous nature of the Tsimane communities sampled.

Thymus depth in Turkish infants was larger at birth than in the South American native infants and became most pronounced in the first 6 months of life. Differences in thymic development between these infant populations may reflect the markedly different prenatal and postnatal environmental conditions they experience. The Turkish pattern of rapid thymic growth in the first 6 months, followed by declining thymic size, has been observed in other populations (Hasselbalch et al., 1999b; Kizilcan et al., 1995). Postnatal thymus growth mirrors infant fat deposition patterning (Kuzawa, 1998; Roberts and Young, 1988) and both seem to be phenotypically correlated in nutritionally adequate environments. However, postnatal thymus growth has also been observed in poor, rural populations in The Gambia and Bangladesh (Collinson et al., 2003; Moore et al., 2009), and so is not restricted to privileged environments. The Gambian and Bangladeshi mothers and infants probably differ from the Tsimane and Pumé in important ways because they have experienced longer term medical interventions to improve maternal and infant health.

We speculate that much population-level variation in postnatal thymic development is driven by differential morbidity rates because infection contributes to transient thymus depletion (Savino and Dardenne, 2010). Turkish infants inhabit an environment with low morbidity and adequate health care, as evidenced by their low national IMR (<1% in 2003, (WHO, 2012)). In contrast, population mean thymus size in the Tsimane and Pumé is more likely to be influenced by concurrent infant morbidity at any given age. Whether infection-induced thymus depletion in resource-scarce environments is adaptive in facilitating immunologic maturation, as suggested by McDade (2003) or a deleterious effect of adverse conditions remains unclear. To evaluate these hypotheses, the respective energetic costs of thymus tissue versus peripheral T-lymphocyte maintenance should be assessed and compared with the risks of infant mortality by infectious disease in a variety of populations.

TABLE 4. Qualitative predictions

1. Variation in thymus size at birth is related to variation in maternal condition
2. Postnatal thymic developmental trajectories are shaped by environmental factors
3. Age-specific population variation in thymus size is driven by differences at birth and concurrent morbidity

Life history theory provides a useful perspective to inform cross-cultural studies of thymic development. Under this framework, thymus physiology should be designed to maximize infant survival in risky environments by optimizing infant immunocompetence and growth in response to different energy-availability and pathogen-stress profiles (McDade, 2003). Variable thymic developmental trajectories are expected across populations due to the diverse challenges associated with different prenatal and postnatal environments. In nutritionally and epidemiologically privileged environments, high levels of investment in growth and thymus maintenance may lead to phenotypic correlations between thymus and body size. We expect a more complex relationship between infection, nutrition, and thymus size in the Tsimane and Pumé because energy allocation trade-offs are more pronounced when resources are scarce (McDade, 2005; Norris and Evans, 2000; Zera and Harshman, 2001).

In light of our findings and above discussion, we generate three general predictions pertaining to patterns of cross-cultural variation in thymus size at birth and in postnatal life (Table 4). Future studies should assess these predictions in an attempt to explain variation in cross-cultural thymic development patterns. Importantly, future research should evaluate our findings through additional measures of thymus function. This can be done by analyzing T-lymphocyte counts and subsets with respect to thymus size or using T-cell receptor excision circles. While several studies have indicated an association between thymus size and aspects of immune function (Chevalier, 1994; Cohen-Stuart et al., 1998; Jeppesen et al., 2004; Ngom et al., 2004), this relationship is not simple and may be variable across populations.

CONCLUSION

We have provided evidence for a complex relationship between nutritional status and thymus size in infants from two small-scale societies in South America. Population-level variation in thymus size is evident in the Tsimane and Pumé infants even after accounting for body size. These differences begin in the first month of life and persist across infancy despite their similar anthropometric trajectories. This study adds comparative data to the few existing studies of thymic development in undernourished infants inhabiting pathogenic environments.

Thymic development is driven by a combination of environmental factors in both prenatal and postnatal life. Variation in thymus size at birth is likely influenced by maternal condition. Cross-cultural differences in thymic development are probably driven by environmental factors including variation in morbidity rates. The

interactions between energy availability and pathogen exposure are complex and play an especially important role in thymic development outside of urban, sanitary environments. Better understanding of thymic developmental influences during infancy is relevant to effective public health programming, especially among South American natives, who suffer negative health outcomes from poverty, infectious disease susceptibility, and chronic parasitism (Hurtado et al., 2004).

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