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RESEARCH ARTICLE

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Disparities in bone density across contemporary Amazonian forager-horticulturalists: Cross-population comparison of the Tsimane and Shuar

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Abstract

Objectives: This study investigates bone density across the life course among Bolivian Tsimane and Ecuadorian Shuar of Amazonia. Both groups are rural, high-fertility forager-horticulturalists, with high lifetime physical activity levels. We test whether Tsimane and Shuar bone density patterns are different from each other, and if both groups are characterized by lower osteoporosis risk compared to U.S. references.

Methods: Anthropometric and calcaneal bone density data, obtained via quantitative ultrasonometry (QUS), were collected from 678 Tsimane and 235 Shuar (13–92 years old). Population and sex differences in QUS values (estimated bone mineral density, speed of sound, broadband ultrasound attenuation) by age group were assessed using Mann–Whitney *U* tests. Age-related change and age at peak QUS value were determined using polynomial regressions. One-way analyses of covariance assessed population-level differences in QUS values by age group adjusting for body mass index. Participants aged 50+ years at elevated osteoporosis risk were identified using a *T* score < −1.8; binomial tests assessed risk compared to U.S. references.

Results: Shuar males and females <50 years old have QUS values 3–36% higher than Tsimane, with differences evident in adolescence. Among Tsimane and Shuar, 49 and 23% of participants aged 50+ years old, respectively, are at high risk for osteoporosis, compared to 34% of Americans; Shuar osteoporosis risk is comparable to Americans, while Tsimane risk is elevated.

Conclusions: Disparate patterns in QUS values are documented for Tsimane and Shuar, with pronounced differences early in life. Potential explanations for differences include gene–environment interactions and/or degree of market integration, which influences diet, activity profiles, pathogen exposures, and other lifestyle covariates. As Tsimane osteoporosis risk is greater than in the United States, findings point to alternative risk factors for low bone density that are not readily discernible in industrialized populations.

KEYWORDS

bone mineral density, osteoporosis, quantitative ultrasonometry, Shuar, Tsimane

1 | INTRODUCTION

Osteoporosis is a progressive disease characterized by low bone mass and microarchitectural deterioration of bone tissue, thereby increasing fracture risk. According to global estimations >150 million people are at risk of an osteoporotic-related fracture; this estimate is expected to double by 2040 (Odén, McCloskey, Kanis, Harvey, & Johansson, 2015; Sànchez-Riera et al., 2014). Most available data, however, are biased toward populations living in Western industrialized countries, such that global incidence and prevalence of osteoporosis are likely gross underestimations. Largely owing to a lack of diagnostic resources in remote regions, there is minimal information on bone density in vivo from rural small-scale populations living in non-industrialized, developing nations (but see e.g., Madimenos et al., 2011; Madimenos, Liebert, Cepon, Snodgrass, & Sugiyama, 2015; Stieglitz et al., 2015; Stieglitz, Madimenos, Kaplan, & Gurven, 2016; Stieglitz, Trumble, Kaplan, & Gurven, 2017; Stieglitz et al., 2019). This gap in the literature is problematic for several reasons. First, many factors known to promote low bone mass and bone loss, including physical inactivity (e.g., Carter, 2014), alcohol consumption and smoking (e.g., Sampson, 2002), and glucocorticoid therapy (e.g., Briot & Roux, 2015) are often intercorrelated, which limits the ability to assess the relative importance of each in affecting bone structural variation. Second, overemphasis on samples from industrialized populations, particularly those with positive energy balance, low pathogen burden, and reliable access to medical care, may bias or obfuscate current understanding of basic bone biology and osteological responses to mechanical and other stress. Data from non-Western, subsistence-based populations can therefore help identify novel risk factors for low bone mass that are not readily discernible in industrialized, nonclinical contexts.

Bone density data from extant subsistence populations can also illuminate the extent to which osteoporosis can be framed within the "mismatch" paradigm from evolutionary medicine (e.g., Gluckman & Hanson, 2006; Lieberman, 2013). The global increase in rates of osteoporosis over time has been attributed to relatively recent, rapid changes in life expectancy and lifestyle, compared to rates of genetic change, that are aberrant from environments in which human morphology evolved (see Madimenos, 2015 for overview). Specifically, modern human skeletons are seen as inadequately or imperfectly adapted to novel conditions associated with industrialized environments, with factors like micronutrient deficient diets and in particular, physical inactivity, viewed as especially risk enhancing for osteoporosis (Lieberman, 2013; Wallace, Rubin, & Lieberman, 2015). Implicit in this perspective is the notion that physically active populations in nonindustrialized settings should be protected from low bone density and osteoporosis. Yet, Ruff et al. (2015) concluded that modern human skeletal gracility (i.e., reduced bone mass, and strength for body size), particularly in the lower limbs, results from increased sedentism (i.e., reduced terrestrial mobility) associated with agricultural intensification beginning in the Neolithic, rather than more recent (i.e., past 2,000 years) transitions toward mechanization and industrialization. While an analysis of bone density among extant forager-horticulturalists and comparison to industrialized populations may not directly address debates over the timing of and mechanisms underlying transition to skeletal gracility in past human populations, such population-level comparisons can illuminate lifestyle factors affecting bone strength that may have been relevant during major socioeconomic transitions in human history (Stieglitz et al., 2019).

Technological advancements in quantitative ultrasonometry (QUS) have resulted in the development of portable, noninvasive methods for in vivo diagnostic assessment, which help address existing gaps in epidemiological and evolutionary understanding of bone strength. QUS devices measure estimated bone mineral density (eBMD; g/cm^2), a widely employed measure for assessing bone quantity and fracture risk (Small, 2005). The present study provides a unique QUS data set from two indigenous Amazonian forager-horticultural populations, the Tsimane of Bolivia and Shuar of Ecuador. The objectives of this study are to compare bone density patterns by age and sex for both populations, and then compare their results to a U.S. reference sample. Specifically, we test the following hypotheses: (a) there is no difference in bone density between Tsimane and Shuar, given numerous similarities in their lifestyle and environment (i.e., both populations forage and practice horticulture for subsistence, reside in rural, tropical lowland environments of the Amazon basin, and have high fertility with prolonged, on-demand breastfeeding); and (2) compared to age- and sex-matched U.S. references, Tsimane and Shuar adults show reduced risk of osteoporosis, given the overall lack of exposure to traditional osteoporosis risk factors found in industrialized countries (e.g., physical inactivity, smoking, heavy alcohol use, glucocorticoid therapy).

2 | BACKGROUND ON STUDY POPULATIONS

Tsimane forager-horticulturalists of lowland Bolivia (Beni Department; population ~16,000) are semisedentary and reside in >90 villages. The Shuar are a lowland Ecuadorian forager-horticulturalist population (population ~60,000–100,000) concentrated in the Morona-Santiago, Pastaza, and Zamora-Chinchipe provinces (CODENPE, 2012; Jokisch & McSweeney, 2011; Rubenstein, 2001; UNICEF, 2004). Both groups inhabit similar neotropical environments where they maintain physically active lifestyles (Gurven, Jaeggi, Kaplan, & Cummings, 2013; Madimenos, Snodgrass, Blackwell, Liebert, & Sugiyama, 2011). Compared to the United States, Tsimane and Shuar subadult growth patterns follow similar trajectories suggesting either similar reaction norms expressed throughout Amazonia, or possibly less flexible shared genetic variants (Blackwell et al., 2017). Furthermore, group similarities in staple foods and fertility patterns (see below), and in climate, minimal smoking and store-bought alcohol consumption, and their limited access to medical care (e.g., Blackwell et al., 2011; Gildner et al., 2016; Gurven et al., 2016; Kraft et al., 2018; Urlacher et al., 2018) collectively contribute to the logic underlying the hypothesis

that bone density will not vary significantly between these two subsistence-based populations.

2.1 | Diet

Despite reported insufficiencies in calcium intake based on Western dietary recommendations (Kraft et al., 2018; Sarti et al., 2015), deficiencies in serum 25-hydroxyvitamin D and D levels in equatorial subsistence populations may be rare because of year-round exposure to sunlight and habitual outdoor work (e.g., Luxwolda, Kuipers, Kema, Dijck-Brouwer, & Muskiet, 2012). Additionally, among Tsimane and perhaps Shuar, dietary consumption of other bone-forming minerals (zinc, magnesium, phosphorus) may be higher than or comparable to Western recommendations (Kraft et al., 2018). Among both Tsimane and Shuar, diets consist of staple cultigens (e.g., manioc, plantains) and lean meat and freshwater fish, with relatively minimal consumption of market foods (although this varies within populations based on residential proximity to the market) (Houck et al., 2013; Liebert, Snodgrass, Blackwell, Madimenos, & Sugiyama, 2013; Urlacher et al., 2016, 2016). Among Tsimane, 8% of calories are market derived (e.g., from pasta, wheat flour) (Kraft et al., 2018). Among Shuar, consumption of highly processed starches or fatty foods is also relatively limited; this is supported by favorable cardiovascular and metabolic health profiles documented across different levels of market integration (Liebert et al., 2013).

2.2 | Fertility

Tsimane and Shuar women rarely use contraception and invest considerable energy in reproduction relative to women in low-fertility societies of the developed world. This is due to the combination of early onset of reproduction, short birth spacing, multiparity, and prolonged on-demand breastfeeding (Madimenos, Snodgrass, Liebert, Cepon, & Sugiyama, 2012; Stieglitz et al., 2015, 2019). These reproductive factors have variable effects on bone density (Kovacs, 2016). Among Tsimane, total fertility rate (TFR) is nine births per woman, mean interbirth interval (IBI) is 30 months, and mean weaning age is ~19 months (Mcallister, Gurven, Kaplan, & Stieglitz, 2012; Veile, Martin, McAllister, & Gurven, 2014). Similar patterns are observed among Shuar women (TFR: 9; mean IBI: ~31 months, weaning age: ~16 months) (Madimenos et al., 2012).

2.3 | Market integration

Market integration structures exposure to socioecological factors known to influence bone strength and health (e.g., nutrition, physical activity level, pathogen exposure, access to medicine, and goods like cigarettes and alcohol) (McDade & Nyberg, 2010; Shephard & Rode, 1996; Stagaman et al., 2018; Stuckler, Siegel, De Vogli, & Basu, 2011; Valeggia & Snodgrass, 2015). While all study participants are from rural villages and maintain a subsistence lifestyle including hunting and foraging (e.g., for honey, insect larvae, palm fruit), both populations are also undergoing integration into the market economy with variable effects at inter- and intra-population levels (e.g., Liebert et al., 2013; Minkin & Reyes-García, 2017; Urlacher, Liebert, et al., 2016). For example, at the time of data collection, no Tsimane villages had running water or electricity, while Shuar villages had some access to an electrical grid and variable access to water pumps; these differences can alter bioenergetic states and modify exposure to pathogenic infections. Heterogeneous effects of market integration on health more broadly among Shuar and Tsimane have been documented elsewhere (Houck et al., 2013; Stieglitz et al., 2012; Urlacher, Liebert, et al., 2016). With respect to Shuar and Tsimane bone density, market integration may be expected to exert opposing effects, mediated by lower physical activity levels (–), greater energetic status (+), and reduced pathogen burden (+). These complex interrelationships and their individual and combined effects on bone density are beyond the scope of this article. Nevertheless, heterogeneity in market integration between Shuar and Tsimane contributes to an alternative hypothesis that population-level bone density may vary despite many similarities in their environment and lifestyle.

All Tsimane data were collected as part of the Tsimane Health and Life History Project (THLHP; http://www.unm.edu/~tsimane/), while Shuar data were collected as part of the Shuar Health and Life History Project (SHLHP; www.bonesandbehavior.org/shuar).

3 | MATERIALS AND METHODS

3.1 | Quantitative ultrasonometry

The right calcaneus was measured using a gel-based Sahara Clinical Bone Sonometer (Hologic, Waltham, MA). Instrumental quality control scans of a phantom provided by the manufacturer were performed daily (see Stieglitz et al., 2015, 2016). The same ultrasound was used for both populations, obviating the need for machine cross-calibration and permitting direct cross-population comparisons. To perform an ultrasound, the heel is positioned between a pair of transducers that are mounted on a motorized caliper, enabling direct contact with the heel through elastomer pads and a coupling gel. One transducer serves as a transmitter and the other as a receiver. In order to ensure consistent placement and maximum reproducibility of measures, foot, ankle, and leg positions are fixed by a foot positioning guide extending from the foot to the shin.

The ultrasound generates the following parameters: speed of sound (SOS, m/s), reflecting transit velocity through the calcaneus, and broadband ultrasound attenuation (BUA, dB/MHz), reflecting attenuation of sound as it passes through the bone (Bartl & Bartl, 2017). Bone mineral mass and elasticity largely influence SOS, while BUA is more related to structural parameters (i.e., connectivity, porosity) and bone density (Gluer, Wu, Jergas, Goldstein, & Genant, 1994). For SOS and BUA, lower values indicate lower bone mineral content per surface area such that young, healthy individuals generally have higher SOS and BUA than older, osteoporotic individuals. The sonometer also estimates calcaneal BMD (eBMD; g/cm^2) from a linear combination of SOS and BUA (eBMD = 0.002592 × [SOS + BUA] – 3.687 g/cm^2) (Frost, Blake, & Fogelman, 2000).

Numerous clinical studies have found that these QUS parameters are significantly associated with fracture risk (Chan, Nguyen, Center, Eisman, & Nguyen, 2012; Hans & Baim, 2017; Komar et al., 2019; Marin, Gonzalez-Macias, Diez-Perez, Palma, & Delgado-Rodriguez, 2006). In large prospective studies, diagnostic sensitivity of calcaneal QUS in the prediction of hip fracture is similar to that of hip BMD measured with dual-energy X-ray absorptiometry (DXA), the preferred method for quantifying bone density. Correlations between BMD from QUS and DXA range from 0.28 to 0.86 (Lee, Hwang, & Lin, 2010; Njeh, Boivin, & Langton, 1997; Trimpou, Bosaeus, Benth-Ake, & Landin-Wilhelmsen, 2010); this variance may be attributed to differences in skeletal sites measured, ultrasounds used, or the fact that ultrasound velocity may be dependent on aspects of bone other than mineral density. While DXA is the recommended method for diagnosing osteoporosis (International Society of Clinical Densitometry [ISCD, 2019]), the portability and low cost of calcaneal QUS devices broaden the range of samples in which low bone mass detection is possible (Chin & Ima-Nirwana, 2013; Rhee et al., 2009; Steiner, Dimai, Steiner, Cirar, & Fahrleitner-Pammer, 2019). Currently, the calcaneus is the only validated skeletal site for the use of QUS in osteoporosis risk assessment and fracture prediction (ISCD, 2019).

Short-term reproducibility of QUS measurements were assessed by duplicate scans (morning, afternoon) performed by F.M. (among Shuar) and J.S. (Tsimane) for 15 THLHP history research assistants (men and nonpregnant, nonlactating women) ranging in age from 23 to 53 years. Mean coefficient of variation (CVs) for Shuar data for BUA and SOS were 5.1 and 0.6%, and for Tsimane were 6.3 and 0.2%, respectively (see Quantitative Ultrasound in Data S1 for more details).

QUS parameters are presented in the text separately, although to avoid redundancy as these parameters are highly correlated, eBMD results are presented in the main text, with SOS and BUA details in Data S1.

3.2 | Demographics

This study includes 678 Tsimane (372 females, 306 males; 13–92 years old) and 235 Shuar (147 females, 88 males; 13–86 years old). For both populations, birth years were assigned based on a combination of methods including employing known ages from written records, relative age lists, and cross-validation of information from independent interviews of kin. Each method provides an independent estimate of age, and when estimates yielded a date of birth within a 3-year range, the average was generally used. Individuals for whom reliable ages could not be ascertained are not included in analyses.

As most reproductive-aged females are pregnant and/or lactating in these two natural fertility populations, we include pregnant and lactating women in analyses. Physiological changes characteristic of various reproductive states (e.g., pregnancy-related weight gain) invariably influence bone density (see Kovacs, 2016 for a comprehensive review), and as such, both pregnant and lactating women are typically excluded from reference data sets in other populations. However, describing bone density patterns in natural fertility populations without including women experiencing heterogenous reproductive states is problematic for several reasons. First, within a given natural fertility population, women are pregnant or lactating for much of their reproductive life span (Eaton et al., 1994; Sperling & Beyene, 1997; Strassman, 1999; Weaver, 1998). Exclusion of pregnant or lactating women dramatically reduces sample size (here by 27–28%), specifically for critical age intervals (20–30 years old) when peak bone density is realized (Chew & Clarke, 2018). Furthermore, data on exclusively nulliparous women of reproductive age are biased because these women may be suffering from fertility or health problems that may affect bone structure. Omission of pregnant and lactating women would thus result in a biased and unrepresentative sample. In this study, 36 Tsimane and 9 Shuar pregnant women (mean ages: 26.9 ± 7.3 and 24.6 ± 7.4 years old, respectively) and 66 Tsimane and 32 Shuar lactating women (mean ages: 25.0 ± 8.2 and 28.4 ± 9.1 years old, respectively) were included. The sample includes only one Shuar woman (no Tsimane) who was both pregnant and lactating at the time of data collection.

3.3 | Anthropometrics

Stature was measured while barefoot and recorded to the nearest millimeter using a Seca stadiometer. Participant weight was measured while wearing minimal clothing and recorded to the nearest 0.1 kg using a Tanita digital scale.

3.4 | Statistical analyses

Because population-level data sets (including U.S. references) on bone density generally exclude subadults, we performed initial statistical analyses on Tsimane and Shuar participants aged 20+ years. Initial descriptive statistics are therefore presented on a subset of the sample, 298 Tsimane females, 270 Tsimane males, 120 Shuar females, and 75 Shuar males, and exclude the <20-year-old cohort. As anthropometric and QUS variables deviated from normality (based on Shapiro–Wilk tests), population-level differences were evaluated using Mann–Whitney *U* tests. Males and females were analyzed separately for all analyses.

To examine QUS parameters across the life course and control for the effect of age on bone density, Tsimane and Shuar were further divided into 6 categories, ~10 years of age (with deviations in the youngest and oldest groupings): 13–19, 20–29, 30–39, 40–49, 50–59, and ≥60 years. Mann–Whitney *U* tests were used to evaluate population differences in anthropometric variables within each age category. Because body size influences areal bone density (Bartl & Frisch, 2004), one-way analyses of covariance (ANCOVA) tests were used to assess population differences in QUS parameters for each age group adjusting for body mass index (BMI). Given that Tsimane and Shuar sample sizes are asymmetrical and smaller in some age groups (e.g., 60+ years), data violated multiple ANCOVA assumptions. For this reason, we used bias-corrected and accelerated (BCa) bootstrapping in the ANCOVA analysis to obtain empirically determined estimated marginal means and standard errors (*SE*). This approach reduces sample bias due to violation of distributional assumptions and

has been applied in prior osteological studies (Cheverko, Downey, & Hubbe, 2016).

We also compared Tsimane and Shuar by analyzing age-related change in QUS values using polynomial regressions and identifying the model with the best fit (i.e., highest coefficient of determination [R^2]). Age at peak QUS value (i.e., eBMD, SOS, BUA) was estimated from the quadratic (for Tsimane females, Shuar females and males) and cubic (for Tsimane males) regressions. After peak QUS value attainment, rate of decline was determined after standardizing age and eBMD values (by sex) and including the *z*-scored variables in the general linear model. In order to determine if the rate of change of postpeak varied significantly between groups by sex, we compared extent of Confidence Interval (CI) overlap following Cumming (2009).

T scores are presented to facilitate comparisons with a U.S. reference population. A *T* score compares an individual's eBMD to an average young adult (i.e., aged 20–29 years) reference population and is expressed in *SD* units (*T* score = [participant eBMD – young adult reference mean eBMD]/*SD* of young adult mean). Following ISCD (2019) guidelines, *T* scores are calculated for individuals aged 50 + years using as a baseline a healthy adult Caucasian female sample for both females and males. In the current study, we use the ultrasound-generated *T* scores, which compares Tsimane and Shuar participants to a U.S. reference population and standardizes the analysis. As only measures derived by DXA can lead to a clinical diagnosis of osteoporosis based on current WHO definitions (Rothenberg, Boyd, & Holcomb, 2004), QUS of the calcaneus may only ascertain those with elevated osteoporosis risk should their *T* score fall below the QUS-specific threshold of −1.8 (Frost et al., 2000). Because DXA and QUS do not generate equivalent measures and employ different technology in assessing bone health, this threshold necessarily differs from the WHO DXA-specific threshold of −2.5 (see Statistical Analyses in Data S1).

Finally, a binomial test was performed on participants aged 50+ years with *T* scores less than −1.8 to determine if the proportions of Tsimane and Shuar at elevated osteoporosis risk vary significantly from the U.S. reference population (Wright et al., 2014). U.S. data are based on a noninstitutionalized population aged 50+ years from the National Health and Nutrition Examination Survey III. Statistical estimates of osteoporosis or osteopenia (i.e., low bone mass) from clinical U.S. populations are typically DXA rather than QUS based, which complicates comparisons with QUS results. Recognizing this methodological issue in cross-population comparisons, our analysis incorporates only prevalence estimates of osteopenia based on femoral neck measurements from a U.S. population (calcaneal QUS is more strongly correlated with density in the proximal femur than other skeletal sites; Zhang et al., 2015). U.S. participants were identified with osteopenia based on DXA-derived *T* scores between −1.0 and −2.5. Individuals with osteoporosis (DXA-derived *T* score ≤ −2.5) were not included in this comparison. This approach, in combination with our use of a conservative QUS-specific *T* score threshold of −1.8 (also see Statistical Analyses in Data S1), facilitates comparison between populations that are at-risk of osteoporosis, but are not diagnosed with the condition.

All statistical analyses were performed using R software version 3.4.4 (R Core Team, 2018) and IBM SPSS Version 24.0 (SPSS Inc., Chicago, IL).

3.5 | Ethics statement

Research approval was obtained from village leadership and study participants, as well as the Shuar Federation and Tsimane government, both of which oversee research projects conducted in their respective regions. Institutional Review Board approval was granted by the University of New Mexico (THLHP) and the University of Oregon (SHLHP).

4 | RESULTS

4.1 | Are Tsimane and Shuar QUS values different? Yes, and population-level differences are greater earlier in life

QUS and anthropometric values by sex and population are presented in Table 1 for all participants aged 20+ years. Mean QUS values are higher for Shuar than Tsimane (SOS—both females and males: 3% difference; BUA—females: 22% difference; males: 31%; eBMD—females: 32% difference; males: 36%). Tsimane aged 20+ years are taller (females: 1.5% difference; males: 1.9%) and have lower BMI than Shuar (both females and males: 8.9% difference) (all *p* values ≤0.01); we therefore adjust for BMI in subsequent analyses.

4.1.1 | Age-associated trends

QUS values decline with age for both sexes in both populations (Figures 1a,b). Tables 2 and 3 present descriptive statistics by age category and sex for each population. One-way ANCOVAs show that among both females (Table 2) and males (Table 3) <50 years old, QUS values for Shuar are significantly higher than Tsimane after adjusting for BMI (SOS—both females and males: 1–3% difference; BUA—females: 16–21% difference; males: 17–40%; eBMD—females: 29–31% difference; males: 15–41%). Among women aged 60+ years, higher QUS values among Shuar are observed only for SOS; higher SOS and eBMD in Shuar males are observed among 50–59-year olds but not in the oldest age category.

Figure 1a,b shows scatterplots of eBMD by age fitted with a quadratic or cubic regression (see Figures S1 and S2 in Data S1 for SOS and BUA). Age-related eBMD decline is curvilinear for Tsimane females (Std. β_{Age} = 0.82, $p \leq .001$; Std. β_{Age2} = −1.29, $p \leq .001$; adj. R^2 = .27), Shuar females (Std. β_{Age} = .70, $p \leq .05$; Std. β_{Age2} = −1.12, $p \leq .001$; adj. R^2 = .21), Tsimane males (Std. β_{Age} = 2.87, $p \leq .01$; Std. β_{Age2} = −6.53, $p \leq .01$; Std. β_{Age3} = 3.35, $p \leq .01$; adj. R^2 = .19), and Shuar males (Std. β_{Age} = 1.08, $p \leq .01$; Std. β_{Age2} = −1.59, $p \leq .001$; adj. R^2 = .33).

Table 4 shows age at peak QUS value for each population by sex. Similar age at peak eBMD is found for Tsimane and Shuar females (27.6 vs. 25.5 years old, respectively) and males (26.6 vs. 29.0 years

TABLE 1 Tsimane and Shuar sample means (M), *SD*, and % differences by sex and population for participants aged ≥20 years

	Female						Male						Total					
	Tsimane (T), *n* = 298		Shuar (S), *n* = 120		Mann–Whitney	T vs. S[a,b]	Tsimane (T), *n* = 270		Shuar (S), *n* = 75		Mann Whitney	T vs. S[a,b]	Tsimane (T), *n* = 568		Shuar (S), *n* = 195		Mann Whitney	T vs. S[a,b]
Variables	*M*	*SD*	*M*	*SD*	*Z*	% Difference	*M*	*SD*	*M*	*SD*	*Z*	% Difference	*M*	*SD*	*M*	*SD*	*Z*	% Difference
Age (years)	42.0	16.0	36.7	13.1	−2.90	12.6**	44.3	15.5	40.1	13.9	−2.26	9.5*	43.1	15.8	38.0	13.5	−3.82	11.8***
Height (cm)	150.3	4.6	148.1	4.9	−3.61	1.5***	161.8	5.4	158.7	4.7	−4.70	1.9***	155.7	7.6	152.2	7.1	−5.40	2.2***
Weight (kg)	53.3	9.0	56.6	10.3	3.40	−6.2***	61.8	7.6	64.8	7.9	3.09	−4.9**	57.3	9.4	59.8	10.2	2.70	−4.4**
BMI (kg/m^2)	23.6	3.5	25.7	3.9	5.80	−8.9***	23.6	2.6	25.7	2.5	6.29	−8.9***	23.6	3.1	25.7	3.5	8.42	−8.9***
SOS (m/s)	1,525.7	25.5	1,567.4	32.3	11.25	−2.7***	1,527.8	24.7	1,570.2	32.5	9.41	−2.8***	1,526.7	25.2	1,568.5	32.4	14.64	−2.7***
BUA (dB/MHz)	64.5	13.9	78.8	18.7	7.86	−22.2***	66.4	14.2	87.0	17.8	8.50	−31.0***	65.4	14.1	82.0	18.8	11.19	−25.4***
Estimated BMD (g/cm^2)	0.44	0.10	0.58	0.13	10.51	−31.8**	0.45	0.10	0.61	0.13	9.34	−35.6***	0.44	0.10	0.59	0.13	13.94	−34.1***

Abbreviations: BMI, body mass index; BMD, bone mineral density; BUA, broadband ultrasound attenuation.

[a]*p* Values from Mann–Whitney *U* tests which were performed on all variables as they deviated from normality (based on Shapiro–Wilk tests).

[b]Tsimane versus Shuar.

*$p \leq .05$; **$p \leq .01$; ***$p \leq .001$.

old, respectively). In both populations, SOS is the first and BUA is the last QUS parameter to peak. Regarding rate of decline postpeak QUS value, among Tsimane females, each *SD* increase in age is associated with a 0.57 *SD* (95% CI: −0.68 to −0.46) decrease in eBMD (−0.004 g/cm^2) compared to a 0.52 *SD* (95% CI: −0.71 to −0.34) decrease among Shuar females (−0.006 g/cm^2). Among Tsimane males, eBMD decreases by 0.40 *SD* (95% CI: −0.52 to −0.28; −0.003 g/cm^2) and by 0.66 *SD* among Shuar males (95% CI: −0.87 to −0.45; −0.007 g/cm^2) for each *SD* increase in age. Across populations, degree of CI overlap between males and females indicates no significant difference in rate of postpeak eBMD decline (see Figure S3 in Data S1).

4.2 | Do Tsimane and Shuar show reduced risk of osteoporosis compared to age- and sex-matched Americans? Shuar risk is comparable while Tsimane risk is elevated

Forty-nine percent of Tsimane (95% CI: 42–57%), including 57% of females (95% CI: 46–67%) and 42% of males (95% CI: 32–53%) are considered high risk of osteoporosis based on the calcaneal QUS-specific *T* score threshold of −1.8. Among Shuar, 23% of the population (95% CI: 10–41%), including 22% of females (95% CI: 6–48%) and 23% of males (95% CI: 5–54%) are considered high risk (Figure 2a,b). In comparison, 34% of age-matched Americans are characterized as osteopenic (26% of females and 13% of males) based on the DXA-derived *T* score value of −2.5 to −1.0 at the femoral neck (see *T* score Analyses in Data S1 for additional details).

Binomial test results show elevated risk among Tsimane vs. Americans ($Z = 4.31$, $p \leq .001$) for both sexes (Tsimane females: $Z = 6.56$, $p \leq .001$; males: $Z = 8.28$, $p \leq .001$). Among Shuar, no difference in risk is observed compared to Americans ($Z = -1.15$, $p = .13$) for both sexes (Shuar females: $Z = -0.10$, $p = .48$; males: $Z = 0.67$, $p = .23$).

5 | DISCUSSION

Tsimane and Shuar calcaneal bone density patterns are strikingly disparate, particularly at younger ages, which may be surprising given that both of these populations live in neotropical environments, engage in similar subsistence-based forager-horticultural lifestyles, share similar growth trajectories, remain active throughout life, and have high fertility (Blackwell et al., 2017; Madimenos et al., 2012; Madimenos, Snodgrass, Blackwell, Liebert, & Sugiyama, 2011; McAllister et al., 2012; Stieglitz et al., 2015; Urlacher, Blackwell, et al., 2016). While some of the observed disparity may be attributed to differences in median age between samples (Tsimane vs. Shuar females: 34 vs. 31 years; Tsimane vs. Shuar males: 43 vs. 36), these differences persist when participants are matched for age. Tsimane bone density is consistently lower than Shuar across most ages after also adjusting for BMI, although this disparity in bone density diminishes at advanced ages. It should be noted that the comparatively small sample

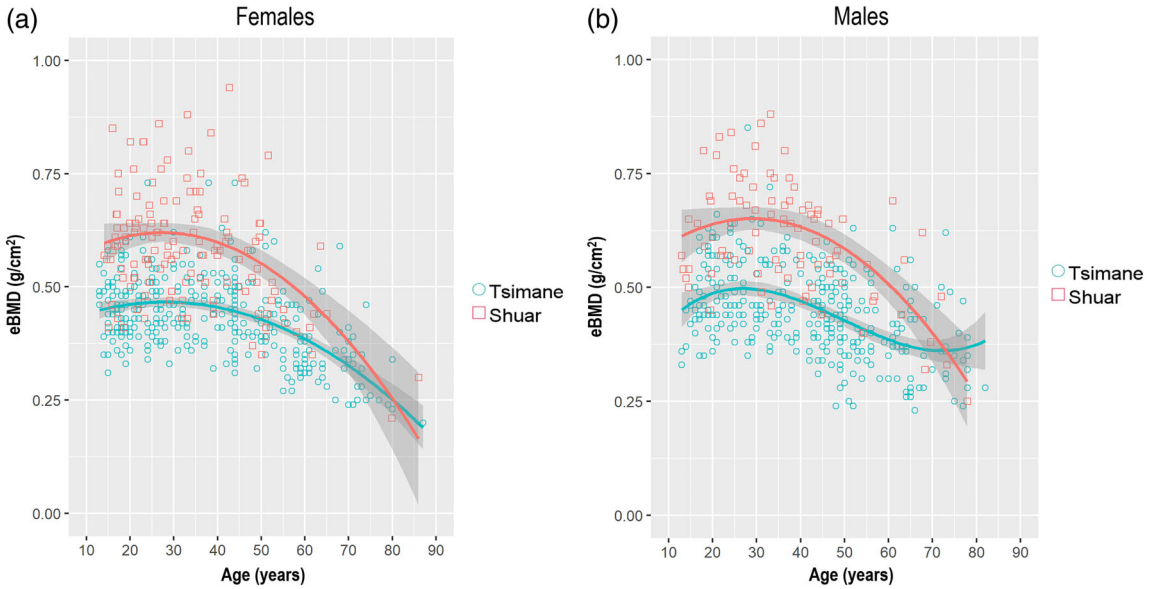

FIGURE 1 (a,b) Scatter plots and fitted curves for age-related change in estimated calcaneal BMD (eBMD) by population, sex, and age. Figure shows a quadratic fit for Shuar males, females, and Tsimane females, and a cubic fit for Tsimane males with 95% CIs. The regression equations between eBMD and age are as follows: (a) Tsimane females: 0.411 + 0.004 × age ± 0.00008 × age^2; Shuar females: 0.537 + 0.006 × age ± 0.0001 × age^2. (b) Tsimane males: 0.311 + 0.016 × age ± 0.0004 × age^2 × 0.000003 × age^3; Shuar males: 0.525 + 0.009 × age + –0.0001 × age^2.

size among older Shuar, coupled with the cross-sectional study design, precludes strong inferences about how data analyzed here accurately reflect age-related change in bone density among the Shuar. The lack of a difference in our sample between Shuar female and male osteoporosis risk (22% and 23%, respectively) is surprising, as risk is typically higher among females, although this result may simply be an artifact of small sample size.

Similar to other studies, we find that calcaneal bone density declines with age and differs by sex (e.g., Looker, Melton 3rd, Harris, Borrud, & Shepherd, 2010; Wright et al., 2014). Generally, within the first three decades of life, bone turnover is coupled tightly to maintain a steady state between bone resorption and formation. After achievement of peak bone mass, bone turnover proceeds at a slower rate with bone resorption outpacing formation (Chew & Clarke, 2018; Demontiero, Vidal, & Duque, 2012). In the current study, age at peak QUS value occurs between 20 and 36 years old in both populations, with some variability across QUS parameters. This finding is consistent with clinical and epidemiological literature demonstrating bone mineral accrual into the third and fourth decade of life, with variability in peak age across skeletal sites (Berger et al., 2010; Chew & Clarke, 2018). After the fourth decade, there is typically a continuous and gradual process of age-related bone loss characterized by cortical thinning, increased cortical porosity, trabecular thinning and decrease in trabecular connectivity serving to compromise structural integrity and increase skeletal fragility and fracture risk (Demontiero et al., 2012). While fragility fracture data were not reliable for current study participants (i.e., some individuals were not certain they experienced a fracture; others confused fractures with general injuries), recent work by Stieglitz et al. (2019) shows that thoracic vertebral BMD is inversely associated with fracture risk, particularly for women, based on computed tomography; these findings highlight that heightened susceptibility to fractures is a potential and real outcome for older adults in these subsistence groups.

Ample research on age-related bone loss shows that rate of decline is typically more pronounced among older women compared to men (Hunter & Sambrook, 2000; Seeman, 2001). This heightened rate of bone loss in women, coupled with lower peak bone mass, accounts in large part for the greater frequency of fragility fractures among women in most populations (Cawthon, 2011). In the present study, we similarly find that the rate of female bone loss exceeds that of males among Tsimane (also see Stieglitz et al., 2016). No significant population-level difference in the rate of decline postpeak eBMD was identified for either males or females. This is notable in light of the higher eBMD peak among Shuar and indicates that attainment of a higher peak value in this population does not predict reduced rate of postpeak decline nor does it appear to protect against low bone density in later life.

Among women in this study, the onset of postpeak bone loss coincides with an age of great reproductive burden (i.e., multiparity, pregnancy, and lactation), with complex effects on calcium homeostasis and estrogen levels (Kovacs, 2016). During pregnancy, approximately 2–3% of maternal calcium is transferred to the fetus, primarily in the second and third trimester (Salari & Abdollahi, 2014). Heightened calcium demands increase the rate of intestinal calcium absorption and bone turnover and contribute to an approximate 3% decrease in total bone density, with variable effects across skeletal sites (Salari & Abdollahi, 2014; Shahtaheri, Aaron, Johnson, & Purdie, 1999). During lactation, 300–400 mg of calcium is transferred to breast milk daily

TABLE 2 Descriptive statistics for Tsimane and Shuar women by age category[a,b,c]

Age (years)	Height (cm)				Weight (kg)				BMI (kg/m^2)				SOS (m/s)[c]			BUA (dB/MHz)[c]			Estimated BMD (g/cm^2)[c]		
	Tsimane (T)	Shuar (S)	T vs. S[d]	Mann–Whitney	Tsimane (T)	Shuar (S)	T vs. S[d]	Mann–Whitney	Tsimane (T)	Shuar (S)	T vs. S[d]	Mann–Whitney	Tsimane (T)	Shuar (S)	T vs. S[d]	Tsimane (T)	Shuar (S)	T vs. S[d]	Tsimane (T)	Shuar (S)	T vs. S[d]
	M (*SD*)	*M* (*SD*)	% Difference	*Z*	*M* (*SD*)	*M* (*SD*)	% Difference	*Z*	*M* (*SD*)	*M* (*SD*)	% Difference	*Z*	*M* (*SE*)	*M* (*SE*)	% Difference	*M* (SE)	*M* (SE)	% Difference	*M* (SE)	*M* (SE)	% Difference
13–19	150.8 (5.0)	148.8 (4.9)	1.3	−1.65	50.8 (7.0)	50.9 (5.1)	−0.2	0.53	22.3 (2.8)	22.9 (1.9)	−2.7	1.69	1,535.1 (2.0)	1,576.9 (5.7)	−2.7***	63.8 (1.4)	76.4 (3.0)	−19.7***	0.46 (0.01)	0.60 (0.02)	−30.4***
20–29	150.2 (4.4)	149.4 (4.6)	0.5	−0.75	52.3 (7.2)	55.6 (10.7)	−6.3	1.94	23.2 (2.8)	24.9 (4.3)	−7.3**	2.86	1,536.0 (2.6)	1,576.4 (3.9)	−2.6***	67.3 (1.5)	81.7 (2.5)	−21.4***	0.47 (0.01)	0.61 (0.02)	−30.0***
30–39	150.4 (5.2)	148.2 (4.4)	1.5*	−2.00	54.3 (8.8)	59.4 (10.0)	−9.4[b]	2.10	23.9 (3.4)	27.0 (3.8)	−13.0***	3.39	1,535.7 (2.7)	1,577.6 (5.9)	−2.7***	69.4 (1.7)	80.3 (4.0)	−15.7**	0.48 (0.01)	0.62 (0.02)	−29.2***
40–49	151.3 (4.3)	147.5 (4.8)	2.5**	−3.06	56.2 (11.4)	58.3 (10.0)	−3.7	1.15	24.5 (4.7)	26.7 (3.5)	−2.2**	2.86	1,528.2 (2.8)	1,567.6 (6.7)	−2.6***	68.6 (1.4)	80.0 (3.4)	−16.6***	0.45 (0.01)	0.59 (0.03)	−31.1***
50–59	150.9 (4.7)	147.3 (4.9)	2.4	−1.62	53.3 (8.3)	56.2 (7.9)	−5.4	1.09	23.4 (3.1)	25.9 (3.1)	−10.7*	2.09	1,516.7 (3.2)	1,532.3 (11.4)	−1.0	61.9 (1.9)	60.7 (6.0)	1.9	0.41 (0.01)	0.44 (0.04)	−7.3
≥60	148.4 (3.9)	142.4 (6.6)	4.0*	−2.42	50.1 (8.3)	46.1 (6.8)	8.0	−1.02	22.7 (3.4)	22.7 (2.0)	0.0	−0.29	1,500.7 (2.9)	1,521.6 (13.6)	−1.4*	51.0 (1.8)	51.8 (9.9)	−1.6	0.34 (0.01)	0.39 (0.06)	−14.7

Note: Means (*M*) and standard deviation (*SD* are presented for anthropometric variables and estimated marginal means (*M*) and standard errors (*SE*) for QUS parameters.
Abbreviations: BMI, body mass index; ANCOVA, analyses of covariance; QUS, quantitative ultrasonometry.
[a]Number of participants for Tsimane age categories: 13–19 (*n* = 74); 20–29 (*n* = 84); 30–39 (*n* = 60); 40–49 (*n* = 62); 50–59 (*n* = 43); ≥ 60 (*n* = 49).
[b]Number of participants for Shuar age categories: 13–19 (*n* = 27); 20–29 (*n* = 46); 30–39 (*n* = 31); 40–49 (*n* = 25); 50–59 (*n* = 11); ≥60 (*n* = 7).
[c]ANCOVA-derived estimated marginal means (*M*) and *SE* adjusting for BMI. *p* values obtained through bias-corrected and accelerated (BCa) bootstrap procedure with 1,000 replications.
[d]Tsimane versus Shuar.
*$p < .05$; **$p \leq .01$; ***$p \leq .001$.

TABLE 3 Descriptive statistics for Tsimane and Shuar men by age category[a,b,c]

Age category (years)	Height (cm)				Weight (kg)				BMI (kg/m^2)				SOS (m/s)[c]			BUA (dB/MHz)[c]			Estimated BMD (g/cm^2)[c]		
	Tsimane (T)	Shuar (S)	T vs. S[d]	Mann–Whitney	Tsimane (T)	Shuar (S)	T vs. S[d]	Mann–Whitney	Tsimane (T)	Shuar (S)	T vs. S[d]	Mann–Whitney	Tsimane (T)	Shuar (S)	T vs. S[d]	Tsimane (T)	Shuar (S)	T vs. S[d]	Tsimane (T)	Shuar (S)	T vs S[d]
	M (*SD*)	*M* (*SD*)	% Difference	*Z*	*M* (*SD*)	*M* (*SD*)	% Difference	*Z*	*M* (*SD*)	*M* (*SD*)	% Difference	*Z*	*M* (*SE*)	*M* (*SE*)	% Difference	*M* (*SE*)	*M* (*SE*)	% Difference	*M* (*SE*)	*M* (*SE*)	% Difference
13–19	158.9 (7.4)	150.5 (14.0)	5.3*	−2.20	53.6 (8.2)	48.5 (13.5)	9.5	−1.18	21.1 (2.4)	20.9 (2.7)	0.9	−0.27	1,539.1 (3.1)	1,578.2 (6.5)	−2.5***	63.0 (2.3)	73.9 (4.9)	−17.3*	0.47 (0.01)	0.60 (0.03)	−27.7***
20–29	162.3 (5.2)	159.9 (4.5)	1.5	−1.91	60.7 (7.3)	63.8 (5.9)	−5.1	1.77	23.0 (2.4)	24.9 (1.2)	−8.3***	3.55	1,546.7 (3.4)	1,597.5 (9.5)	−3.3***	71.5 (2.0)	99.9 (5.3)	−39.7***	0.51 (0.01)	0.72 (0.04)	−41.2***
30–39	162.7 (7.4)	158.6 (4.2)	2.5***	−3.77	61.2 (8.4)	65.4 (6.0)	−6.9*	2.03	23.0 (2.0)	26.0 (2.0)	−13.0***	4.63	1,538.2 (3.1)	1,569.9 (7.7)	−2.1**	70.6 (2.1)	86.1 (5.0)	−22.0***	0.48 (0.01)	0.61 (0.03)	−27.1**
40–49	162.4 (3.9)	159.3 (3.9)	1.9***	−3.22	64.3 (6.4)	67.6 (9.9)	−5.1	1.16	24.4 (2.4)	26.6 (3.2)	−9.0**	2.88	1,527.0 (2.3)	1,561.8 (4.9)	−2.3***	68.6 (1.5)	81.4 (2.6)	−18.7***	0.45 (0.01)	0.57 (0.02)	−26.7***
50–59	162.0 (5.3)	162.5 (5.8)	−0.3	0.36	62.9 (8.2)	63.4 (3.9)	−0.8	0.53	23.9 (2.6)	24.1 (2.5)	−0.8	0.34	1,517.2 (3.2)	1,538.1 (21.3)	−1.4*	63.0 (2.0)	66.2 (13.6)	−5.1	0.41 (0.01)	0.47 (0.07)	−14.6*
≥60	159.3 (4.8)	153.6 (4.8)	3.6**	−2.73	59.5 (7.4)	60.1 (10.7)	−1.0	0.01	23.5 (3.1)	25.4 (3.6)	−8.1	1.49	1,510.9 (3.0)	1,521.2 (11.9)	−0.7	59.2 (2.0)	60.8 (33.7)	−2.7	0.38 (0.01)	0.42 (0.20)	−10.5

Note: Means (*M*) and standard deviations (*SD*) are presented for anthropometric variables and estimated marginal means (*M*) and standard errors (*SE*) for QUS parameters.
Abbreviations: BMI, body mass index; ANCOVA, analyses of covariance; QUS, quantitative ultrasonometry.
[a]Number of participants for Tsimane age categories: 13–19 (*n* = 36); 20–29 (*n* = 60); 30–39 (*n* = 46); 40–49 (*n* = 69); 50–59 (*n* = 43); ≥60 (*n* = 52).
[b]Number of participants for Shuar age categories: 13–19 (*n* = 13); 20–29 (*n* = 20); 30–39 (*n* = 21); 40–49 (*n* = 21); 50–59 (*n* = 4); ≥60 (*n* = 9).
[c]ANCOVA-derived estimated marginal means (*M*) and *SE* adjusting for BMI. *p* values obtained through bias-corrected accelerated bootstrap procedure with 1,000 replications.
[d]Tsimane versus Shuar.
*$p < .05$; **$p \leq .01$; ***$p \leq .001$.

(Van Houten, 2005). Most of the calcium for breast milk production is drawn from bone, accounting for a temporary 3–9% decrease in bone density during lactation (Kovacs, 2016). Lactation-induced bone loss even occurs among women with high calcium intake, due to the hypoestrogenic state associated with prolonged postpartum amenorrhea. As estrogens have physiological and biomechanical effects on bone including inhibiting resorption by affecting osteoclast formation (Riggs, 2000; Devlin, 2011), reduced estrogen concentration combined with loss of calcium in milk explains most of the bone density loss among lactating women. After lactation ceases, bone mineral status is often restored to prepregnancy values, until the perimenopausal period when the rate of bone loss accelerates in conjunction with changes in mineral regulating hormones (e.g., estrogen, progesterone) (Kalkwarf, 2004; Riggs, Khosla, & Melton, 1998). Importantly, while postlactation restoration of bone density has been demonstrated in low-fertility populations who often engage in restricted breastfeeding and have long interbirth intervals (IBIs) (Sowers et al., 1993), there is evidence that Gambian women practicing extended, on-demand lactation with shorter IBIs and higher fertility may experience incomplete BMD restoration (Jarjou et al., 2010, 2013; Sowers, Randolph, Shapiro, & Jannausch, 1995).

Because we include pregnant and lactating women in this study, the postpeak eBMD decline observed in this study encompasses both pregnancy and lactation-induced bone loss, along with bone loss associated with menopausal transition (mean ± *SD* age at menopause for Tsimane: 49 ± 4 years, and for Shuar: 46 ± 4; Stieglitz et al., 2019; Madimenos et al., 2019). While the complex effects of reproduction on bone density are examined elsewhere for Tsimane (Stieglitz et al., 2015; Stieglitz et al., 2019) and Shuar (Madimenos et al., 2012), we find that population-level differences in women's QUS values at advanced ages are negligible, which suggest comparable effects of postmenopausal cessation of critical bone maintaining hormones. Approximately half of women lose 10–20% of bone density in the 5–6 years around menopause (Ji & Yu, 2015). Comparable bone diminishing effects of this physiological transition may be experienced across older Tsimane and Shuar females despite high lifetime physical activity levels in both populations and substantially higher (by ~30%) peak bone density values among the Shuar.

Few population-representative bone density data sets are available for other extant subsistence-based groups and therefore how Shuar and Tsimane results compare to other subsistence populations is unknown. However, research from bioarchaeology offers some insight into past human populations, with studies showing temporally and regionally variable patterns of bone loss and fragility (Agarwal, 2008). Using a large sample of European skeletons spanning the Upper Paleolithic to the 20th century, Ruff et al. (2015) found significant declines in anteroposterior bending strength of the femur and tibia beginning in the Neolithic; this finding is interpreted as a consequence of increased sedentism tied to intensive agriculture with only minimal effects on skeletal robusticity related to more recent cultural and technological advancements such as increasing mechanization and industrialization. Other paleoanthropological studies have demonstrated deleterious skeletal effects (e.g., low trabecular density, thinner trabeculae) related to economic shifts from hunting and gathering to agriculture, particularly in the lower limbs, attributed in major part to reduced mobility that manifests in osteologically distinct ways (Chirchir et al., 2015; Nelson, Agarwal, & Darga, 2015; Ryan & Shaw,

TABLE 4 Age (years) at peak QUS value, estimated from polynomial regressions

	eBMD (g/cm^2)	SOS (m/s)	BUA (dB/MHz)
Tsimane females	27.6	22.0	34.7
Shuar females	25.5	19.8	31.0
Tsimane males	26.6	23.3	31.9
Shuar males	29.0	21.7	36.1

Abbreviations: eBMD, estimated bone mineral density; BUA, broadband ultrasound attenuation; SOS, speed of sound.

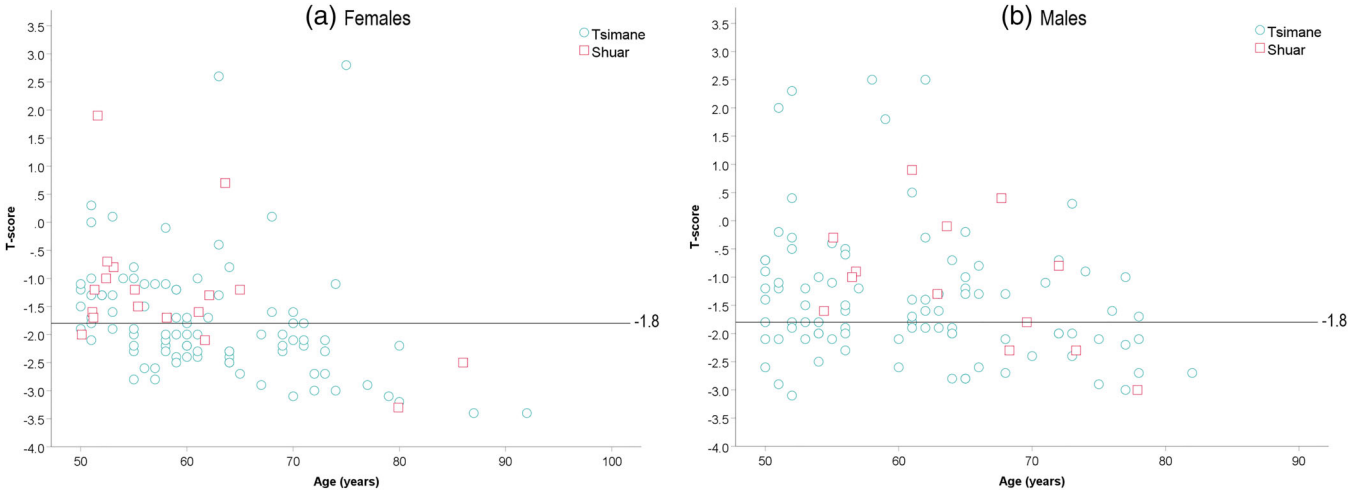

FIGURE 2 (a,b) Scatterplot of *T* score by age for participants aged 50+ years. Plots are presented by sex: (a) female and (b) male. Markers below the −1.8 cutoff line represent individuals at elevated osteoporosis risk based on a calcaneal QUS device-specific threshold. QUS, quantitative ultrasonometry

2015; but see Bridges, 1989). Additional factors, such as chronic malnutrition and increased prevalence of infectious disease, have also been linked to low BMD and long bone cortical thinning among children from bioarchaeological assemblages representing subsistence-level groups (e.g., Martin & Armelagos, 1985; McEwan, Mays, & Blake, 2005); this reduced bone quantity in childhood may be expected to transfer into adulthood, thereby exacerbating later bone density loss. Collectively, these bioarchaeological studies and current study findings suggest potential risk factors for reduced bone quality and quantity that are not as readily discernable in industrialized, nonclinical populations and call into question the extent to which low bone density and osteoporosis have been inextricably tied to industrialized lifestyles (Gluckman & Hanson, 2006; Lieberman, 2013; Wallace et al., 2015).

5.1 | Potential sources of variation in bone mineral density

Shuar QUS values are higher than Tsimane for most age categories. This difference is already apparent early in adolescence, and possible explanations for the disparity must be targeted at factors contributing to bone growth and mineralization. For example, genetic factors may be implicated as the heritability of bone density is estimated to range between 60 and 85% (Harris, Nguyen, Howard, Kelly, & Eisman, 1998; Richards, Zheng, & Spector, 2012). However, despite the identification of 153 genetic variants associated with bone density (Kemp et al., 2017), each variant by itself may convey a small risk or benefit making it difficult to determine who is at risk of compromised bone integrity simply based on random genetic variation. Furthermore, genetic variants interact with multilevel factors (e.g., nutrient intake, physical activity, hormonal status) across developmental stages, from the embryonic stage onwards, with a cumulative impact on bone metabolism (e.g., Ellison, 1982; Bachrach, 2001; Agarwal & Glencross, 2010; Devlin, 2011). Even if genetic data were available, much is still unclear about how gene–environment and gene–gene interactions influence bone density and osteoporosis risk; indeed, research by Wallace et al. (2016) indicates that stochastic genetic diversity across populations does not associate with variation in hip fracture incidence.

Variability in activity patterns is one lifestyle factor that can contribute to variation in bone mineral status between populations. Epidemiological, bioarchaeological, and paleontological research emphasizes the role of sedentary lifestyles as a major contributing factor underlying reduced bone strength. Sedentism is the aspect of the "mismatch" paradigm that has garnered most attention with regard to the skeleton because of the physical activity effects, particularly of the weight-bearing variety, on enhancing bone growth and development (Carter & Orr, 1992; Pitukcheewanont, Austin, Chen, & Punyasavatsut, 2013; Zemel, 2013). Yet, physical inactivity is not characteristic of either subsistence population studied here, as both are reported to have moderate to vigorously active lifestyles (Gurven et al., 2013; Madimenos, Snodgrass, Blackwell, Liebert, & Sugiyama, 2011). Furthermore, osteoporosis risk prevalence is higher for Tsimane vs. the United States, as is thoracic vertebral fracture prevalence (Stieglitz et al., 2019), contrary to predictions from a physical inactivity hypothesis of skeletal fragility. Regarding Tsimane–Shuar differences, published data for adults (e.g., Gurven et al., 2013) indicate that any difference in physical activity levels between the two populations are unlikely to be large enough to satisfactorily explain the pronounced variation in adult QUS parameters (particularly in early adulthood). Whether differences in physical activity levels are found across childhood, when bone density differences are also apparent (see Figure 1a,b) and the magnitude of bone tissue response to mechanical loading is greatest (Warden et al., 2014), is unknown and warrants further study.

Another possible explanation for Tsimane–Shuar bone density differences in early life includes the high pathogen burden documented in these groups, leading to heightened immune activation throughout life (Blackwell et al., 2011; Blackwell, Snodgrass, Madimenos, & Sugiyama, 2010; Urlacher et al., 2018). Heightened immune activity has been associated with increased biomarkers of inflammation (e.g., C-reactive protein) that have been shown to stimulate osteoclastic bone resorption and impede osteoblast function (e.g., Ginaldi, Di Benedetto, & De Martinis, 2005; Schett et al., 2006). Stieglitz et al. (2016) found that among Tsimane adults aged 50+ years, white blood cell count is moderately correlated within individuals over time and inversely associated with QUS values, a finding that is in concordance with literature linking infection or inflammation to reduced bone density (e.g., Ginaldi et al., 2005; Munday, Ginty, Fulford, & Bates, 2006; Schett et al., 2006). Consistent with a tradeoff between energetic investment in growth and somatic maintenance predicted by life history theory, frequent pathogen-driven immune activation could shunt energetic resources away from bone accretion to a more pronounced degree among Tsimane than Shuar and the U.S. population. Blackwell et al. (2011), for example, found that Tsimane produce higher levels of the antibody immunoglobulin E (IgE) at earlier ages and experience earlier IgE peaks compared to the Shuar (age 7 vs. 10 years old). Furthermore, while both Tsimane and Shuar IgE levels are significantly higher than in the United States, Shuar display substantially lower IgE than Tsimane. These data indicate a more rapid and heavy investment in immunocompetence among the Tsimane (than either Shuar or in the United States), presumably at the expense of other investments, such as growth. Urlacher et al. (2018) found support for this trade-off among Shuar subadults, where IgE negatively predicted 20-month growth and height-for-age *z* scores. Increased production of IgE among Tsimane compared to Shuar and the United States may be one pathway through which energy is diverted away from bone development/maintenance, resulting in lower bone density from childhood onward. Interestingly, at the population level, Tsimane are generally taller than the Shuar, suggesting that linear skeletal growth may be maintained at the expense of bone density in the former group.

5.2 | Study limitations

First, the research design is cross-sectional, which limits our ability to document age-related change in QUS parameters. Second, sample

sizes for Shuar at older ages are small, precluding greater certainty in our interpretation of results regarding population-level differences in osteoporosis risk and the rate of postpeak QUS decline. Third, despite the complex effects of pregnancy and lactation on bone density, we chose to include pregnant and lactating women in order to obtain a representative sample. Fourth, the main strength of this study is to establish a reference range and report prevalence estimates of osteoporosis risk from two subsistence-based groups. However, prevalence estimates are defined here by QUS and not DXA and so our estimations may not accurately reflect population-level risk. Additionally, we do not incorporate information on fracture risk for Shuar or Tsimane, which constitutes the major clinical burden of osteoporosis.

As one major objective of this research was to characterize bone density trends across populations, there are no direct comparisons of diet, activity, genetics, immune activation, and other factors that will permit a comprehensive investigation of the factors promoting Tsimane–Shuar differences. For example, lack of potable water or electricity among Tsimane compared to Shuar villages may dramatically alter their energetic state and/or modify exposure to infectious agents with potential downstream effects on bone density.

Another limitation includes the focus on a single skeletal site, the weight-bearing calcaneus. Because different skeletal regions vary in bone density, the disparity documented here may not be translated to other sites. Also, while calcaneal QUS examines factors related to bone density, QUS devices are unable to assess trabecular versus cortical strength, and they lack the resolution to assess various microarchitectural features (e.g., trabecular separation, thickness). Finally, QUS devices may lead to measurement variability as it relates to bone size and shape (e.g., Cheng et al., 2002). Subtle variations in foot placement combined with the heterogeneity in external geometry and internal structure and density of the calcaneus can therefore affect intraindividual and interindividual variability of QUS measures.

6 | CONCLUSIONS

Bone density is scantily described for subsistence-based populations, limiting epidemiological and evolutionary understandings of low bone density and osteoporosis. Here, we document that despite living in similar neotropical environments, engaging broadly in similar subsistence practices, having high fertility, sharing similar subadult growth trajectories, and remaining active throughout their lives, Tsimane and Shuar display distinctive bone density patterns that are most pronounced earlier in life. This difference persists until advanced ages. Based on data presented here, it is unclear what specifically accounts for this early difference, although numerous testable hypotheses are offered for future avenues of research.

Our findings have several implications. First, this study emphasizes how the multifactorial etiology of bone accretion and loss is population- and context-specific and that apparent similarities in lifestyle and environment may not necessarily translate into similar bone densities. This conclusion has application not only for clinical and epidemiological research on modern populations, but it also bears implications for how bone quantity and shape may be interpreted in bioarchaeological and paleontological samples. Related to this first point, this study stresses against oversimplifying human susceptibility to low bone density and osteoporosis as a "mismatch" between the so-called ancestral biology and novel conditions associated with industrialized environments. As low bone density and relatively high osteoporosis risk is identified in at least one of the forager-horticulturalist populations studied here, and because etiology may be linked to challenges not characteristic of industrialized settings, the current "mismatch" paradigm may be a limiting framework for understanding osteoporosis risk among extant subsistence groups.

Finally, consistent with prior research in human biology, the data presented here highlight the importance of early developmental life in shaping adult bone density. If bone loss is inevitable with age, as appears to be the case for humans and other primates (e.g., Didier et al., 2016), then greater consideration of ways to maximize peak bone density early in life, and also reduce bone loss in advanced age will help mitigate osteoporosis prevalence. Developing such strategies requires more research on early developmental life (i.e., in utero and beyond) from cross-cultural and subsistence-based groups with heterogeneous lifestyles to achieve a more comprehensive and inclusionary understanding of global skeletal health.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding authors. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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