TITLE PAGE

Title: Growth curves & the international standard: How children’s growth reflects challenging conditions in rural Timor-Leste

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ABSTRACT

Objectives
Population-specific growth references are important in understanding local growth variation, especially in developing countries where child growth is poor and the need for effective health interventions is high. In this paper, we use mixed longitudinal data to calculate the first growth curves for rural East Timorese children to identify where, during development, deviation from the international standards occurs.

Materials and Methods
Over an eight-year period, 1245 children from two ecologically distinct rural areas of Timor-Leste were measured a total of 4904 times. We compared growth to the WHO standards using z-scores, and modeled height and weight velocity using the SITAR method. Using the GAMLSS method, we created the first growth curves for rural Timorese children for height, weight and BMI.

Results
Relative to the WHO standards, children show early-life growth faltering, and stunting throughout childhood and adolescence. The median height and weight for this population tracks below the WHO 5\textsuperscript{th} centile. Males have poorer growth than females in both z-BMI ($p = 0.001$) and z-height-for-age ($p = 0.018$) and, unlike females, continue to grow into adulthood.

Discussion
This is the most comprehensive investigation to date of rural Timorese children’s growth, and the growth curves created may potentially be used to identify future secular trends in growth as the country develops. We show significant deviation from the international standard that becomes most pronounced at adolescence, similar to the growth of other Asian populations. Males and females show different growth responses to challenging conditions in this population.
INTRODUCTION

The creation and use of growth references has a much wider significance in the study of human variation than simply providing a tool for clinicians to monitor child growth and diagnose malnutrition. The World Health Organization’s creation of international growth standards marked an important development in monitoring child growth on a global level, as they are a tool designed “to assess children everywhere, regardless of ethnicity, socioeconomic status and type of feeding,” (WHO Multicentre Growth Reference Study Group, 2006), and to compare children’s actual growth to how they should be growing (de Onis, 2011). However, characterizing the pattern of growth at a population level can provide important information about differences both within and between populations, for example localizing particular points in development when growth falters. Differences between populations can reveal large-scale trends in over- or under-nutrition, or the presence of local biological differences in growth. Population-specific references can be used to identify and monitor children performing outside the local normal range, a particularly useful instrument for clinicians and public health workers (Kuczmarski et al., 2002). Changes between references created from the same population at different points in time can also reveal secular trends in child growth.

Human populations show great diversity in overall size, growth rates, and body proportions (Bogin, 1999). Studies of small populations, for example the Shuar in South America, show both a reduction in average body size, and a different pattern of adolescent growth compared to the international reference (Urlacher et al., 2016). Indonesian-Malay children fall within European references until 12-13 years of age, and then begin to fall away (Eveleth and Tanner, 1990). Ulijaszek (2001) concludes, given that children are well-nourished, that populations vary little in preadolescent height, with the exception of Asiatic populations¹, which have slightly lower means than any other group. Haas and Campirano (2006) also find little variation in privileged populations during

¹ Includes China, Japan, Korea, Mongolia & Singapore
preadolescence; however, during puberty, variation increases and non-European populations, especially Asian populations², fall below the international population. Both reviews highlight the difficulties of teasing apart whether increasing differences between populations with increasing age of the child are due to genetic differences in growth, or due to secular trends. If a population is in the process of a secular trend toward increasing height, then older children in the population may show different growth, and smaller attained size, than will the younger cohort by the time this younger cohort reaches adulthood. It may be that Asian populations tend to be in the process of a secular trend of increasing height, and over time may reach the same means as European populations, rather than being genetically small.

Among Asian populations, there exists wide variation in child growth (Eveleth and Tanner, 1990). Timor-Leste has one of the highest rates of child malnutrition in the world, as classified by the WHO (von Grebmer et al., 2014); however, there are few data on patterns of child growth within this population. There are also limited data on adult height in Timor-Leste with only two publications of national-level data: one stating that 15% of women are shorter than 145cm (National Statistics Directorate [Timor-Leste], Ministry of Finance, & ICF Macro, 2010), and another that average adult male height is 158.7cm and adult female height is 152.9cm (World Health Organization, 2014). In this paper, we characterize the growth of rural Timorese children through comparison to the WHO standards, and modeling of growth and growth velocity. This allows detailed examination of current child growth patterns in Timor-Leste, and provides a potential reference for future identification of secular trends in growth of rural children. We also describe a methodology well suited to creating growth curves for small populations.

The population of Timor-Leste is genetically complex as a result of multiple migration waves during early human history between Australia, Island South-

² Includes China, Taiwan, Japan, Thailand, Pakistan, India, Iran, Turkey, Jordan, UAE, Saudi Arabia & Native Americans
East Asia, and Melanesia (Gomes et al., 2015), as well as a long history of colonization. Two distinct language groups exist in Timor: Austronesian (East/South East Asia origin) and non-Austronesian (Near Oceania); however, substantial admixture has occurred between the two language groups, resulting in no gene-language correlation (Gomes et al., 2016).

Timor-Leste was colonised by the Portuguese for 400 years, and then invaded by Indonesia in 1975. Following the conflict leading to the nation’s independence from Indonesia in 2002, much of the remaining infrastructure and development of Timor-Leste is centralized around the capital, Dili. As such, the vast majority of areas outside Dili are considered rural. The majority of rural Timorese are subsistence farmers, cultivating the staple crops of corn, rice and cassava. Some families supplement agriculture with cash work, while only a few derive their entire living from salaried work. Reliance on agriculture in Timor-Leste is associated with periodic food shortages due to climate seasonality (Sanders, Judge, Pauli, Amaral, & Schmitt, 2014; Seeds of Life, 2009). Poor road infrastructure, exacerbated by heavy rains in the wet season, means many rural areas are quite isolated from Dili, and therefore reliant on limited local markets for selling produce. National-level epidemiological information is also limited. According to the most recent Demographic and Health Survey, 16% of under-fives experienced diarrhea in the past two weeks, and 19% reported fever (National Statistics Directorate [Timor-Leste], Ministry of Finance, & ICF Macro, 2010). Soil-transmitted helminth infection occurs in 29% of children aged between seven and 16 years (Martins and McMinn, 2012). Poor access to healthcare in rural areas, low per-capita income, and widespread reliance on subsistence agriculture mean that it is highly unlikely children reach their genetic potential for growth.

Understanding the patterns of growth in nutritionally stressed children adds to our understanding of human variation in that pre-agricultural human development likely took place under challenging conditions. Observed patterns of growth suppression and catch-up growth indicate that humans evolved
capacities to respond to periods of scarcity and resource flushes. In rural Timor-Leste, scarcity is at least seasonal and resource flushes rare. Understanding how children's growth responds, especially in terms of expected growth spurts and prolongation of the growth period, provides new insights on the nature and constraints on plasticity in this life history stage. It also sets the stage to investigate the importance of conditions in early life to later responses to environmental conditions.

**MATERIALS & METHODS**

**Study population**

Data were collected at least annually from 2009 to 2016 in rural Timor-Leste. This study is based in two rural areas – the Ossu sub-district of the Viqueque district in the mountains of the central east (600-1000m above sea level), and the Natarbora sub-district of the Manatuto district in the flat coastal plains of the south (5-50m above sea level; Figure 1). Both sites have a basic health center, local market and schools. Ossu is situated on a main road and is more populous than Natarbora. For a more detailed description of the field sites, see Thu and Judge (2017).

[Figure 1 here]

Ossu was first visited in 2009, and Natarbora in 2012. Both sub-districts’ samples include four local communities within the area. Subjects were recruited through approaching households on a nearest neighbour basis, and gaining consent from households with resident children (Reghupathy, Judge, Sanders, Amaral, & Schmitt, 2012). Households were then re-visited on a six-monthly to yearly basis, with some additional recruitment of participants each year (Supplementary Table 1).

On each visit, all children in participating households aged from birth to 19 years were measured for height and weight (de Onis, Onyango, Van den...
This resulted in a total of 1245 children measured on 4904 occasions (Table 1). Note that the lower number of children measured seven or more times is largely due to children being recruited or born later in the study period. For children too young to stand upright unassisted, recumbent length was measured. All children were measured wearing a light layer of clothing (shorts/skirt and a t-shirt).

[Table 1 here]

Data manipulation and analyses

In Timor-Leste, not all families know the official birth dates of their children, so ages given by parents or guardians are not always accurate. If the date of birth of a child was not known, a year of birth was estimated from the child’s reported age on each visit. If the child’s age differed by more than two years over a one-year period, and could not be corrected via documentation, that child was removed from the sample. This resulted in a small number of exclusions as most measurements requiring estimation could be corrected to within one year. Age at measurement was then calculated from known date of birth and measurement date, or estimated year of birth and measurement date, with the default month of birth being June.

WHO growth comparison

For comparison to the WHO growth standards, growth measures were converted to z-scores using the WHO Anthro and AnthroPlus software in IBM SPSS Statistics version 22. Children with calculated z-scores of greater than five and less than -5 were removed (n = 22). To examine sex and age differences in this population beyond those built into the WHO z-scores, analyses of z-scores were performed using linear mixed models with child ID as a random factor to account for repeated measures of the same child.
Growth velocity

Growth velocity was modeled for height and weight using the SITAR (SuperImposition by Translation And Rotation) method (Cole, Donaldson, & Ben-Shlomo, 2010), following the R code provided by Blackwell et al. (2017). This method fits a random effects model to individual growth curves. Individuals with fewer than three measurements were excluded from this analysis. Outliers were removed using the velout procedure in the SITAR package in R. Models including infancy and early childhood (before five years of age) would not converge. Remeasures of children occurred at approximately yearly intervals; however, not all children could be measured every year. In early life, when growth velocity changes rapidly, infrequent measurements mean averaging over time blunts these changes in velocity, and this may explain models not converging. In future data collection, shorter measurement intervals and birth measurements for anchoring models may resolve this issue. Models from ages five to 18 are presented here.

Growth curve modelling

The GAMLSS package in R allows for fitting of growth curves using a BCPE (Box-Cox power exponential) distribution, and for smoothing degrees of freedom to be determined in a step-wise manner (Rigby and Stasinopoulos, 2001; 2004). Smoothing was performed using cubic splines, a function that adds 3 degrees of freedom to each model parameter (Cole and Green, 1992). The BCPE model has four parameters: $\mu$ (median), $\sigma$ (coefficient of variation), $\nu$ (the Box-Cox transformation power, and $\tau$ (parameter related to kurtosis).

The following protocol is based on the WHO (World Health Organization, 2006), and a more recent use of the GAMLSS procedure to model growth curves on the Shuar of South America (Urlacher et al., 2016). The starting model was entered as df($\mu$) = 10 and df($\sigma$) = 5, with $\tau$ fixed at 2 and $\nu$ fixed at 1. The best transformation power ($\lambda$) for age was determined by trialling a range of values ranging in 0.05 intervals from 0.05 to 1.00, and selecting the
transformation power giving the lowest global deviance value for the model. For both male and female BMI, and female weight, the lowest global deviance occurred with no age transformation. From this model, outliers (predicted z-scores were less than -3 or greater than 3) were removed. The model was then fit for the best number of degrees of freedom for μ and σ, with df(σ) ranging from 1-10 and df (μ) ranging from 1-15. Following the WHO, the best value for σ was determined using the lowest value of GAIC(3), where 3 is the penalty term, and the best value for μ was determined using the lowest value of AIC. Selecting models based on only one criteria (e.g. only GAIC for both σ and μ) gave two conflicting ‘best’ models. Following this, the degrees of freedom for ν, then τ, were varied from 1-8 and again the best model was selected based on GAIC(3). For all models except female BMI, unfixing the τ parameter slightly increased the GAIC of the models, but led to better fit worm plots and centiles, so τ was allowed to vary by one degree of freedom. For female BMI, df(τ) = 2 had the lowest GAIC. Final model age transformations, sample sizes and parameter degrees of freedom are provided in Supplementary Table 2.

Residual plots, worm plots and Q statistics were used to test goodness of fit of the models. Example tests of model fit are provided (Supplementary Table 3, Supp. Figure 1 and Supp. Table 4), using the model formula for the female height data. All models were tested using this procedure. Following the WHO, to account for data being longitudinal, children were grouped into one-year age groups to minimize the effects of repeated measures of the same child in the same interval. For example, those aged between 0.5 years to 1.49 years were placed in the one-year-old group. Due to some data being collected at six-month intervals, some individuals were repeated within an age group (on average 14.7% of each age group is due to repeat measurements; n = 743 measurement incidences out of total n = 4904). For those age groups with a high percentage of repeated individuals (>18%), we compared the mean height for the whole age group to that of a subset with one randomly selected repeat measure for each repeated individual removed. There were no significant
differences, so the entire dataset including repeats within age groups was used when testing model fit.

Residual plots were used to detect deviation from normality in the fitted model, where normality is given as a mean of zero, variance of one, a coefficient of skewness of zero and a coefficient of kurtosis of three. Worm plots allow one to check fitted model residuals for different ranges of the independent variable; that is, to determine if the model fits adequately across all ages. If the majority of points in the worm plot for each age range fall within the 95% confidence intervals, the model fits well (van Buuren and Fredriks, 2001). Q-statistics are another test of normality of the residuals for each age range. Significant Q statistics indicate an inadequacy of the model (Royston and Wright, 2000). Increasing the degrees of freedom for that particular parameter can ameliorate such inadequacies. Within any age range, if the Q statistics Z value is greater than 2, this also indicates an inadequacy in the model; however, if the overall Q statistic is not significant, it may be due to a biological difference, rather than due to the fit of the model (WHO Multicentre Growth Reference Study Group, 2006).

RESULTS

Characteristics of the sample
Mean age at measurement was 8.98 (SD = 5.03) years (Supp. Figure 2). Almost the same number of individual males (617; 49.3%) were measured as were females (635; 50.7%), with the same percentage split when considering all measurement incidences. Mean age at measurement was slightly higher for females (9.27 years) than for males (8.69) (Independent t-test: t = 4.04, p < 0.001).
Growth velocity

The WHO only provides standards for growth velocity until 24 months, so growth velocity data is presented unstandardized. For comparison, models of Timorese growth velocity are presented with the 50th centile of growth increments from healthy, white participants of the Fels Longitudinal Study (Baumgartner, Roche, & Himes, 1986). The peak of modeled growth velocity curve was 8.67 cm/year at 14.26 years in males, while female peak height velocity occurred earlier and at a lower magnitude (6.96 cm/year at 11.72 years) (Figure 2). Male peak weight velocity occurred at a similar time to peak height velocity (5.36 kg/year at 14.29 years), whereas female peak weight velocity occurred relatively later (5.05 kg/year at 12.55 years) (Figure 3). Relative to the Fels children, pubertal growth in height was similar in Timorese children. However, there are pronounced differences in pre-pubertal growth, particularly immediately preceding the adolescent growth spurt, where Timorese children showed slower growth velocities in both height and weight. In weight, Timorese males had a peak velocity of reduced magnitude, and Timorese female peak velocity was slightly delayed.

Comparison to WHO standards

Growth was standardized for age and sex to compare to the World Health Organization’s standards for normal growth. This process produces z-scores to show deviation from the international median, trends in local variations from the biological optima, and allows children to be compared to each other regardless of their age and sex. Following standardisation, children were grouped into five age groups. All z-scores were significantly associated with child age group (Table 2), meaning that after standardising for expected differences in growth with age, further differences remain in this population. In z-weight-for-age, older children had poorer growth than did younger children. Z-body mass index (BMI) for age was highest for 0-2 year olds, and lowest for...
10-15 year olds. For z-height-for-age, older children (>15 years) had the best
growth, with the poorest being 2-5 year olds.

[Table 2 here]

Given that there were age differences beyond expected biological differences,
we tested whether sex also influenced growth beyond what the WHO standards
incorporate. Of the three growth measures, only z-BMI and z-height-for-age
showed a sex difference beyond expected biological differences, with females
having better growth than males (Table 3).

[Table 3 here]

As age group and sex were independent predictors of z-height-for-age and z-
BMI, the relationship between the two variables was examined. In a combined
model, the interaction only showed a trend for z-height (F = 2.177, p = 0.069;)
but was significant for z-BMI (F = 9.780, p < 0.001). As BMI is a calculated
measure from height and weight, and z-height was not independently
correlated, this indicates the interaction effect is due to the contribution of
weight rather than height. The lack of significance of z-weight-for-age with sex
is likely because standards are only available until the age of 10, whereas z-BMI
considers children until age 19, and differences in growth beyond the growth
standards emerge during adolescence. When considering the two age groups
after 10 years of age, males and females have significantly different growth as
measured by z-height-for-age and z-BMI-for-age (Table 4). This indicates that
in this population, adolescent Timorese females gain more height and weight
relative to the WHO reference than do males.

[Table 4 here]
Centile modelling of Timorese children’s growth

Growth curves were modeled to further characterize the growth pattern of rural Timorese children and to examine where the shape of growth trajectories and timing of major events differ from the curves of privileged populations. Centile curves were estimated for males and females for height, weight and body mass index. Centiles in one-year age groups and LMS tables are presented in supplementary material (Supp. Table 5).

Height

From the final height model, the M curve (50\textsuperscript{th} centile), and 5\textsuperscript{th} and 95\textsuperscript{th} centiles were plotted against the same centiles from the WHO data for males and females (Figure 4). The values at age zero for the WHO are birth measurements, whereas due to the lack of birth length measures in our sample, age zero included birth to 0.49 years, artificially inflating Timorese birth weight. For comparison with the WHO data, Timorese data is plotted without age zero. Both rural Timorese males and females follow an approximately linear pattern of height gain until adulthood. Females show a flattening of growth from approximately age 14, whereas males appear to continue growing until after age 19. The female curve unexpectedly shows a slight decline in height from age 17, which is not biologically possible and may be due to sampling.

For rural Timorese females, the pattern of height increase is similar to the international reference, with the 50\textsuperscript{th} centile closely following the 5\textsuperscript{th} centile of the WHO across all ages. The shape of the male growth curve in Timorese children is also fairly similar to the international reference; however, the mean growth for Timorese children is displaced downward and tracks below the 5\textsuperscript{th} centile, rather than along the international median. Adolescent growth for Timorese males is slower than the reference; at age nine the difference between the Timorese 50\textsuperscript{th} centile and the WHO 50\textsuperscript{th} centile is 11.8cm, and by age 14 has increased to 18.4cm. Timorese males also show evidence of continued growth after age 19, whereas the WHO reference curves have flattened out.
Weight
The WHO model weight only until age 10, because variation around adolescence may result in children mistakenly being diagnosed as overweight, when they are in fact undergoing an earlier growth spurt than average (de Onis et al., 2007). Infant Timorese males begin closer to the international median for weight than they do for height, tracking just above the 5th centile until approximately four years of age, and then gradually shifting down (Figure 5). Females follow almost the exact same pattern, starting just above the 5th centile at age one, then dropping below by age five. For both males and females, variation in weight increases with age, shown by the increasing distance between the 5th and 95th centiles. The decline in weight for females from age 17 to age 19 is not biologically likely.

Body mass index
As with the height data, Timorese fitted centiles were compared with the WHO starting from age one (Figure 6). Body mass index is a composite measure of body mass relative to height (kg/m²) and it does not follow the same pattern as do height and weight (increasing from birth to adulthood). Both females and males show a decrease in BMI from birth until age six in females and age seven in males, and a subsequent increase. Female BMI appears to decline after age 17. Both males and females begin at 1 year very close to the WHO reference, with the exception of the 5th centile, which falls below the WHO. BMI then falls, with the mean Timorese BMI becoming closer to the 5th WHO centile. Females recoup during adolescence, whereas the males remain close to the 5th centile until adulthood.
DISCUSSION

This paper characterizes the growth of rural Timorese children from two sub-districts by examining growth velocity, WHO-calculated z-scores, and modeled growth curves. Each method provides some new understanding of the way Timorese children grow. As an anthropometric measurement, growth velocity has an advantage over attained body size in that a downshift in growth rate precedes a decrease in relative size; thus abnormal growth velocity can be used as an earlier diagnostic of possible health issues (Tanner, 1952). However, creation of growth velocity standards requires longitudinal rather than cross-sectional data, and thus there are very few growth velocity references (World Health Organization, 2009). Creating population-specific growth velocity references should therefore be a focus for public health researchers and physical anthropologists alike. Evaluation using the WHO international standard (to age five) and reference (ages five to 19) is a useful tool for comparing specific child growth in a population relative to the international optima, as well as comparing children within a population to each other. Standardizing data in this way controls for normal biological differences in growth with age and sex; thus, any effects found within the population for age or sex reveal local differences. The timing of these differences in the developmental period can indicate when to look for causes of poor growth and thus where to target interventions. The GAMLSS-modeled growth curves present both the average growth pattern and the way the extremes of the population are growing, providing more nuanced information on the population’s growth than either the WHO method or growth velocity. We use these three methodologies in combination to provide the most comprehensive study to date on the growth of rural Timorese children.
Early life growth

Relative to the WHO, rural Timorese children are small at most ages throughout development. Body mass index patterns are common to those in other developing countries; both males and females are closer to the international median early in life, then show growth ‘faltering’ by age two (Shrimpton et al., 2001). The Timorese median and 95th centile for both male and female one-year-olds closely matches the equivalent WHO BMI centiles; however, the 5th centile is clearly below the WHO 5th centile. This reveals the presence of two co-occurring growth patterns within this population: children who begin with appropriate BMI and then decline, and children who are born with lower than expected BMI and remain small. Body mass index declines from about 12 months of age in normal, healthy children, reflecting changes in body composition (Figure 6); however, the magnitude of this decline is far greater in Timorese children. Early life growth is associated with maternal conditions, for example, maternal under-nutrition is linked to foetal growth restriction and stunting by age two (Black et al., 2013). The 5th centile of Timorese children may thus represent those with poorer in-utero conditions. The children who are born with better BMI (and possibly better in-utero conditions) show a larger decline in BMI, likely due to poor postnatal environmental conditions, such as high rates of infectious disease or poor infant feeding practices (Jason, Nieburg, & Marks, 1984; Motarjemi, Käferstein, Moy, & Quevedo, 1993). Those children born smaller (the 5th centile) would also be exposed to similar environmental conditions; however, it is not possible for them to decrease their BMI any further and still survive. This pattern highlights the need for both pre- and postnatal interventions into maternal and child health, to ensure better in-utero growth and to buffer against poor conditions in the critical postnatal period. Continuing work on this population is investigating whether birth season is a contributing factor to differences in early life growth as it is in other populations facing seasonal food availability (Rayco-Solon, Fulford, & Prentice, 2005).
During preadolescence, wealthy Asian children’s growth in height begins to diverge from that of European children (Haas and Campirano, 2006; Ulijaszek, 2001) (Asian data included children from China, Japan, Singapore, Korea & Tibet). Asian children have an earlier age at peak height velocity than do European children, but the growth spurt is of similar magnitude (Ulijaszek, 2001). Less stature gained before the growth spurt leads to shorter adult stature, and by 17 years, Japanese are the shortest population group (Haas and Campirano, 2006). While this population of rural Timorese children are not well off, and therefore have poorer growth than their well-nourished Asian counterparts, they also begin life relatively closer to the WHO median and move away during preadolescence. Comparison to the Fels children (Figures 2 & 3) shows these rural Timorese children have lower pre-pubertal growth velocity, which is especially pronounced immediately preceding the adolescent growth spurt. It is possible that, under good conditions, Timorese children may still not reach the heights of non-Asian populations, due to genetic differences in Asian populations. The WHO reference may therefore overestimate the severity of malnutrition in the older age groups of children in this population.

**Timing and magnitude of pubertal growth**

In most populations, the timing and magnitude of the adolescent growth spurt shows greater variation than that of the childhood period (Hauspie, 2002). During this period of high variation amongst children, some patterns do exist; for example, chronic malnutrition during childhood has been linked to a delayed age at maturation (Kulin, Bwibo, Mutie, & Santner, 1982). Normally growing children who experience a later growth spurt generally have one of a reduced magnitude (Sherar, Mirwald, Baxter-Jones, & Thomis, 2005). When compared to the 50th centile of growth increments of children from the Fels Longitudinal Study (Baumgartner, Roche, & Himes, 1986), Timorese height velocity at adolescence appears very similar. However, the 50th centile reflects the mean growth increment for each age group, rather than the growth velocity of the average individual throughout the adolescent growth spurt, and as such, comparisons must be made with caution. In a review of studies of healthy
American children (including the Fels study), male peak pubertal height velocity (PHV) is 9.5 cm/yr at age 13.5 (Abbassi, 1998), whereas the peak of the fitted velocity curve for Timorese males is 8.7 cm/yr at age 14.3, indicating that on average, male pubertal growth is slightly delayed and decreased in magnitude. For female American children, peak pubertal height velocity is 8.3 cm/yr at age 11.5 (Abbassi, 1998), and 7.0 cm/yr at age 11.7 in Timorese children, indicating a decrease in magnitude but similar timing. Timorese girls thus appear to reach maturity at a similar time to well-nourished girls, but gain less height, which leads to smaller adult stature. In Japan, a population towards the end of a secular trend for earlier puberty, PHV for girls is 8.3 cm/yr at age 11.05 and 9.9 cm/yr at age 13.05 for boys, slightly earlier than for American children (Suwa, Tachibana, Maesaka, Tanaka, & Yokoya, 1992). If a secular trend in age at puberty is yet to occur in Timorese children, we would expect that, with time, the PHV of both girls and boys will fall, and that Timorese girls will approach a mean age at PHV more similar to that of Japanese girls than American girls. It must be noted, however, that longer intervals between measurements will blunt any rapid changes in velocity. Given that the majority of measurements included in the Timor-Leste SITAR models occurred at approximately yearly intervals, results should be interpreted cautiously. It must also be noted that some children's ages are estimated, and as such this may affect model precision.

Adolescent male growth

The modeled growth curve indicates that Timorese males experience a delayed, reduced adolescent growth spurt in height compared to the international population, but continue growing into adulthood (past 19 years). Similarly to Timorese males, Datoga males in East Africa and Quechua males in the Peruvian highlands also have poor growth and a delayed pubertal growth spurt (Frisancho and Baker, 1970; Leatherman, Carey, & Thomas, 1995; Sellen, 1999). Shuar males, while having a clear growth spurt that is only slightly delayed compared with the international reference, also exhibit extended growth into
adulthood (Urlacher et al., 2016). Continued growth of males into their early twenties is well documented (Coly et al., 2006; Steegmann Jr, 1985), and indeed is shown by the WHO male height curve continuing upwards at age 19 (Figure 4). However, the greater rate of growth of older Timorese males compared with the WHO (between ages 18 and 19 the 50\textsuperscript{th} centile is steeper; Figure 4) suggests they are further away from growth cessation, and thus likely have a taller adult stature than shown at age 19. Slow, continued growth in height in Turkana pastoralists results in both males and females catching up to the 50\textsuperscript{th} centile of US children by adulthood (Little and Johnson Jr, 1987). While catching up to the 50\textsuperscript{th} centile is unlikely for this population, we now continue to measure males older than age 19 to determine both the timing of cessation of growth, and the mean achieved adult height for males.

Adolescent female growth

In females, the shape of the height growth curve closely matches that of the WHO throughout the developmental period, but shifted down. Unlike height, the variation in weight within the sample of Timorese females increases with increasing age. This may be due to ecological factors, for example the accumulation of differences in physical activity levels, and therefore energy expenditure, or differences in household resource levels. Similarly to males, the effects of malnutrition on female growth patterns at puberty also vary; Shuar females have an earlier age at peak height velocity (10.2 years old) than do American girls, while male peak velocity is similar to the international reference (Urlacher et al., 2016). In the Andes, Nuñoa females show similar responses to conditions in adolescent growth as do males, with both showing reduced and delayed growth spurts (Frisancho and Baker, 1970; Leatherman, Carey, & Thomas, 1995). Adolescent girls in Kenya, in areas with a high prevalence of stunting and thinness, have delayed puberty but show catch-up growth, with rates of apparent malnutrition decreasing with increasing age (Leenstra et al., 2005). Female growth in this population appears to decline after age 17. This is similar to what occurs when a population is undergoing a
secular increase in growth, whereby those born earlier are not exposed to the better conditions associated with the secular increase and show a different pattern of growth and thus a smaller adult size than their later born counterparts (Hauspie, Vercauteren, & Susanne, 1997). For these Timorese females, the growth decline is likely due to a cohort effect, rather than a secular trend. Females aged 17 and older in the sampling period were born, at latest, in 1999, and thus developed during a period of Timorese history characterized by Indonesian occupation and civil unrest prior to the establishment of independence. Early life exposure to psychosocial stress, or the decline in available resources linked with conflict, may have a negative effect on child growth (Alderman, Hoddinott, & Kinsey, 2006). Therefore, the smaller achieved height of females born in the 1990s may be a result of their exposure to conflict that the later-born children did not experience. This remains a hypothesis pending growth to adulthood in the post-independence cohort. Alternatively, the decrease in female growth centiles with age may be due to selection bias in sampling. The decline in height in the older age groups is more pronounced in the 95th centile, possibly indicating the presence of fewer relatively taller females. Taller girls are more likely to be from families of higher socio-economic status than shorter girls, a relationship that is stronger in older children (Currie and Stabile, 2003). These girls might be more likely to move away from the rural areas during their late teens in order to marry, or go to university in Dili, the country’s capital. If taller girls are over-represented in the attrition in sample size of the older age groups, they could contribute to the lower average height of older girls in the modeled growth curves.

**Sex differences in growth beyond normal sexual dimorphism**

Relative to the optima, older Timorese male children have poorer growth than do females in both height and BMI, as in Sanders et al., (2014) and Spencer, Sanders, Canisio Amaral, & Judge, (2016). While growth velocity for these children is higher in males than in females (fitting the normal biological pattern), the sex differences indicated by the z-scores confirm that the
magnitude of this difference between males and females is less than that of normal sexual dimorphism. Adolescent Timorese females are relatively larger than males in both z-height-for-age and z-BMI-for-age. As taller stature reduces BMI (calculated as kg/m$^2$), the fact that females also have a higher z-BMI indicates that they are both relatively heavier and relatively taller than males.

That males and females differ in their growth responses to poor environmental conditions is well documented. In sub-Saharan Africa, males under age 5 have higher rates of stunting that do females (Wamani, Åstrøm, Peterson, Tumwine, & Tylleskär, 2007). Stunted boys in rural Senegal show less catch-up growth during adolescence than do girls (Coly et al., 2006). Similarly in America, very low birth weight females show more catch-up growth by age 20 than do males (Hack et al., 2003). It is hypothesized that males are more susceptible to hostile environments than females are; however, evidence is mixed and the strongest evidence in support of this hypothesis is from studies of prenatal growth and mortality (Stinson, 1985). Male mortality is consistently higher than female mortality in early life, with differences in mortality disappearing by approximately four years of age (Wells, 2000). In rural Bangladesh, male mortality is higher in the neonatal period, while females have higher mortality during infancy and childhood; however, this is attributed to better nutrition and healthcare being provided to male children, rather than inherent biological differences (Chen, Huq, & D’Souza, 1981). Barbadian girls who experienced early life malnutrition have poorer growth in adolescence than do boys, indicating that contrary to other evidence, the effects of early deprivation may be more persistent in females (Galler, Ramsey, & Forde, 1986). In this population, we do not observe sex differences in growth until adolescence, where females have better heights relative to the international reference than do boys, and show more catch-up growth in body mass index. There are two possible explanations for this difference: firstly, that inherent differences in susceptibility to poor conditions such as disease and food shortage are indeed persisting in this population, or secondly, that there is buffering of females
through cultural practices against these environmental conditions. We must now look at illness-related data to look for sex differences in disease rates, and at seasonal growth rates, to determine if males lose proportionally more growth during the food-scarce season than do females. In India during famines, male mortality from adolescence through adulthood increased relative to female mortality, indicating males were more affected by food shortage than females (Dyson, 1991). We are also currently examining if there are differences in physical activity level for male and female children. If males have higher activity levels than females, and thus greater energy expenditure, this may explain the poorer growth of older male children.

**Applications**

The growth curves created here using the GAMLSS method are based on *children in two rural areas*, and as such present growth patterns specifically relevant to rural children, who make up the majority of the young East Timorese population (National Statistics Directorate [Timor-Leste] et al., 2010). Rural Timorese children under five years show higher rates of stunting and wasting than do urban children (National Statistics Directorate [Timor-Leste] et al., 2010), so understanding the growth pattern of rural children is of particular importance. This paper presents the first curves that could be used as a reference for rural children, and could be used to identify, relative to the environment, those who are growing the most poorly. To confirm if these curves are suitable as a reference for all rural children, there must be a nationwide focus on collecting anthropometric information to gain a better understanding of the country’s child growth profile. Previous work using a subset of this data has shown differences in growth between the two rural locations, with children in Ossu shorter at all ages than those in Natarbora (Spencer et al., 2016). **Presenting a curve comprised of the children in both locations thus covers at least some of the range of growth variation across ecologically different locations in rural Timor-Leste.**
Conclusions

By using three different methods to examine the growth of this population, we have gained a clearer understanding of the way Timorese children grow, and how this growth differs from that of well-nourished children. Future work must examine the environmental conditions children face at the points of deviation from normal growth in order to help guide health interventions. The growth curves produced here now provide a baseline reference for these two populations, and potentially of rural children’s growth, and as such may be able to be used to track future growth for evidence of secular trends as the country develops. The methodology described in this paper can be applied to other small populations for the creation of population-specific growth references. The production of these references is important in order to understand the full extent of variation in growth among human populations.

Some of the clear differences of the modeled Timorese growth curves to that of the WHO are doubtless due to the difficult environmental conditions faced by the Timorese, and provide evidence for the plasticity of growth in consistently poor resource environments. However, evidence in the literature for differences between growth in wealthy Asian populations and those populations comprising the WHO growth curves suggests that some of this variation may have a genetic basis (Haas and Campirano, 2006; Ulijaszek, 2001). We show that this population follows growth of other Asian populations with greater differences becoming pronounced at adolescence, following a relatively normal (albeit shifted down) pattern of preadolescent growth. In order to confirm this hypothesis of genetic differences, it is vital we continue to monitor the growth of Timorese children over time for evidence of secular trends so we can determine this population’s genetic potential for growth.

ACKNOWLEDGEMENTS

This research was undertaken with the permission of the Ministry of Health, Timor-Leste. We are grateful for the participation of the families in Ossu and
Natarbora, and for the support of local leaders and health clinics. We thank all our local and Australian research assistants (especially Raimundo da Costa, Lucia Hornai, Isaias Monteiro, Ivonia Correia, Carolyn Skorupa and Dr. Natasha Pauli) for their invaluable contributions to this project. We also thank three anonymous reviewers for their constructive comments on the original version of this paper. Human Ethics approval was granted by the University of Western Australia (RA/4/1/2401) and the Ministry of Health, Timor-Leste. The research was funded by the Australian Research Council (DP 120101588 to Debra Judge et al.), and by the School of Anatomy, Physiology and Human Biology, University of Western Australia.

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velocity based on weight, length and head circumference: methods and

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Leste - 2014.* Retrieved from India:
Figure legends

Figure 1: Map of Timor-Leste. The study sites Ossu and Natarbora are shown in rectangles. Map adapted from United Nations (2011).

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70x61mm (300 x 300 DPI)
Table 1: Number of children (%) measured each possible number of times, and total number of measurements taken over the study period (2009-2016).

<table>
<thead>
<tr>
<th>Times measured</th>
<th>Number of children (%)</th>
<th>Measurement incidents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>266 (21.4)</td>
<td>266 (5.4)</td>
</tr>
<tr>
<td>2</td>
<td>162 (13.0)</td>
<td>324 (6.6)</td>
</tr>
<tr>
<td>3</td>
<td>156 (12.5)</td>
<td>468 (9.5)</td>
</tr>
<tr>
<td>4</td>
<td>147 (11.8)</td>
<td>588 (12.0)</td>
</tr>
<tr>
<td>5</td>
<td>189 (15.2)</td>
<td>945 (19.3)</td>
</tr>
<tr>
<td>6</td>
<td>143 (11.5)</td>
<td>858 (17.5)</td>
</tr>
<tr>
<td>7</td>
<td>62 (5.0)</td>
<td>434 (8.9)</td>
</tr>
<tr>
<td>8</td>
<td>59 (4.7)</td>
<td>472 (9.6)</td>
</tr>
<tr>
<td>9</td>
<td>61 (4.9)</td>
<td>549 (11.2)</td>
</tr>
<tr>
<td>Total</td>
<td>1245</td>
<td>4904</td>
</tr>
</tbody>
</table>
Table 2: Estimated marginal mean z-score (SE) and sample size of three growth measures; test of significant differences between age groups using a linear mixed model with individual ID as random factor accounting for repeat measures.\(^b\)

<table>
<thead>
<tr>
<th></th>
<th>0-2 yrs</th>
<th>2.1-5 yrs</th>
<th>5.1-10 yrs</th>
<th>10.1-15 yrs</th>
<th>&gt;15 yrs</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>z-BMI</td>
<td>-0.12 (0.053)</td>
<td>-0.40 (0.040)</td>
<td>-1.00 (0.032)</td>
<td>-1.23 (0.033)</td>
<td>-0.92 (0.043)</td>
<td>130.089</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>n</td>
<td>361</td>
<td>750</td>
<td>1541</td>
<td>1406</td>
<td>617</td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-height-for-age</td>
<td>-2.13 (0.051)</td>
<td>-2.26 (0.040)</td>
<td>-1.89 (0.035)</td>
<td>-2.00 (0.036)</td>
<td>-1.76 (0.043)</td>
<td>50.852</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>n</td>
<td>372</td>
<td>751</td>
<td>1544</td>
<td>1408</td>
<td>618</td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-weight-for-age</td>
<td>-1.45 (0.047)</td>
<td>-1.75 (0.040)</td>
<td>-1.93 (0.037)</td>
<td>a</td>
<td>a</td>
<td>59.036</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>n</td>
<td>462</td>
<td>808</td>
<td>1549</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) WHO standards not available

\(^b\) All age groups are different from each other except 5-10 yrs and >15 yrs for z BMI
Table 3: Estimated marginal mean z-score (SE) and sample size of three growth measures for males and females; test of significant differences between sexes using a linear mixed model with individual ID as a random factor accounting for repeated measures.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>Females</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>z-BMI</td>
<td>-0.94 (0.038)</td>
<td>-0.76 (0.037)</td>
<td>11.886</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>n = 2372</td>
<td>n = 2372</td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-height-for-age</td>
<td>-2.06 (0.042)</td>
<td>-1.92 (0.042)</td>
<td>5.603</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>n = 2382</td>
<td>n = 2311</td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-weight-for-age (0-10 yrs)</td>
<td>-1.80 (0.049)</td>
<td>-1.76 (0.051)</td>
<td>0.508</td>
<td>0.476</td>
</tr>
<tr>
<td></td>
<td>n = 1482</td>
<td>n = 1337</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4: Estimated marginal mean z-score (SE) and sample size for z height-for-age and z BMI for males and females, and differences in z-growth by sex within age groups (Linear mixed model, individual ID as random factor accounting for repeated measures).

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male</th>
<th>Female</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.1-15 yrs</td>
<td>-2.26</td>
<td>-1.98</td>
<td>11.831</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>(0.059)</td>
<td>(0.059)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-height</td>
<td>n = 705</td>
<td>n = 703</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;15 yrs</td>
<td>-2.19</td>
<td>-1.82</td>
<td>16.288</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0.069)</td>
<td>(0.059)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 264</td>
<td>n = 354</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.1-15 yrs</td>
<td>-1.42</td>
<td>-1.07</td>
<td>23.421</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0.051)</td>
<td>(0.052)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-BMI</td>
<td>n = 704</td>
<td>n = 702</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;15 yrs</td>
<td>-1.26</td>
<td>-0.59</td>
<td>53.379</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0.070)</td>
<td>(0.061)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 264</td>
<td>n = 353</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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120x61mm (300 x 300 DPI)