

# Normative calcaneal quantitative ultrasound data for the indigenous Shuar and non-Shuar *Colonos* of the Ecuadorian Amazon

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Received: 24 March 2011 / Accepted: 21 April 2011

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## Abstract

**Summary** Minimal data on bone mineral density changes are available from populations in developing countries. Using calcaneal quantitative ultrasound (QUS) techniques, the current study contributes to remedying this gap in the literature by establishing a normative data set on the indigenous Shuar and non-Shuar *Colonos* of the Ecuadorian Amazon.

**Purpose** The paucity of bone mineral density (BMD) data from populations in developing countries partially reflects the lack of diagnostic resources in these areas. Portable QUS techniques now enable researchers to collect bone health data in remote field-based settings and to contribute normative data from developing regions. The main objective of this study is to establish normative QUS data for two Ecuadorian Amazonian populations—the indigenous Shuar and non-Shuar *Colonos*. The effects of ethnic group, sex,

age, and body size on QUS parameters are also considered. **Methods** A study cohort consisting of 227 Shuar and 261 *Colonos* (15–91 years old) were recruited from several small rural Ecuadorian communities in the Upano River Valley. Calcaneal QUS parameters were collected on the right heel of each participant using a Sahara bone sonometer. Three ultrasound generated parameters were employed: broadband ultrasound attenuation (BUA), speed of sound (SOS), and calculated heel BMD (hBMD).

**Results** In both populations and sexes, all QUS values were progressively lower with advancing age. Shuar have significantly higher QUS values than *Colonos*, with most pronounced differences found between pre-menopausal Shuar and *Colonos* females. Multiple regression analyses show that age is a key predictor of QUS while weight alone is a less consistent determinant. Both Shuar males and females display comparatively greater QUS parameters than other reference populations.

**Conclusions** These normative data for three calcaneal QUS parameters will be useful for predicting fracture risk and determining diagnostic QUS criteria of osteoporosis in non-industrialized populations in South America and elsewhere.

**Keywords** Bone mineral density · Shuar · *Colonos* · Calcaneal ultrasound · Developing country

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## Introduction

Bone mineral density (BMD) data are limited for non-industrialized, natural fertility populations, a gap that obscures our current understanding of the prevalence of osteoporosis in developing countries. In part, the lack of

epidemiological data on osteoporosis in developing countries reflects the limited availability of diagnostic resources in these regions. However, relatively non-invasive and portable techniques have recently facilitated the collection of BMD data in remote field settings. Calcaneal quantitative ultrasound (QUS) techniques in particular have proven to be clinically useful as a screening tool for early signs of low BMD, and are highly correlated with dual X-ray absorptiometry (DXA) measures [1–3]. Additional reasons for the lack of attention to bone health in developing countries are assumptions that osteoporosis is a disease of developed nations and an inevitable consequence of aging that cannot be effectively treated or prevented [4]. Indeed, key environmental risk factors for the development of osteoporosis are typically associated with a Westernized lifestyle, characterized by low levels of physical activity and poor diets that include processed foods, coffee, and carbonated soda beverages. Yet demographic transition and the aging of the population, as well as increasing global trends towards Westernization, appear to be contributing to a dramatic rise in the burden of osteoporosis prevalence in developing countries [5]. The paucity of data on bone density in these countries limits our ability to assess osteoporosis prevalence and risk, and complicates prevention and treatment efforts. In order to begin addressing these issues, it is critical to gather and present population-specific normative data on BMD from non-urbanized groups living in developing countries.

The current study was designed to assess bone quality, using calcaneal QUS techniques, among the indigenous Shuar and non-Shuar mestizo colonists (*Colonos*) of Ecuadorian Amazonia. As with other indigenous populations, BMD information for the Shuar is not presently available. Further, published data are virtually non-existent for any subsistence-based, natural fertility population. These type of data are critical because diet, activity, and reproductive patterns all contribute to BMD, but it is currently unclear whether the BMD trajectories observed in low-fertility Western populations will be similar to those of subsistence-based groups. Further, an investigation of the key factors that structure BMD variation with age among subsistence groups can provide valuable information on the myriad of factors that shape bone health and disease risk in industrialized populations. Inclusion of the *Colono* population was intended to provide a comparative sample of individuals from the same geographical region who also participate in agricultural-based activities but who are not closely related to the Shuar. The cross-sectional data reported here are part of a larger, multi-year study on health and market integration, the Shuar Health and Life History Project (<http://www.bonesandbehavior.org/shuar>).

## Methods

### Study populations

#### *Shuar*

The Shuar are a large indigenous Amazonian forager-horticulturalist group (~50,000 persons) concentrated in the Morona-Santiago and Zamora provinces of Ecuador. Many Shuar live in scattered households across the Upano River Valley, between the eastern Andean foothills and the Cordillera de Cutucu mountain range. Traditional Shuar economy is based on hunting, fishing, and swidden-horticulture [6]. While these subsistence activities remain common, recent infrastructural development has led to an increasingly rapid lifestyle change for many Shuar, especially in the Upano River Valley. In this region, recently paved roads extending to many Shuar communities have resulted in increased access to commercial goods and a lifestyle associated with market integration (e.g., electricity). These lifestyle changes are most apparent in large town centers where some Shuar work wage-labor jobs. However, many Shuar living in rural communities continue to depend on and actively participate in mixed subsistence and agricultural production.

From an epidemiological perspective, the Shuar in the current study are not a particularly high-risk group for the development of osteoporosis. In the participant communities, Shuar engage in consistent levels of moderate subsistence-based activities year-round [7] and consume a diet that is rich in yuca, plantains, and rice with minimal inclusion of foods that are typically associated with poor bone health (e.g., caffeine, alcohol, processed foods, excessive protein) [8–11]. Additionally, deficiencies in vitamin D, a key nutrient for promoting intestinal calcium absorption, are rarely found in equatorial populations because of year-round exposure to sunlight (e.g., Refs. [12, 13]). While the Upano Valley Shuar are currently shifting towards an increased reliance on a market economy, including economic development and lifestyle changes similar to those observed in Western countries, the participant communities continue to be highly dependent on a subsistence-based lifestyle.

#### *Colonos*

The settlement of non-Shuar *Colonos* into Morona Santiago began during the mid-20th century at the height of the oil boom. While Upano River Valley *Colonos* and Shuar often reside in communities that are in close proximity, they are largely socially and politically divided, a schism that can be attributed to traditional Shuar antagonism toward outside colonization of their land and recurrent land feuds [6].

Similar to the Shuar, *Colonos* in the Upano River Valley live in communities with varying degrees of market dependence. *Colonos* who live in town, particularly in the Sucua canton, typically engage in wage labor, attend school, and have minimal participation in the subsistence economy. Conversely, in rural areas, many *Colonos* are strongly dependent on household-level animal husbandry and agricultural production. The *Colonos* included in this study are primarily from small rural communities scattered across the Upano River Valley where varying degrees of dependence on an agriculture-based lifestyle exist across individuals.

### Subjects

Typically, there is a high degree of inter-relatedness within rural Shuar communities. Potential issues can arise in assessing bone health for a highly related group of individuals because this can increase frequencies of population-specific alleles responsible for maintaining bone integrity. To avoid these issues, Shuar participants were recruited from four Upano Valley communities. Rural *Colono* communities do not exhibit the same degree of relatedness as the Shuar. Nevertheless, *Colono* participants were recruited from across a large canton in the Upano Valley comprised of several smaller rural communities. In total, 227 Shuar (136 females and 91 males; 15–86 years old) and 261 *Colonos* (157 females and 104 males; 15–91 years old) participated in this study.

Although conditions in this remote part of Ecuador prevented us from obtaining a random sample of participants, we made every effort possible to enroll a sample of participants that was representative of each community in terms of age and sociodemographics. This study used a cross-sectional design and while this approach is inherently limited for assessing individual bone loss through time, it is useful for determining prevalence of low bone density across a population.

All participants gave individual informed verbal consent, with both parental consent and child assent for individuals under 18 years old. The study protocol was approved by community leaders, the Instituto Ecuatoriano de Seguridad Social (IESS), the Federación Interprovincial de Centro Shuar (FISCH) as appropriate for FISCH-associated communities, and the Office for Protection of Human Subjects at the University of Oregon.

### Bone mineral density measurements

Calcaneal QUS parameters were collected on the right heel of each participant using the gel-based Sahara<sup>®</sup> bone sonometer (Hologic, Inc., Waltham, MA). Two primary QUS values were used for this analysis: broadband

ultrasound attenuation (BUA; decibels per megahertz), which is the slope of the ultrasonic attenuation versus frequency as it passes through bone; and speed of sound (SOS; meters per second), a value determined by the width of the heel and time delay between initial transmission and subsequent receipt of sound waves. BUA is more closely related to the micro-architecture of bone such as trabecular connectivity, while SOS is greatly influenced by the elasticity and mineral constituents of the bone matrix [14, 15]. A third parameter, heel bone mineral density (hBMD), a device-generated measure calculated from BUA and SOS values, was also used. Instrumental quality control scans of the manufacturer-provided phantoms were performed daily.

### Additional measures of body size and reproduction

Participant stature (measured to the nearest millimeter [mm]) and weight (measured to the nearest 0.1 kilogram [kg]) were recorded using a field stadiometer (Seca, Hanover, MD) and digital scale (Tanita BF-558 electronic scale, Tokyo, Japan), respectively. Body mass index (BMI) was calculated as weight (kg)/height (in square meters).

Female participants were asked additional questions regarding current reproductive status. Women were considered post-menopausal if they reported not having experienced a menstrual cycle within the last 12 months and were neither pregnant nor lactating.

### Statistical analyses

Participants were divided into six groups based on age (15–20, 21–30, 31–40, 41–50, 51–60, >60 years) with a 10-year age span within each group (except in the 15–20 and over 60 age intervals). Two-tailed Student's t-tests were used to assess differences between ethnic groups and sexes for bone density measures and anthropometric variables. Stepwise multiple linear regression analyses were conducted to estimate the variation of QUS explained by ethnicity, age, body weight, height, and BMI. All statistical analyses were conducted using SPSS 17.0 (Chicago, IL).

### Issues with normative data sets in developing countries

Reproductive patterns among women, including parity, breast-feeding behaviors, and age at first pregnancy are known to contribute to bone density changes throughout the lifespan (e.g., Refs. [16–18]). For example, physiological changes characteristic of various reproductive states such as pregnancy-related weight gain, cumulative estrogen exposure, and fluctuations in bone turnover rates invariably influence bone integrity. As a result, pregnant and/or lactating women are typically excluded from normative datasets on bone health. However, presenting normative

data on a natural fertility, subsistence-dependent population in a developing country without including women who are pregnant and/or lactating is problematic for several reasons. First, women in many developing countries are pregnant and/or lactating for much of their reproductive life spans [19–21]. Exclusion of these women dramatically reduces the size of the participant pool, specifically for critical age intervals when peak bone mass is realized. Further, in developing countries, particularly in the type of rural settings described here, nulliparous women of reproductive age do not represent the norm, as they may be suffering from health or fertility problems. Therefore, presenting normative data on the indigenous Shuar and *Colono* populations without inclusion of this female demographic is problematic. However, in order to maximize our ability to compare across populations, data on the subset of females who were not pregnant and/or lactating at the time of this study are also presented separately.

## Results

### Characteristics of study participants

Anthropometric and QUS measures by ethnic group and sex, and menopausal status are presented in Table 1. Although height, weight, and BMI of *Colono* participants are higher than for Shuar participants, QUS values are significantly higher among the Shuar ( $p < 0.001$ ). While this trend is consistent across the Shuar group when compared to *Colonos*, the most pronounced difference is found between pre-menopausal Shuar and *Colono* females (BUA:  $p < 0.05$ ; SOS and hBMD:  $p < 0.001$ ); the only exception is shown among post-menopausal women where there is no discernable difference in bone health measures between ethnic groups. In both populations, all QUS values are progressively lower in advanced age groups irrespective of sex.

### Shuar

Overall, Shuar males exhibit significantly higher bone density parameters than their female counterparts for all bone health measures except SOS (BUA:  $p < 0.001$ ; hBMD:  $p < 0.05$ ). Table 2 illustrates Shuar anthropometric and calcaneal QUS results by age cohort and sex. For both sexes, all bone health measures are highest in the 21–30 age cohort and are progressively lower in each subsequent age group. One exception is found in males from the 51- to 60-year-old cohort where all QUS parameters are greater than among males in the preceding 41–50 age interval. This difference may be attributed to small sample size in the older age interval, biased by one outlier with extremely

**Table 1** Anthropometric and QUS data by group, sex, and menopausal status (mean [SD])

	Shuar	<i>Colono</i>	<i>p</i> Value
<i>Sexes Combined</i>			
<i>N</i>	227	261	
Age (years)	34.97 (14.72)	47.43 (19.53)	**
Height (cm)	152.09 (7.23)	153.83 (10.91)	*
Weight (kg)	58.49 (10.50)	64.72 (12.50)	**
BMI (kg/m <sup>2</sup> )	25.19 (3.51)	27.28 (5.18)	**
BUA (dB/MHz)	82.0 (19.3)	69.4 (19.0)	**
SOS (m/s)	1,571.4 (33.2)	1,545.1 (32.9)	**
hBMD	0.600 (0.131)	0.499 (0.130)	**
<i>Males</i>			
<i>N</i>	91	104	
Age (years)	37.26 (14.76)	47.30 (20.15)	**
Height (cm)	157.99 (6.27)	161.13 (8.45)	*
Weight (kg)	63.19 (9.32)	69.49 (13.38)	**
BMI (kg/m <sup>2</sup> )	25.20 (2.73)	26.90 (5.99)	*
BUA (dB/MHz)	87.5 (19.54)	74.1 (20.38)	**
SOS (m/s)	1,574.4 (34.65)	1,550.8 (33.96)	**
hBMD	0.622 (0.137)	0.527 (0.134)	**
<i>Females</i>			
<i>N</i>	136	157	
Age (years)	33.44 (14.54)	47.52 (19.18)	**
Height (cm)	148.32 (4.9)	149.00 (9.6)	**
Weight (kg)	55.51 (10.14)	61.57 (10.8)	**
BMI (kg/m <sup>2</sup> )	25.19 (3.95)	27.53 (4.6)	**
BUA (dB/MHz)	78.4 (18.4)	66.2 (17.43)	**
SOS (m/s)	1,569.4 (32.14)	1,541.3 (31.7)	**
hBMD	0.586 (0.126)	0.481 (.124)	**
<i>Pre-menopausal women</i>			
<i>N</i>	118	81	
Age (years)	29.52 (10.55)	31.75 (10.44)	<i>Ns</i>
Height (cm)	148.86 (11.31)	152.34 (6.07)	**
Weight (kg)	56.17 (10.57)	61.76 (11.13)	**
BMI (kg/m <sup>2</sup> )	25.33 (4.51)	26.58 (4.43)	*
BUA (dB/MHz)	80.6 (15.3)	74.3 (15.3)	*
SOS (m/s)	1,574.2 (27.9)	1,559.2 (23.6)	**
hBMD	0.605 (0.110)	0.548 (0.095)	**
<i>Post-menopausal women</i>			
<i>N</i>	13	76	
Age (years)	61.60 (11.35)	64.32 (9.68)	<i>Ns</i>
Height (cm)	144.66 (6.23)	145.40 (11.31)	<i>Ns</i>
Weight (kg)	49.63 (8.75)	61.37 (10.57)	**
BMI (kg/m <sup>2</sup> )	23.57 (2.85)	28.56 (4.51)	**
BUA (dB/MHz)	58.7 (21.2)	57.5 (15.3)	<i>Ns</i>
SOS (m/s)	1,532.7 (35.8)	1,522.2 (27.9)	<i>Ns</i>
hBMD	0.439 (0.144)	0.409 (0.110)	<i>Ns</i>

*Ns* not significant

\* $p < 0.05$ ; \*\* $p < 0.001$  (significant difference between ethnic groups)

**Table 2** Age-specific anthropometric and QUS parameters by ethnic group and sex (mean [SD])

Ethnic Group	Age	Sex	<i>n</i>	Height (cm)	Weight (kg)	BMI (kg/m <sup>2</sup> )	BUA (dB/MHz)	SOS (m/s)	hBMD
Shuar	15–20	Female	28	148.77 (4.94)	50.52 (5.03)	22.80 (1.77)	77.5 (17.0)	1,577.6 (29.2)	0.604 (0.115)
		Male	12	154.83 (11.6)	54.05 (12.32)	22.20 (2.56)	77.9 (15.6)	1,580.4 (23.9)	0.612 (0.098)
	21–30	Female	38	149.63 (4.51)	55.49 (11.52)	24.81 (4.64)	83.0 (17.2)	1,578.4 (28.3)	0.620 (0.112)
		Male	21	158.93 (5.16)	62.53 (6.22)	24.69 (1.21)	95.7 (12.8)	1,590.3 (25.4)	0.684 (0.096)
	31–40	Female	30	149.31 (3.63)	61.44 (9.44)	27.53 (3.81)	80.4 (17.9)	1,571.8 (29.2)	0.599 (0.117)
		Male	25	158.38 (3.99)	66.16 (5.97)	26.35 (1.85)	96.1 (15.7)	1,582.6 (27.5)	0.665 (0.110)
	41–50	Female	23	146.86 (4.69)	57.66 (10.44)	26.60 (3.61)	81.2 (14.7)	1,567.5 (30.6)	0.591 (0.114)
		Male	19	159.07 (4.02)	68.14 (9.84)	26.85 (3.13)	83.3 (11.5)	1,563.6 (20.3)	0.583 (0.077)
	51–60	Female	9	145.79 (5.57)	55.71 (7.54)	26.15 (2.57)	67.4 (19.8)	1,538.6 (29.7)	0.476 (0.125)
		Male	5	162.40 (4.58)	63.97 (3.56)	24.33 (2.25)	86.1 (44.4)	1,573.4 (79.0)	0.615 (0.319)
>60	Female	8	143.14 (7.06)	45.79 (6.32)	22.23 (1.20)	56.4 (23.1)	1,529.4 (33.5)	0.424 (0.145)	
	Male	9	153.38 (4.58)	58.30 (10.45)	24.68 (3.58)	66.5 (20.9)	1,529.8 (36.6)	0.452 (0.149)	
Colono	15–20	Female	16	151.76 (5.66)	54.73 (13.0)	23.66 (5.04)	66.7 (9.2)	1,557.9 (22.5)	0.525 (0.732)
		Male	13	164.41 (7.72)	61.18 (10.39)	22.68 (3.94)	69.6 (21.6)	1,556.5 (30.4)	0.535 (0.135)
	21–30	Female	22	153.96 (5.68)	62.23 (10.22)	26.20 (3.77)	79.5 (16.3)	1,563.9 (24.6)	0.574 (0.101)
		Male	13	165.67 (5.85)	68.95 (8.85)	25.17 (3.47)	86.1 (21.0)	1,583.9 (36.7)	0.641 (0.141)
	31–40	Female	22	152.52 (6.52)	63.86 (9.32)	27.57 (4.55)	77.3 (15.1)	1,562.8 (24.2)	0.565 (0.981)
		Male	14	159.98 (14.24)	72.46 (11.99)	29.49 (10.98)	91.0 (16.8)	1,580.9 (24.7)	0.647 (0.106)
	41–50	Female	23	149.96 (6.91)	63.89 (10.37)	28.30 (3.19)	70.7 (14.5)	1,549.4 (21.5)	0.513 (0.089)
		Male	21	163.19 (5.39)	80.16 (14.90)	30.04 (5.03)	73.8 (15.5)	1,545.5 (19.5)	0.511 (0.086)
	51–60	Female	27	145.48 (17.75)	67.60 (10.39)	30.65 (4.44)	63.1 (14.0)	1,530.9 (22.6)	0.448 (0.092)
		Male	13	160.40 (6.42)	68.86 (13.31)	26.70 (4.48)	70.9 (12.1)	1,538.8 (19.6)	0.488 (0.754)
	>60	Female	47	145.58 (5.67)	58.04 (9.34)	27.41 (4.31)	54.3 (16.1)	1,516.9 (30.3)	0.386 (0.118)
		Male	30	157.23 (8.45)	65.10 (13.38)	26.28 (4.0)	64.8 (21.0)	1,528.8 (30.6)	0.448 (0.123)

high bone density (BUA: 164.2 dB/MHz; SOS: 1,712.0 m/s hBMD: 1.177).

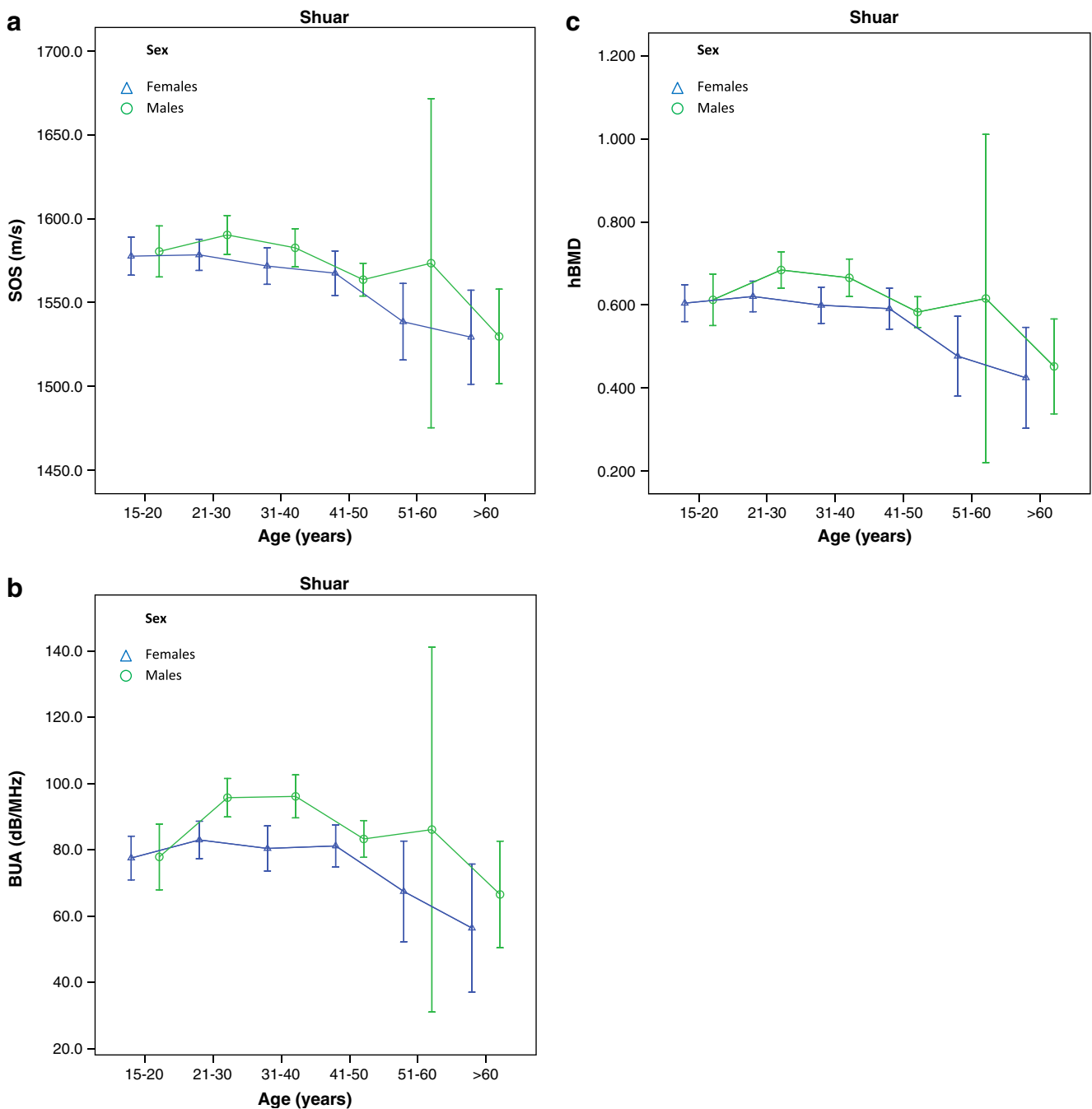
Figure 1a–c presents clustered scatterplots of BUA, SOS, and hBMD by age for Shuar females and males. In both sexes, there is a significant inverse relationship between age and each of the QUS parameters ( $p < 0.001$ ). From ages 21 to 86, females show a cumulative decrease of 34% in BUA, 3.1% in SOS, and 32% in hBMD. Although males begin with higher peak bone mass values than females, Shuar males from ages 21 to 80 show greater total decrease in bone density: 37% in BUA, 3.8% in SOS, and 38% in hBMD. Among females, the most pronounced decrease in BUA, SOS, and hBMD by age group occurs after 50 years of age, whereas among males the greatest decline is seen among individuals over 60.

In the stepwise multiple linear regression analyses (Table 3), weight was a less important factor for calcaneal QUS than age in both Shuar men and women. In men, all QUS parameters were significantly related to age (BUA:  $\beta = -0.543$ ,  $p < 0.001$ ; SOS:  $\beta = -1.288$ ,  $p < 0.001$ ; hBMD:  $\beta = -0.005$ ,  $p < 0.001$ ) and BMI (BUA:  $\beta = 2.768$ ,  $p < 0.001$ ; SOS:  $\beta = 2.682$ ,  $p < 0.05$ ; hBMD:  $\beta = 0.014$ ,

$p < 0.01$ ). However, in women, SOS was dependent only on age ( $\beta = -0.889$ ,  $p < 0.001$ ), while BUA was dependent on age ( $\beta = -0.284$ ,  $p < 0.05$ ), height ( $\beta = 0.740$ ,  $p < 0.05$ ), and BMI ( $\beta = 1.288$ ,  $p < 0.05$ ). Height was not a predictive variable for hBMD, although age ( $\beta = -0.003$ ,  $p < 0.001$ ) and BMI ( $\beta = 0.006$ ,  $p < 0.05$ ) were significant. Weight alone was not a significant predictor for any QUS value among the Shuar.

#### Colonos

Among *Colonos*, sex differences for all QUS measures are more pronounced than among Shuar, and higher bone density measures are found among *Colono* males than females (BUA:  $p = 0.001$ ; SOS:  $p < 0.05$ ; hBMD:  $p < 0.01$ ). Analysis of QUS values by age cohort (Table 2) shows that similar to Shuar, *Colono* females display the highest ultrasound measures in the 21- to 30-year-old cohort, with lower values in each successive age cohort. *Colono* males also have highest SOS in the 21- to 30-year-old cohort, although 31- to 40-year-olds exhibit the highest BUA and hBMD values. Among *Colono* females, as with their Shuar



**Fig. 1** Sex differences in the Shuar for SOS (a), BUA (b), and hBMD (c) values based on age. Values represent means for the age category with confidence intervals

counterparts, the most pronounced reduction in BUA, SOS, and hBMD is apparent after 50 years of age. In contrast, for *Colono* males, all QUS parameters are sharply lower after 40 years of age.

Clustered scatterplots for BUA, SOS, and hBMD by age for *Colono* females and males (Fig. 2a–c) show a significant negative relationship between age and QUS parameters for both sexes ( $p < 0.05$ ). Among *Colono*

females between 21 and 90 years old, total decline in QUS values with age are 38%, 3.0%, 36% for BUA, SOS, and BMD, respectively; among *Colono* males who are 21 to 91 years old, there is a 31% decrease in BUA, 3.5% decrease in SOS, and 36% decrease in hBMD

The stepwise multiple regression analysis in Table 3 shows that age is the sole predictive factor for SOS in *Colono* males ( $\beta = -0.832$ ,  $p < 0.001$ ) and females

**Table 3** Stepwise multivariate linear regression model between QUS values and age (years), weight (kg), height (cm), and BMI (kg/m<sup>2</sup>)

Ethnicity	QUS	Stepwise multiple regression model <sup>a</sup>	R <sup>2</sup>	Adjusted R <sup>2</sup>	p Value
Shuar	Male				
	BUA	38.04–0.543 * age+2.768 * BMI	0.224	0.204	0.000
	SOS	1,554.9–1.288 * age+2.682 * BMI	0.269	0.250	0.040
	hBMD	0.443–0.005 * age+0.014 * BMI	0.252	0.233	0.007
	Female				
	BUA	–53.7–0.284 * age+0.74 * height+1.288 * BMI	0.192	0.172	0.029
	SOS	1,560.0–0.889 * age	0.159	0.152	0.000
	hBMD	0.56–0.003 * age+0.006 * BMI	0.151	0.138	0.042
	Total				
	BUA	–63.1–0.35 * age+0.8 * height+1.431 * BMI	0.236	0.224	0.000
	SOS	1,497.5–0.94 * age+0.705 * height	0.198	0.190	0.016
	hBMD	0.03–0.003 * age+0.004 * height+0.006 * BMI	0.202	0.191	0.017
Colono	Male				
	BUA	62.7–0.289 * age+0.363 * weight	0.143	0.126	0.012
	SOS	1,590.2–0.832 * age	0.244	0.236	0.000
	hBMD	0.175–0.003 * age+0.003 * height	0.226	0.210	0.049
	Female				
	BUA	68.1–0.486 * age+0.773 * BMI	0.282	0.272	0.005
	SOS	1,588.9–1.001 * age	0.370	0.366	0.000
	hBMD	0.567–0.004 * age+0.004 * BMI	0.357	0.348	0.043
	Total				
	BUA	–29.0–0.321 * age+0.609 * height+0.726 * BMI	0.253	0.244	0.000
	SOS	1,489.7–0.837 * age+0.617 * height	0.335	0.330	0.001
	hBMD	0.195–0.003 * age+0.002 * height+0.001 * weight	0.322	0.314	0.039

<sup>a</sup> QUS parameters that are not in the regression equation are not significant in the model.

( $\beta=-1.001$ ,  $p<0.001$ ). In *Colono* females, BUA and hBMD were a function of both age (BUA:  $\beta=-0.486$ ,  $p<0.001$ ; hBMD:  $\beta=-0.004$ ,  $p<0.001$ ) and BMI (BUA:  $\beta=0.773$ ,  $p<0.01$ ; hBMD:  $\beta=0.004$ ,  $p<0.05$ ). In *Colono* males, BUA was a function of age ( $\beta=-0.289$ ,  $p<0.01$ ) and weight ( $\beta=0.363$ ,  $p<0.05$ ) while hBMD was dependent on age ( $\beta=-0.003$ ,  $p<0.001$ ) and height ( $\beta=0.003$ ,  $p<0.05$ ).

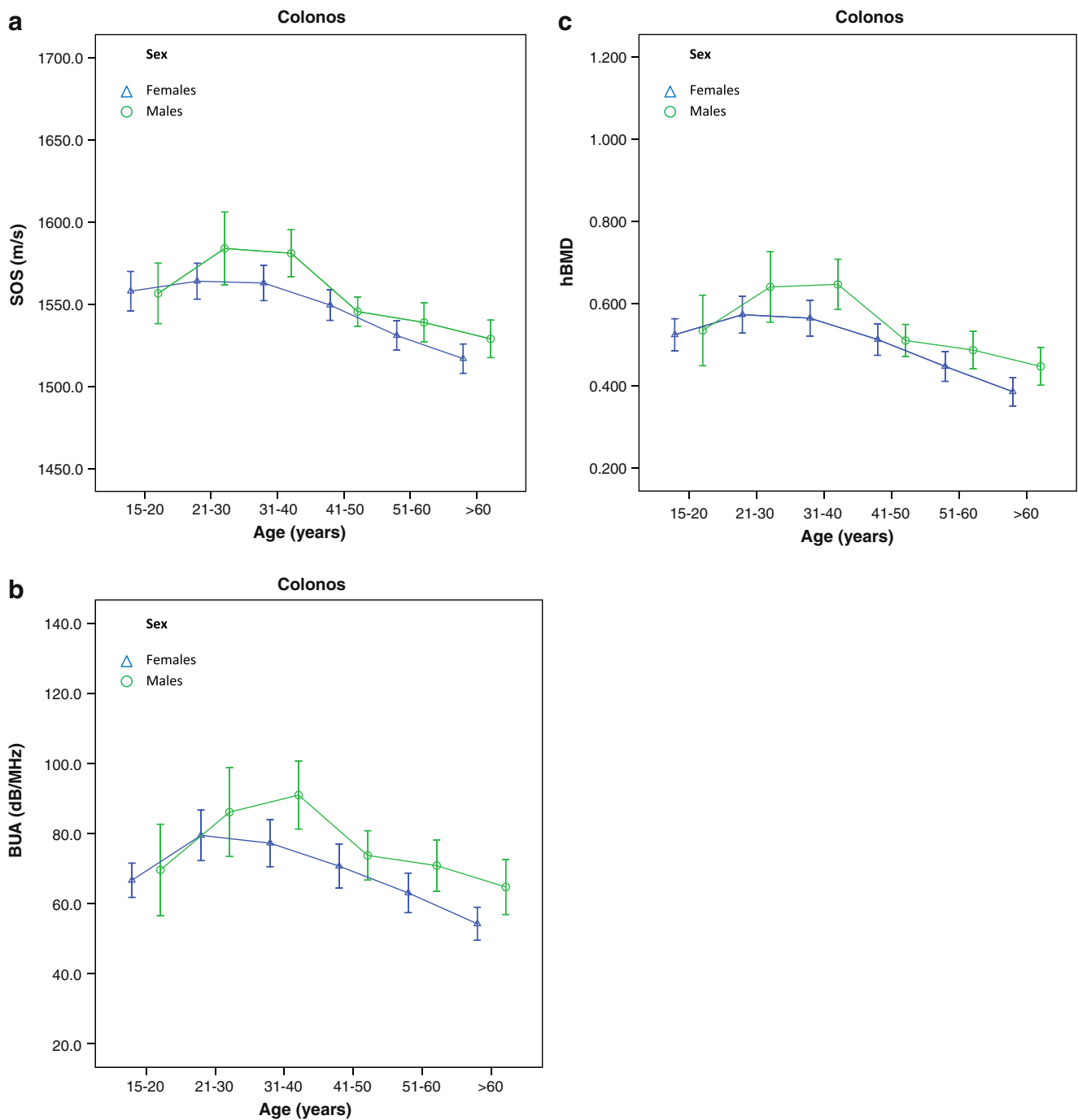
#### Reproductive status

Table 4 shows descriptive statistics of QUS and anthropometric measures for Shuar and *Colono* females who were neither pregnant nor lactating at the time of the study. Among the Shuar, nine participants (4%) were pregnant, 30 (13.2%) were lactating, and one (0.4%) was both pregnant and lactating at the time of the study. Half of Shuar women aged 21–30 years reported being either pregnant and/or lactating at the time of the bone scan; all women who reported being in a lactating state were from this age cohort. Among *Colono* women, four (1.5%) were pregnant and eight (3.1%) were lactating at the time of the study. Student's t-tests were performed comparing QUS param-

eters between Shuar and *Colono* women based on reproductive status—that is, whether they were pregnant, lactating, both, or neither. Significant ethnic group differences in bone health measures were found only for non-pregnant/non-lactating participants, with Shuar women showing significantly higher bone density than *Colono* women for BUA, SOS, and hBMD ( $p<0.001$ ).

#### Menopausal status

Analysis by menopausal status revealed that among premenopausal women there are significant ethnic differences for all QUS parameters ( $p<0.001$ ). Menopausal status was not reported for four Shuar women (aged 52–55 years old) and thus could not be included in these analyses. Thirteen Shuar (9.6%) and 76 *Colono* women (48.4%) were postmenopausal at the time of the study (Table 1). Among *Colono* and Shuar postmenopausal women, there is no significant difference in BUA, SOS, and hBMD values, which suggests that the highest degree of variation in bone health between ethnic groups is present in the premenopausal lifespan.



**Fig. 2** Sex differences in the *colonos* for SOS (a), BUA (b), and hBMD (c) values based on age. Values represent means for the age category with confidence intervals

## Conclusions

The main purpose of this paper was to present population-specific normative data for bone density in a non-industrialized, natural fertility population from a developing country. Minimal bone health data are available from these contexts and this has inhibited efforts to quantify the burden

of osteoporosis in developing nations, especially among rural groups [4, 5]. Additionally, bone health information from diverse populations is necessary to determine the degree to which normative data from developed countries may reflect sub-optimal bone density levels. The skeletal system and mechanisms of bone turnover evolved under subsistence-based, natural fertility conditions with dietary,



**Table 4** Descriptive statistics for non-pregnant and non-lactating women by ethnic group (mean [SD])

	Shuar (n=96)	Colono (n=145)	p Value
Age (years)	35.81 (15.37)	48.50 (19.17)	**
Height (cm)	148.17 (5.19)	148.84 (9.68)	Ns
Weight (kg)	55.30 (9.17)	61.51 (10.92)	**
BMI (kg/m <sup>2</sup> )	25.13 (3.55)	27.54 (4.61)	**
BUA (dB/MHz)	77.5 (19.4)	64.8 (16.7)	**
SOS (m/s)	1,566.4 (32.6)	1,539.1 (30.8)	**
hBMD	0.576 (0.130)	0.472 (0.119)	**

Ns not significant

\*\* $p < 0.001$  (significant difference between ethnic groups)

activity, and reproductive patterns that likely produce a vastly different representation of normative bone health than data garnered from industrialized, urban populations. The paucity of epidemiological data from non-urbanized populations in developing countries can be partly attributed to the lack of diagnostic resources in these regions. However, portable QUS technology now enables the collection of BMD data from remote field settings. Calcaneal sonometers, in particular, are increasingly being utilized to obtain data from populations worldwide and to compare this information with existing reference populations. The current study focused on the calcaneus in order to maximize future inter-population comparisons, and because the calcaneus is a weight-bearing site rich in trabecular bone that is therefore an ideal single-site measure of bone density parameters.

The present study found that *Colonos* are taller, heavier, and have greater BMI values than the Shuar. However, despite this result, and the finding that age-related bone loss occurs in both groups, values for BUA, SOS, and hBMD are consistently higher in the Shuar population. Higher peak bone mass among the Shuar may partially explain the higher QUS values exhibited in all age groups when compared to *Colonos*. Although both ethnic groups participate to a varying degree in an agriculture-based subsistence regime, population-specific variability in dietary and lifestyle factors may account for the significant QUS differences reported here. Individual and community-level variation in diet, activity levels, and smoking/drinking behaviors have been reported elsewhere for these populations [7, 22] and future research will explore the specific effects of these factors on Shuar and *Colono* bone health. Genetic differences between ethnic groups have been established (e.g., African-American versus Caucasian-American populations) and these factors may also explain the lower BMD documented across *Colono* age cohorts (e.g., Refs. [23–26]).

The most pronounced ethnic group difference in QUS values was documented between Shuar and *Colono* premenopausal women who were not pregnant or lactating. In post-menopausal women, no significant difference was found, suggesting that menopause onset has similar negative effects on bone integrity across ethnic groups. For both Shuar and *Colono* females, the lowest bone health values were found after 50 years old. This finding is consistent with other studies (e.g., Refs. [27, 28]) that show that the most profound bone loss occurs for similar age intervals, typically at the onset of menopause.

We also observed the effect of age and body size (height, weight, and BMI) on QUS by ethnic group using a stepwise multiple regression analysis. Age is a consistent predictor of all QUS parameters, with declining values in older age groups. Among female cohorts, the second most reliable predictor of higher QUS values is greater BMI. In the current study, the highest QUS values among females are found among 21- to 30-year-olds, which coincides with a period when peak bone mass is generally achieved. It is also important to recognize that half of the Shuar women in this age cohort reported being pregnant and/or lactating which may be variously contributing to calcaneal QUS values. However, when comparing females who are not pregnant and lactating, differences between the two ethnic groups are maintained.

Males in both populations show consistently higher QUS parameters than females and, similar to women, calcaneal QUS values are a primary function of age. BMI also predicts SOS, BUA, and hBMD among Shuar males. However, in *Colono* males QUS values, specifically BUA and hBMD, are not a function of BMI and are better predicted by the independent variables, weight and height, respectively. Furthermore, one of the earliest and more pronounced declines in QUS values occurs in *Colono* males after 40 years old. In contrast, Shuar male QUS data do not show this steep decline until after 60 years old, more typical of the pattern documented in other studies (e.g., Refs. [29, 30]). While the *Colono* result does not follow the typical trend, a similar decrease in heel BMD is reported among Roma males from Croatia after 35 years old [31]. Potential causes for this apparently early decrease in QUS parameters are not addressed in the Roma study, but the authors state that increased sedentism and diminutive body size are possible explanations for the low BMD characteristic of the entire group. The reason for an early decline among *Colono* males is unclear, but may be related to sampling biases. A larger sample size for each age interval is necessary to address this issue.

Shuar men and women both display comparatively greater QUS parameters than other populations whose reference values were determined using the same calcaneal QUS instrument. In South Korea, in a study by Rhee and

colleagues [32], all QUS values are lower than the Shuar population but higher than the *Colono* group. In a German study on an exclusively female cohort [33], BUA and SOS parameters are again lower than Shuar women but higher than *Colono* females in our study for similar age intervals. Normative data from the Croatian Roma population discussed earlier show markedly lower hBMD values when compared to Ecuadorian data; however this disparity is minimized after 40 years old for both female cohorts and for *Colono* males. Shuar males maintain among the highest ultrasound values for any comparative population presented here. From this cross-study comparison, the Shuar exhibit some of the highest QUS values, while *Colonos* as a group show slightly lower bone density measures than other global reference populations. The high values among the Shuar are likely related to a suite of lifestyle and activity patterns that are reflective of greater dependency on a subsistence-based economy than the *Colono* participants.

### Study limitations

The present study has several important limitations. While field-based research is a necessary and critical approach to gathering information from populations in remote settings, this type of research presents issues when aiming to establish a controlled setting for data collection. One study limitation is that participant sample sizes for certain age intervals are small, particularly among older cohorts, an issue that is more pronounced in the Shuar group. The small sample sizes and non-random selection of participants within age categories may inhibit our interpretations of the age effects on BMD that have been reported for other reference populations. Also, participants in the current study were recruited from a sample pool of individuals who may have been inclined to seek out health information provided by this study. Although this may inherently bias the dataset towards individuals who demonstrate greater health-related issues, this recruitment strategy is not atypical for field-based research in remote settings. Also, as previously mentioned, normative data on bone health generally exclude pregnant and lactating females, because the modifiable (e.g., diet, activity) and non-modifiable (e.g., hormones) factors affecting bone integrity are constantly fluctuating. Yet in many developing countries most females are pregnant and lactating for much of their reproductive life spans, so inclusion of this demographic was an effort to present a normative representation of bone health in these two groups. This inclusion, however, can present problems with cross-population comparative studies. Further research on bone health in developing countries is necessary in order to determine the most effective approach to remedying this issue. Finally, cross-sectional data prevent us from making definitive statements on age-related changes in skeletal

health, but is a necessary first step in determining patterns of bone loss.

The current study established a preliminary dataset for three calcaneal QUS parameters in the Shuar and non-Shuar *Colono* populations of Amazonian Ecuador. It investigated ethnic and sex differences for the effect of age and body size variables on QUS parameters. The study also addressed relevant issues in gathering bone health data in a remote field-based setting within a developing country. The Shuar and *Colonos* represent two populations from the same geographical region who have a range of dependency on mixed agricultural and subsistence-based economy. Differences in QUS values between the two groups may be attributed to genetics as well as to individual- and community-level variation in diet and lifestyle. Future research will examine the specific ways in which dietary composition and lifestyle behaviors contribute to Shuar and *Colono* bone health profiles.

**Acknowledgments** We express gratitude to Ruby Fried, Michael Fernandez, Betsy Ruth, Paula Tallman, Cesar Kayap, Oswaldo Mankash, Luzmila Jempekat, Estela Jempekat, and Marilu Utitaj for their assistance with data collection. We also thank our funding sources: Wenner-Gren Foundation for Anthropological Research (Grant number: 7970); NSF (Grant number: BCS-0824602); Evonuk Foundation; L.S.B. Leakey Foundation; Ryoichi Sasakawa Young Leaders Fellowship; NIH (5DP1OD000516-5 via UCSB Center for Evolutionary Psychology); University of Oregon. Finally, we express our sincerest gratitude to the participants in this study.

**Disclosures** None

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