3-27-2017

Horticultural Activity Predicts Later Localized Limb Status in a Contemporary Pre-Industrial Population

Jonathan Stieglitz
Institute for Advanced Study in Toulouse

Benjamin C. Trumble
Arizona State University

Hillard Kaplan
Chapman University, hkaplan@chapman.edu

Michael Gurven
University of California, Santa Barbara

Recommended Citation

HORTICULTURAL ACTIVITY PREDICTS LATER LOCALIZED LIMB STATUS IN A CONTEMPORARY PRE-INDUSTRIAL POPULATION
Forager-farmer activities and bone strength

Jonathan Stieglitz[a,b,*], **Benjamin C. Trumble**[c,d], **Hillard Kaplan**[b], and **Michael Gurven**[e]

[a]Institute for Advanced Study in Toulouse, Université de Toulouse, FRANCE

[b]Department of Anthropology, University of New Mexico, Albuquerque, USA

[c]Center for Evolution and Medicine, Arizona State University, Tempe, USA

[d]School of Human Evolution and Social Change, Arizona State University, Tempe, USA

[e]Department of Anthropology, University of California, Santa Barbara, USA

Abstract

Objectives—Modern humans may have gracile skeletons due to low physical activity levels and mechanical loading. Tests using prehistoric skeletons are limited by the inability to assess behaviour directly, while modern industrialized societies possess few socio-ecological features typical of human evolutionary history. Among Tsimane forager-horticulturalists, we test whether greater activity levels and, thus, increased loading earlier in life are associated with greater later-life bone status and diminished age-related bone loss.

Materials and Methods—We used quantitative ultrasonography to assess radial and tibial status among adults aged 20+ years (mean±SD age=49±15; 52% female). We conducted systematic behavioural observations to assess earlier-life activity patterns (mean time lag between behavioural observation and ultrasound=12 years). For a subset of participants, physical activity was again measured later in life, via accelerometry, to determine whether earlier-life time use is associated with later-life activity levels. Anthropometric and demographic data were collected during medical exams.

Results—Structural decline with age is reduced for the tibia (female: -0.25 SDs/decade; male: 0.05 SDs/decade) versus radius (female: -0.56 SDs/decade; male: -0.20 SDs/decade), which is expected if greater loading mitigates bone loss. Time allocation to horticulture, but not hunting, positively predicts later-life radial status ($\beta_{\text{Horticulture}}$=0.48, p=0.01), whereas tibial status is not significantly predicted by subsistence or sedentary leisure participation.

[*]Corresponding author Contact information: Institute for Advanced Study in Toulouse, 21 allée de Brienne, ME 603.2, 31015 Toulouse Cedex 6, FRANCE, Telephone: +33 06 24 54 30 57, jonathan.stieglitz@iast.fr.

Authors' contributions:

All authors designed the study. JS collected qUS data and MG collected demographic data. JS conducted analyses and wrote the manuscript. All authors commented on and approved the manuscript.

Competing interests:

We have no competing interests.

Discussion—Patterns of activity- and age-related change in bone status indicate localized osteogenic responses to loading, and are generally consistent with the logic of bone functional adaptation. Non-mechanical factors related to subsistence lifestyle moderate the association between activity patterns and bone structure.

Keywords

Bone functional adaptation; mechanical loading; physical activity; Tsimane

Introduction

Modern human osteoporosis susceptibility may result from a postcranial skeleton that is more gracile (i.e. low bone mass for body size) compared to other hominoids and extinct hominins (see Ryan and Shaw [2015] and references therein). Human thoracic vertebrae, which are commonly subject to fragility fracture, show reduced trabecular bone volume fraction and thinner vertebral shells compared to wild-shot apes, with differences emerging in early adulthood (Cotter et al. 2011). Reductions in trabecular bone volume fraction of the metatarsal head (Griffin et al. 2010), calcaneus (Maga et al. 2006), femoral head (Shaw and Ryan 2012) and metacarpal head (Tsegai et al. 2013) have similarly been documented among modern humans compared to other extant apes. While earlier studies document declining postcranial skeletal strength (i.e. ability to withstand an applied load) among *Homo* throughout the Pleistocene (Ruff 2005; Ruff et al. 1993), recent studies of trabecular and cortical bone, particularly in the lower limbs, suggest this decline was limited to the later Pleistocene or Holocene (Chirchir et al. 2015; Ruff et al. 2015; Ryan and Shaw 2015). The leading explanation for modern human skeletal gracility proposes that low levels of physical activity and mechanical loading reduce bone deposition (Cotter et al. 2011; Ruff 2005; Ruff et al. 1993; Ryan and Shaw 2015). As part of a process called "bone functional adaptation" (see Carter and Orr [1992] and references therein), bone responds to demands of physical activity by adding tissue and altering its cross-sectional distribution in the direction of highest bending strains, although this response is not always straightforward (Demes et al. 2001; Lieberman et al. 2004; Lovejoy et al. 2003; Pearson and Lieberman 2004). Higher activity levels in childhood and early adulthood thus result in higher peak bone mass, which then protects against age-related bone loss and risks of osteoporosis and fragility fracture. Fragility fractures indeed appear to be relatively uncommon among older adults in physically active subsistence-level populations (Aspray et al. 1996; Wallace et al. 2014).

Empirical tests of the relationship between human activity patterns and bone structure have been conducted either among modern industrialized populations – often with professional athletes (Haapasalo et al. 1994), in clinical settings or through exercise interventions (Polidoulis et al. 2012) – or using skeletal remains of prehistoric populations (Ruff 1999; Stock and Pfeiffer 2001). In industrialized populations, the combination of energy-rich diets, low pathogen burden, and low fertility with reduced lactation duration and greater birth spacing relaxes energetic constraints on bone growth and maintenance that were likely present over human evolutionary history. On the other hand, excessive sedentism, smoking and alcohol consumption are well-known risk factors for low bone mass and osteoporotic fractures (Kanis 2002), contributing to the idea that osteoporosis and other degenerative

musculoskeletal conditions are "diseases of modernity" (Lieberman 2013). Additional factors associated with modern lifestyles (e.g. glucocorticoid therapy) can obscure the relationship between physical activity and bone structure. Whether low bone mass is prevalent in physically active pre-industrial populations with lower life expectancies and greater nutritional stress, pathogen burden and fertility is an open question. Several recent studies of Tsimane forager-horticulturalists of lowland Bolivia, the population studied here, found reduced indices of calcaneal stiffness among Tsimane compared to more sedentary American matched controls, and more rapid age-related stiffness decline among older adult Tsimane compared to Americans, Chinese, Dutch and Germans (Stieglitz et al. 2015; Stieglitz et al. 2016). Relatedly, analysis of skeletal remains reveals that Australian hunter-gatherers exhibit reduced limb cortical area compared to more sedentary populations (i.e. Khoi-San, Zulu, African American) (Carlson et al. 2007), indicating that increased postcranial thickness is not a hallmark of hunter-gatherers. Together these results are inconsistent with the hypothesis that higher activity levels earlier in life lead to greater peak bone mass and diminished rate of bone loss later in life (all else equal), and suggest that low bone mass characteristic of various degenerative musculoskeletal conditions is not only a disease of modernity (Larsen et al. 2008; Ruff et al. 2015).

Analysis of prehistoric human skeletal remains offers a complementary perspective on the relationship between physical activity and bone structure. Variation in cortical and trabecular morphology has often been used to infer past behaviour (e.g. mobility) of Pleistocene hominins, as bone geometric properties indicate the ability to resist bending and torsion. Many aspects of human skeletal morphology may be constrained by phylogeny or function, but long bone diaphyses are developmentally plastic, changing their relative thickness (robusticity) and shape in response to changes in loading regime (Trinkaus et al. 1994). Population-level comparisons suggest subsistence strategy (e.g. pre-agricultural vs. agricultural), geographical terrain (e.g. mountain vs. coast), and activity level each affect limb structure (Ruff 1999; Ruff et al. 2015; Ryan and Shaw 2015; Stock and Pfeiffer 2001). Variable terrain is more strongly linked to femoral than humeral robusticity and shape (Larsen et al. 2008; Ruff 1999), which is expected because adult human humeri are free from locomotor-induced mechanical loads. Similarly, Shaw and Stock (2013) find high tibial relative to humeral rigidity among Pleistocene hominins compared to early Holocene foragers or modern athletes, perhaps as an adaptation to traversing variable terrain. However, while some studies show less robust limbs among agriculturalists than pre-agriculturalists (May and Ruff 2016; Ruff et al. 1984; Saers et al. 2016), others show no differences by subsistence mode (e.g. Bridges 1989; Ruff 1999), contributing to uncertainty over the precise timing of and major selection pressures underlying the transition to modern human skeletal gracility (Ruff et al. 2015; Scherf et al. 2016). For example, declining subsistence-related mobility and associated declines in skeletal robusticity are widely believed to characterize post-Pleistocene humans, but there is evidence that such declines, particularly in the lower limb, occurred prior to the advent of agriculture, presumably in response to climatic events of the Last Glacial Maximum (Holt 2003; Shackelford 2007). Evidence for a relationship between subsistence involvement and bone structure in an extant pre-industrial population can help reconcile these mixed findings and improve the ability to infer mobility patterns from analyses of prehistoric skeletal remains.

Despite providing fundamental insights into the association between activity patterns and bone structure, prehistoric human skeletal analysis is limited in several ways. First, skeletal samples often span hundreds or thousands of years (Larsen et al. 2008; Ruff 1999), during which climate, topography, diet and other lifestyle factors known to affect bone structure (e.g. disease ecology) likely changed, and thus can confound effects of activity and loading. Second, bio-archaeological studies often dichotomize subsistence strategies (e.g. hunter-gatherer vs. agricultural) even though mixed strategies are often utilized within pre-industrial societies. Third, bio-archaeological studies often rely on imprecise age estimates, and, fourth, lack direct measures of physical activity and anthropometric status. Fat mass, for example, can affect bone structure independently of loading patterns (e.g. via aromatization of androgens to estrogen) (Ho-Pham et al. 2014) and may be more strongly associated with bone structure for women than men (Stieglitz et al. 2016). Fifth, multiple skeletal sites are rarely measured for the same individual, and thus individuals cannot act as their own controls, increasing the likelihood that factors other than activity patterns influence bone structure. *In vivo* studies in contemporary pre-industrial populations can overcome these limitations to advance our understanding of how activity patterns influence bone structure. Such studies are critical to understanding whether modern human skeletal gracility and associated degenerative musculoskeletal conditions are outcomes of an evolutionary mismatch between long-standing adaptations and relatively novel sedentary lifestyles.

Study goals

Here we use quantitative ultrasonography (qUS) to assess age- and activity-related differences in radial and tibial status among Tsimane forager-horticulturalists. We test whether greater activity levels and, thus, increased loading earlier in life are associated with greater later-life bone status and diminished age-related bone loss. This hypothesis predicts that age-related structural decline is greater for the radius than tibia due to habitually greater loading of the tibia (**P1**), and that more time spent in physically intensive subsistence activities earlier in life is associated with greater later-life bone status (**P2**). We examine the effect of earlier-life time allocation to different subsistence and leisure activities (e.g. hunting, horticulture, socializing) on later-life bone status. Comparison of effects of different subsistence and leisure activities informs current evolutionary debates about the relative impact of hunting, farming and sedentism on bone structural variation. We also examine whether earlier-life time allocation is correlated with later-life activity patterns; if there is no correlation, then any association between earlier-life time allocation and later-life bone status is likely due to earlier rather than later-life habitual activities or recent changes in activity.

Moreover, we test whether associations predicted by P2 are stronger for the radius versus tibia (**P3**), since the tibia is more consistently and highly loaded and therefore less determined by variability in activity level and type. P3 contrasts with an alternative prediction that activity-related changes in skeletal robusticity are more marked in the lower versus upper limb because of effects of mobility on lower limb loading (Ruff et al. 2015). Osteogenic responses in the radius should be especially strong for common subsistence activities entailing frequent and intense upper limb loading (Stock and Pfeiffer 2001). For example, Tsimane horticultural field clearance and maintenance, which are routinely

performed by both sexes, involves regular use of machetes that are swung while leading with the elbow and alternating arms when fatigued. Finally, we examine age and sex as moderators of the relationship between activity patterns and bone status. Osteogenic responses to activity may be greater among children and young adults that have not yet attained peak bone mass, and among women due to their lower body and peak bone mass, maternal depletion of mineral reserves from high fertility and prolonged on-demand breastfeeding (Stieglitz et al. 2015), and declining sex steroid hormone concentrations accompanying menopause.

Materials and methods

Study population

The Tsimane of lowland Bolivia are semi-sedentary forager-horticulturalists living in >90 villages, nearly all of which lack running water and electricity. Tsimane have relatively short life expectancy (from 1990-2002, life expectancy at birth, age 15, and age 45: e_0=55 years, e_{15}=53, and e_{45}=29, respectively) (Gurven et al. 2007). Their diet consists of cultigens grown in small swiddens (66% of calories; mostly rice, plantains, sweet manioc and corn), lean meat from hunting (17%), freshwater fish (7%), and fruits and nuts gathered from the forest (6%) (Martin et al. 2012). Calcium intake is low (~320 mg/day, unpublished data) relative to recommendations established by the US Institute of Medicine of the National Academies (Ross et al. 2011), and energetic limitation is apparent from short adult stature relative to World Health Organization growth reference data from the US (Blackwell et al. In press; also see Table 1). Tsimane display high physical activity levels (PALs) typical of other subsistence populations (Gurven et al. 2013). Women's PAL is in the "moderate to active" range (PAL=1.73–1.85) and does not change throughout adulthood. Men's PAL is considered "vigorously active" (PAL=2.02-2.15), and declines by 10-20% from the peak (achieved in the late 20s) to older adulthood (age 60+). PALs vary by season (see ESM for additional details), which can be broadly categorized into "wet" (November to April) and "dry" (May to October).

Participants

Nine hundred and twenty-two adults aged 20+ years (mean±SD age=48.8±15.0; maximum=85; 52% female) from 65 villages participated between October 2014 and October 2015 during routine screening as part of the Tsimane Health and Life History Project (THLHP; see ESM). Protocols were approved by the institutional review boards of the University of New Mexico and University of California-Santa Barbara. Informed consent occurred at three levels: (1) Tsimane government that oversees research projects, (2) village leadership, and (3) study participants.

Quantitative ultrasonography (qUS) of the radius and tibia

qUS has been validated against gold standard methods: several prospective studies demonstrate that qUS predicts fragility fracture as well as or better than dual-energy X-ray absorptiometry (DXA)-derived BMD (Bauer et al. 1997; Hans et al. 1996; Khaw et al. 2004) and that this association is independent of BMD (Olszynski et al. 2013). DXA tests are preferred before administering osteoporosis treatment, but qUS is commonly used in remote

settings lacking DXA access. qUS has several advantages over DXA: lower cost, greater portability, ease of use and lack of ionizing radiation. Besides these advantages, qUS measures bone properties that X-ray-based methods do not (Njeh et al. 1999). Both methods measure attenuation of energy (mechanical wave or ionizing electromagnetic radiation) within tissue, but the interaction with tissue varies greatly across methods. X-ray attenuation depends on elementary composition (effective atomic number) and physical density of measured tissue, but not as much on structural details nor material properties, while ultrasound attenuation depends on material properties, architecture, anatomic composition (e.g. cortical to trabecular ratio), and size of tissue (Njeh et al. 1997).

While most qUS devices are limited to measuring only one skeletal site (typically the calcaneus), the Sunlight MiniOmni ultrasound (BeamMed Ltd., Petah Tikva, Israel) is designed to measure multiple sites. The MiniOmni – used here to assess radial and tibial status – measures acoustic wave velocity along and under the cortex. When a thin cortical shell is present in reference to the wavelength, then the ultrasound waves also travel in the trabecular bone layer immediately under the cortical shell. The velocity or speed of sound (SOS, meters/second [m/s]), which is calculated by dividing propagation distance by transit time (Njeh et al. 1997), is related to cortical thickness, density and porosity (Sievanen et al. 2001). Njeh et al. (1999) observed that when cortical thickness is greater than the ultrasound wavelength, SOS is independent of thickness, but when thickness is smaller than the wavelength, SOS is dependent on thickness. SOS declines rapidly at thicknesses below 3 - 4 mm, with the deflection point depending on bone shape: in the case of circular bone (e.g. the radial wall), SOS decline occurs at thicker wall than is the case with a plate-like structure or plane surface (e.g. the tibial wall). SOS values may therefore reflect either wholly cortical bone, or a composite of cortical and trabecular bone (Njeh et al. 1999). Cortical thickness and the ratio of trabecular to cortical bone vary in the radius and tibia, and this will be reflected in the SOS values (e.g. Sievanen et al. 2001). The MiniOmni uses a set of transducers to measure SOS along a fixed distance of bone, parallel to its long axis. The system has a transducer arrangement designed to eliminate effects of underlying soft tissue on wave velocity.

Additional qUS details (e.g. on probe placement [see Figure S1], measurement and precision) can be found in the ESM.

Demographics, anthropometrics and behavioral observation

Birth years were assigned based on a combination of methods described elsewhere (see Gurven et al. [2007] and ESM).

During medical exams conducted by the Tsimane Health and Life History Project (THLHP), height and weight were measured using a Seca Road Rod 214 stadiometer and Tanita scale (BF680). The scale also recorded body fat percentage by bioelectric impedance, which was used to calculate fat-free mass based on proprietary prediction equations using age, sex, height and weight (see ESM). Correlations between Tanita-based and isotope dilution methods for fat-free mass and weight are 0.91 and 0.91, respectively (p's<0.0001) (Gurven et al. 2016).

Behavioural observation data were collected during instantaneous scans conducted every 30 minutes in 2- or 3-hour blocks from 7am to 7pm throughout the year from July 2002-November 2005. Behavioural data exist for 116 individuals who also received qUS screening (49% female, n=6 villages) totalling 10,267 scans (3,743 scans for 41 individuals 35 years old; 6,524 scans for 75 adults >35 years old; mean±SD number of scans/person=89±28; mean±SD time lag between behavioural observation and ultrasound=12±1 years). Activities are grouped into selected work (i.e. food acquisition [e.g. horticulture, hunting] and domestic activities [e.g. food processing]) and sedentary leisure (i.e. personal [e.g. eating], social [e.g. talking] and idle activities [e.g. sitting]). Given a wide age range that includes pre-reproductive individuals (mean±SD age at onset of behavioural observation=38.4±15.9 [range: 8-76]), time allocation to childcare is not analysed. Percentage of time individual i spent in activity j is the percentage of all of i's person-scans during which i engaged in j. If two activities were performed simultaneously, both were recorded and given equal weighting. The risk set omits from the numerator and denominator behavioural scans in which individuals are coded as accompanying others and thus not directly observed.

For a subset of 32 participants contributing behavioural data, physical activity later in life was measured via accelerometry counts using an Actigraph wGT3X accelerometer (Actigraph LLC, Pensacola, FL), to determine whether earlier-life time allocation is associated with later-life activity levels (mean±SD years after behavioural observation=10.4±1.7, min=8, max=13; mean±SD years before qUS=1.2±0.7, min=0, max=2) (see Gurven et al. [2013] and ESM for additional details regarding the accelerometry method).

Data analysis

For descriptive analyses, Mann-Whitney U and Wilcoxon signed rank tests were used to compare study variables within and across sexes. Generalized additive models (GAMs; see Wood [2006]) were used to examine age profiles of radial and tibial SOS due to their non-linearity with age. Models were fit with the *gam* procedure in R (package *mgcv*) using thin plate regression splines. Smoothing parameters were generated automatically according to *gam* defaults. Models were fitted separately for each sex and include an intercept and a spline for age. To better isolate age trajectories of bone status, we used segmented linear regression (package *segmented*) to estimate: i) age of peak status (or breakpoint if age-related decline was not apparent), ii) the slope of increase before peak status or breakpoint, and iii) the slope after peak status or breakpoint to test P1. Segmented linear regression permits identification of transition points that may otherwise be obscured using nonlinear functions.

Radial and tibial SOS are reported as either raw values or z-scores (see Figure S2 for z-score distributions by sex and age). Weighted least-squares regressions were used to model the association between earlier-life time allocation and later-life bone status (P2). Time allocation data are not normally distributed, so to compare individuals by activity patterns we created a binary variable indicating membership in the upper quartile (sex-specific) of time spent in a given task (see ESM). Models are weighted by number of instantaneous

scans per person and control for age (including age^2 if p 0.1), sex, sex*age^2, and time lag (in years) between behavioural observation and qUS measurement. Height and weight at the time of qUS are each associated with tibial but not radial SOS in the combined sample of men and women (see ESM) and are therefore also included as controls in models of tibial SOS. Multivariate analyses of covariance (MANCOVA) models, similarly weighted by number of scans per person and using the same controls to test P2, are used to test P3. To test P2 and P3, p-values are not adjusted using a Bonferroni or other correction because tests are not independent (i.e. time allocation to work and leisure negatively co-vary, see ESM), and there is no standard correction involving dependent p-values. Participants with missing SOS values (missing completely at random) were removed from analyses.

Results

Bone status descriptives by sex, age and anthropometrics

Descriptive statistics for study variables are presented by sex in Table 1, and radial and tibial SOS reference values by sex and age in Table S1. Within sexes, radial significantly exceeds tibial SOS from ages 20-59 (by 2.3% for men, 3.0% for women). After age 60 there is no significant SOS difference between bones for men, whereas for the oldest women aged 70+ tibial exceeds radial SOS by 2.3%. Radial and tibial SOS are higher for men than women (p's<0.001), but this difference is not apparent until the 50s (there is no significant sex difference before the 50s, despite greater activity levels among men). Among adults aged 50+ radial and tibial SOS is 3.4% and 2.8% higher, respectively, for men than women. These sex differences are smaller than the sex differences in height (6.9%), weight (15.3%), upper limb length (8.8%) and lower limb length (8.0%), and remain significant controlling for these anthropometrics and age (see ESM for additional details, including Table S2).

Is age-related SOS decline greater for the radius versus tibia (P1)? Yes

Male radial SOS increases until the late 30s (peak=39 years) before declining, whereas male tibial SOS increases into the 60s without exhibiting subsequent decline (Figure 1A). Female radial SOS also increases until the late 30s (with an identical peak of 39 years, Figure 1B) but subsequently exhibits accelerated age-related decline compared to males. Unlike males, female tibial SOS increases until the mid-40s (peak=43 years) before declining; this decline occurs at a lower rate than female radial SOS.

Based on segmented linear regression (Table 2; Figure S3), men's radial SOS peaks at age 35 (four years earlier than predicted from the GAM shown in Figure 1A) and declines linearly thereafter (-0.02 SDs/year, adj. R^2=0.10), whereas male tibial SOS exhibits a breakpoint at age 34 without subsequent decline (0.005 SDs/year, adj. R^2=0.05) as also shown in Figure 1A. Comparison of men's radial versus tibial SOS age slopes reveals a significant difference for post- but not pre-breakpoint slopes; while positive, the post-breakpoint slope for the tibia is not significantly greater than zero (Table 2). Women's radial SOS peaks at age 40 (one year later than predicted from the GAM shown in Figure 1B), and tibial SOS peaks at age 41 (two years earlier than predicted from the GAM). Unlike men, both bones for women exhibit significant age-related decline (radius: -0.056 SDs/year, adj. R^2=0.42; tibia: -0.025 SDs/year, adj. R^2=0.09). Like men, comparison of women's radial

versus tibial SOS age slopes reveals a significant difference for post- but not pre-breakpoint slopes.

Is greater time allocation to physically intensive subsistence activities earlier in life associated with greater later-life bone status (P2)? Partially

Weighted least-squares regressions of bone status on time allocation to work and leisure activities are presented in Table S3 (also see Figure 2, and Table S4 for time allocation sample descriptives). For work activities, horticultural time allocation is the only significant predictor of radial SOS ($\beta_{\text{Horticulture}}$=0.483, 95% CI: 0.112-0.855, p=0.01, controlling for age, age^2, sex, sex*age^2 and time lag). This effect size represents the equivalent effect of aging, post-breakpoint, 23 years for men or 9 years for women (Table 2). Because horticultural time allocation is not associated with time spent in any other work activity (Table S5), the positive association between radial SOS and horticultural time allocation is not likely due to involvement in other subsistence tasks. Time spent idle (see Table S3 for task descriptions) is the only type of sedentary leisure that significantly predicts radial SOS in the predicted negative direction (β_{Idle}= -0.333, 95% CI: -0.642 - -0.025, p=0.034, same controls). This effect size represents the equivalent effect of aging, post-breakpoint, 16 years for men or 6 years for women. In contrast, tibial SOS is not predicted by time allocation to any work or leisure activity.

We find no significant interaction effect of age and time allocation to any work or leisure activity on radial or tibial SOS (not shown), suggesting that osteogenic responses to physical activity are not specific to younger individuals that have not yet attained peak bone mass. To test for these interactions, age at onset of behavioural observation was included as either a continuous variable, or as binary indicating membership in the pre- or post-breakpoint age category specific to each sex and bone (Table 2; Figure S3).

We also find no significant interaction effect of sex and time allocation to any work activity on radial or tibial SOS. However, the negative association between time spent socializing and SOS is stronger for women than men (radius: interaction p=0.021, controlling for age, age^2, sex*age^2 and time lag; tibia: interaction p=0.089, controlling for age, sex*age, height, sex*height, weight and time lag, see Figure S4). The negative association between idle time allocation and radial but not tibial SOS is also stronger for women than men (radius: interaction p=0.057; tibia: interaction p=0.125).

Are predicted associations from P2 stronger for the radius versus tibia (P3)? Yes

MANCOVA models confirm that earlier-life time allocation is more strongly associated with later-life radial versus tibial SOS, as suggested but not formally tested in the previous section (Table S6). MANCOVA models similarly show that radial SOS is significantly predicted by time allocation to horticulture and idling, whereas tibial SOS is not predicted by time allocation to any work or leisure activity.

Is earlier-life time allocation associated with later-life activity levels? No

Earlier-life time allocation is not associated with later-life physical activity energy expenditure (PAEE, either 12-hour daytime, 12-hour non-daytime, or 24-hour) as assessed

by accelerometry (Table S7). Despite a small sample size, the positive effect of earlier-life horticultural time allocation on later-life radial SOS strengthens after controlling for later-life 24-hour PAEE ($\beta_{\text{Horticulture}}$=1.103, 95% CI: 0.538-1.667, p<0.001, also controlling for age, age^2, sex, sex*age^2 and time lag, n=32) (see ESM for additional details). PAEE is not significantly associated with radial or tibial SOS (not shown), so any association between earlier-life time allocation and later-life bone status is not likely due to later-life habitual activities, or recent (<3 years) changes in activity.

Discussion

This study used quantitative ultrasonography in a contemporary pre-industrial population to test whether greater activity level and, thus, increased mechanical loading earlier in life is associated with greater later-life bone status and diminished age-related bone loss. To test predictions, we measured multiple bones varying in loading and examined how bone status co-varies with recent and past activity patterns, age and anthropometrics. Consistent with the logic of bone functional adaptation and P1, we found that age-related structural decline is mitigated for the tibia versus radius for both sexes (Table 2; Figure 1 and S3). Following a peak in the mid to late 30s, male radial SOS declines by 0.2 SDs/decade (0.5% of the adult mean), whereas male tibial SOS exhibits no significant age-related decline. This latter result is striking and to our knowledge represents the only documented case in which an indicator of human bone strength *in vivo* shows no age-related decline in adulthood. While we lack the ability to completely rule out a cohort effect underlying this result given the cross-sectional study design, we observed no such age pattern for women (radius or tibia), nor for men's radial SOS, which declines with age as in other qUS studies using Sunlight technology (Huang et al. 2015; Olszynski et al. 2014). Moreover, Tsimane activity levels do not tend to vary by geographic proximity to the market town of San Borja (Gurven et al. 2013); it is therefore unlikely that cohort differences in activity levels associated with "modernization" account for the maintenance of male tibial SOS – an indicator of cortical thickness, density and porosity (Sievanen et al. 2001) – in older adulthood.

Compared to men, women's radial and tibial SOS exhibits greater age-related decline following their respective peaks in the late 30s and early 40s. Women's SOS undergoes accelerated declines around the age at menopause, presumably due to associated changes in mineral regulating hormones (e.g. estrogen, progesterone). Steeper age-related declines in women's bone strength appears to be a species-typical pattern (Khosla 2013; Stieglitz et al. 2016), although whether women in contemporary pre-industrial populations present different cross-sectional bone geometric properties and increased fragility fracture risk remains unexplored. Consistent with P1 we found that women's radial SOS declines by 0.56 SDs/ decade (2.5%), which is greater than their tibial SOS decline of 0.25 SDs/decade (0.9%). A study of age-matched Chinese women also using the MiniOmni ultrasound found a slightly greater decline with age for radial SOS compared to Tsimane women (Huang et al. 2015), and studies of more sedentary Londoners, Israelis and North Americans similarly found greater decline with age for tibial SOS (Drake et al. 2001; Knapp et al. 2001; Weiss et al. 2000). These population-level differences are expected if greater earlier-life activity levels and loading result in diminished later-life bone loss (all else equal). Tsimane women's age-related decline in radial SOS exceeds decline in calcaneal SOS over a similar age range

(unpublished data, but see Stieglitz et al. [2016] for a calcaneal SOS age profile from ages 50-85), which is also expected given greater loading of the calcaneus. Yet women's age-related decline in calcaneal SOS exceeds decline in tibial SOS; the highly trabecular calcaneal structure makes it more porous, metabolically active and presumably prone to bone loss than the cortical structure of the midshaft tibia.

Broadly speaking, our finding that age-related structural decline is mitigated for the tibia versus radius is consistent with bio-archaeological studies showing associations between rugged geographical terrain and greater femoral but not humeral robusticity (Larsen et al. 2008; Ruff 1999). Both sets of findings suggest localized osteogenic responses to loading rather than systemic skeletal responses (e.g. related to diet or pathogen burden). Similarly, among baboons, Havill et al. (2008) show that low bone mass is site-specific (with a higher prevalence at the radius than thoraco-lumbar vertebrae), and that there is a higher correspondence in low bone mass prevalence between bones of the same anatomical region than of the same bone type in different regions. Chimpanzees who, like humans, experience age-related BMD decline and manifestations of skeletal pathology unrelated to traumatic injury at older ages – albeit at lower frequencies and intensities (Carlson et al. 2008; Carter et al. 2008; Gunji et al. 2003) – similarly show greater age-related bone loss in upper compared to lower limb diaphyses, despite their habitual quadrupedality (Morbeck et al. 2002).

We find partial support for P2, as horticultural time allocation earlier in life is the only work activity positively predicting later-life radial SOS. Some bio-archaeological studies in North America similarly show an increase in upper limb strength corresponding to an increase in nutritional reliance on native seed crops from ~50 BC –1050 AD, particularly for women, who may have been primarily responsible for growing and processing these crops (see Bridges et al. [2000] and references therein). While horticultural work entails frequent upper limb loading (e.g. machete swinging, tree chopping, carrying fallen tree trunks), strain amplitude and rate associated with such activities remain unclear as most human kinesiological studies focus on lower limb loading and bio-mechanical adaptations to bipedality. Tsimane staple crops also require substantial processing (e.g. grinding, mashing, pounding, degraining, scraping, cutting), often with tools (e.g. large stones, mortar and pestle), thus further contributing to upper limb loading, although we find no significant association between food processing time and later-life radial SOS (Figure 2; Table S3).

The diversity and dynamics of behaviour in subsistence economies likely play a major role in shaping human lower and upper limb morphology (Stock and Pfeiffer 2001), yet much research on the relationship between known activity patterns and bone structure is conducted among competitive athletes. Athletes engaging in sports producing high peak strains (e.g. weightlifting, gymnastics) or unusual and varied patterns of movement (e.g. squash, cricket) – which also characterizes machete swinging, tree felling and other horticultural tasks – show increased diaphyseal bone mass and BMD at various skeletal sites compared to either sedentary matched controls or athletes engaging in endurance sports (e.g. long-distance running) (Heinonen et al. 1995; Shaw and Stock 2009). Our study represents the first to identify specific subsistence activities contributing to the maintenance of bone structure in a contemporary pre-industrial population. Given that adult bone strength is hypothesized to

reflect skeletal loads incurred earlier in life, our study provides a more direct test than bio-archaeological studies lacking direct behavioural measures, or kinematic studies of modern athletes, clinical samples or exercise interventions that do not generalize to pre-industrial populations whose lifeways are more representative of human evolutionary history. Yet despite their physically active lifestyle generally free of processed foods, their high fertility and lack of public health infrastructure, Tsimane are not pure hunter-gatherers and may differ in important ways from ancestral human populations in terms of residential mobility, diet and disease exposures. Nevertheless, no single population represents the range of experiences across different environments that shaped the evolution of our species over the millennia in which global climates and ecologies fluctuated.

Greater time spent hunting earlier in life does not predict Tsimane men's later-life bone status (Figure 2; Table S3), which is puzzling given the widely held view that greater activity levels and mechanical loading associated with hunting are responsible for the increased bone mass and strength characteristic of hunter-gatherers relative to more sedentary agriculturalists (Ruff 1999). Prior comparisons of adult skeletal remains of hunter-gatherers and either full- or part-time agriculturalists indeed reveal accelerated age-related decline in radial bone mineral content (Perzigian 1973) and reduced femoral strength among agriculturalists (Ruff et al. 1984; Ryan and Shaw 2015). While behavioural and morphological changes associated with the shift from hunting and gathering to agriculture were spatially and temporally heterogeneous (Bridges 1989), hunter-gatherers consistently show greater sexual dimorphism in femoral rigidity than agriculturalists (Larsen et al. 2008; Ruff 1999), presumably due to a more rigid sexual division of labour. Our finding that Tsimane men's tibial SOS exhibits no age-related decline may be linked to men's hunting effort, which amounts to 36 hours per month on average (Gurven et al. 2012), and often involves use of hunting dogs and either low calibre firearms or bow and arrow. Despite reasonably consistent findings regarding the association between activity patterns and bone strength, it is possible that activity-related strain patterns impact bone structure in other ways (Demes et al. 1998; Lieberman et al. 2004; Pearson and Lieberman 2004). Experimental evidence from avian models shows that only a few high magnitude strain cycles suffice to induce cortical modeling (Rubin and Lanyon 1984), with little additional gain beyond a threshold. Accordingly, repetition of low magnitude strains involved in endurance activities common to hunting (e.g. walking) may not necessarily result in significant diaphyseal strength gains compared to higher intensity activities (Frost 1997). If this view is correct, then increased mobility may not consistently result in stronger limb bones, and the common view that modern human skeletal gracility was caused by transition form a mobile hunting and gathering lifestyle toward more sedentary agriculture would need refinement.

Theoretical and experimental evidence indicates greater selective pressure on tissue economy in distal versus proximal limb segments, where there is a greater energetic trade-off between bone strength and mass (see Saers et al. [2016] and references therein). Therefore, not all regions of limb bone diaphyses respond identically to loading. This logic may explain why, in the bio-archaeological record, group-level differences (e.g. mobile foragers vs. sedentary agriculturalists) in bone structural properties are less pronounced more distally along the lower limb, and why we find no association between men's time allocation to hunting and later-life tibial SOS. Our relatively modest sample size of 59 men

also lowers statistical power to detect an association. Nevertheless, we find that variability in tibial status is not explained by variability in activity patterns. An implication of this finding is that failure to reveal group-level differences in tibial morphology in prehistoric skeletal remains does not by itself justify the conclusion that group-level activity patterns were minimal or non-existent (Trinkaus and Ruff 1999). The presumed association between lower limb morphology and mobility has shaped inferences of major trends throughout human evolution (Ruff et al. 1993), but the strength of those inferences ultimately depends on our ability to correlate known patterns of behaviour with bone structure.

Energetic trade-offs between somatic growth, repair and reproduction also likely affect the magnitude and rate of bone modelling and remodelling. Tsimane skeletal gracility relative to matched U.S. controls (Stieglitz et al. 2015) and diminished osteogenic responses to high activity levels (e.g. from hunting) may be due to several aspects of lifestyle that were likely common over human evolutionary history, including energetic limitation (e.g. high energy expenditure relative to consumption, micro-nutrient deficiency), high fertility and short birth spacing, and high pathogen burden (Stieglitz et al. 2016). Mobilization of skeletal mineral stores (e.g. calcium, phosphorus, magnesium) may be an adaptive physiological response to support increased mineral demands of pregnancy, lactation and acute inflammatory responses to infection (Madimenos et al. 2012; Stieglitz et al. 2015; Stieglitz et al. 2016; Straub et al. 2015). Using similar logic, the higher fertility and pathogen burden of full-time agriculturalists relative to hunter-gatherers may explain the former's decreased skeletal robusticity independently of changes in physical activity or diet, although the relative contribution of each factor remains unclear (Nelson et al. 2002). In contemporary Western populations, however, low skeletal mass characteristic of osteoporosis is more likely a result of physical inactivity rather than the combination of high fertility, pathogen burden and energetic limitation.

Consistent with P3, we found that activity-related effects are stronger for the radius versus tibia (Table S3 and S6; Figure 2), particularly for horticultural activities presumably entailing frequent and intense upper limb loading. We also found that the negative association between sedentary leisure activities (socializing, idling) and bone status, particularly the radius, is stronger for women than men (Figure S4). These sedentary activities are compatible with providing high quality childcare (e.g. on-demand breastfeeding) and may further contribute to bone loss together with extended lactation, given increased maternal mineral requirements (Stieglitz et al. 2015). However, in our sample, reproductive-aged women's time spent parenting was not associated with their time spent socializing or idling (not shown), suggesting that the negative association between time allocation to sedentary activities and bone status for women is not directly linked to maternal time investment.

Conclusion

In a physically active pre-industrial population with minimal exposure to osteoporosis risk factors found in industrialized populations, we found partial support for the hypothesis that greater activity levels and, thus, increased mechanical loading earlier in life lead to greater later-life bone status and diminished age-related bone loss. Energetic constraints on bone

growth and maintenance (e.g. due to low energy balance, greater immune activation from high pathogen exposure, and/or high fertility with prolonged on-demand breastfeeding and short birth spacing), which were likely prevalent over human evolutionary history, may limit osteogenic responses to high activity levels, particularly for women given greater direct energetic investments in reproduction. While reduced bone mass characteristic of various musculoskeletal diseases may have been a common response among physically active ancestral humans to pronounced and habitual mechanical stresses (Bridges 1992; Larsen et al. 2008; Stieglitz et al. 2016), reduced bone mass per se may not increase risk of fragility fracture if bone maintains favourable geometric properties promoting structural resistance to loading (Lazenby 1990). The qUS technique used here to assess bone status does not directly measure bone geometric properties and does not incorporate the full extent of variation in bone strength (Wachter et al. 2002). Therefore, comparisons of qUS bone status with more traditional cross-sectional bone properties may not be equivalent, and these methodological differences can contribute to variation in findings across studies. Future studies that standardize methods (e.g. bone measurement techniques, skeletal sites measured) are needed for valid population-level comparisons of bone properties. Future research among contemporary pre-industrial populations utilizing technological advances (e.g. in peripheral quantitative computed tomography) to explore how cross-sectional bone geometry changes with activity patterns over the life course is likely to reveal novel insights.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Literature Cited

Aspray TJ, Prentice A, Cole TJ, Sawo Y, Reeve J, Francis RM. Low bone mineral content is common but osteoporotic fractures are rare in elderly rural Gambian women. J Bone Miner Res. 1996; 11(7): 1019–1025. [PubMed: 8797124]

Bauer DC, Gluer CC, Cauley JA, Vogt TM, Ensrud KE, Genant HK, Black DM. Broadband ultrasound attenuation predicts fractures strongly and independently of densitometry in older women. A prospective study Study of Osteoporotic Fractures Research Group. Arch Intern Med. 1997; 157(6): 629–634. [PubMed: 9080917]

Blackwell A, Urlacher S, Beheim B, Von Rueden C, Jaeggi A, Stieglitz J, Trumble B, Gurven M, Kaplan H. Growth references for Tsimane forager-horticulturalists of the Bolivian Amazon. American Journal of Physical Anthropology. In press.

Bridges PS. Changes in activities with the shift to agriculture in the southeastern United States. Current Anthropology. 1989; 30(3):385–394.

Bridges PS. Prehistoric arthritis in the Americas. Annual Review of Anthropology. 1992; 21:67–91.

Bridges PS, Blitz JH, Solano MC. Changes in long bone diaphyseal strength with horticultural intensification in west-central Illinois. American Journal of Physical Anthropology. 2000; 112(2): 217–238. [PubMed: 10813704]

Carlson K, Sumner D, Morbeck M, Nishida T, Yamanaka A, Boesch C. Role of nonbehavioral factors in adjusting long bone diaphyseal structure in free-ranging Pan troglodytes. International journal of primatology. 2008; 29(6):1401–1420. [PubMed: 19816545]

Carlson KJ, Grine FE, Pearson OM. Robusticity and sexual dimorphism in the postcranium of modern hunter- gatherers from Australia. American journal of physical anthropology. 2007; 134(1):9–23. [PubMed: 17503447]

Carter DR, Orr TE. Skeletal development and bone functional adaptation. Journal of Bone and Mineral Research. 1992; 7(S2):S389–S395. [PubMed: 1485546]

Carter ML, Pontzer H, Wrangham RW, Peterhans JK. Skeletal pathology in Pan troglodytes schweinfurthii in Kibale National Park, Uganda. American Journal of Physical Anthropology. 2008; 135(4):389–403. [PubMed: 18067120]

Chirchir H, Kivell TL, Ruff CB, Hublin J-J, Carlson KJ, Zipfel B, Richmond BG. Recent origin of low trabecular bone density in modern humans. Proceedings of the National Academy of Sciences. 2015; 112(2):366–371.

Cotter MM, Loomis DA, Simpson SW, Latimer B, Hernandez CJ. Human Evolution and Osteoporosis-Related Spinal Fractures. PLoS ONE. 2011; 6(10):e26658. [PubMed: 22028933]

Demes B, Qin YX, Stern JT, Larson SG, Rubin CT. Patterns of strain in the macaque tibia during functional activity. American journal of physical anthropology. 2001; 116(4):257–265. [PubMed: 11745077]

Demes B, Stern JT Jr, Hausman MR, Larson SG, McLeod KJ, Rubin CT. Patterns of strain in the macaque ulna during functional activity. Am J Phys Anthropol. 1998; 106(1):87–100. [PubMed: 9590526]

Drake WM, McClung M, Njeh CF, Genant HK, Rosen C, Watts N, Kendler DL. Multisite bone ultrasound measurement on North American female reference population. Journal of Clinical Densitometry. 2001; 4(3):239–248. [PubMed: 11740066]

Frost HM. Why do marathon runners have less bone than weight lifters? A vital-biomechanical view and explanation. Bone. 1997; 20(3):183–189. [PubMed: 9071467]

Griffin NL, D'Aout K, Ryan TM, Richmond BG, Ketcham RA, Postnov A. Comparative forefoot trabecular bone architecture in extant hominids. J Hum Evol. 2010; 59(2):202–213. [PubMed: 20655571]

Gunji H, Hosaka K, Huffman MA, Kawanaka K, Matsumoto-Oda A, Hamada Y, Nishida T. Extraordinarily low bone mineral density in an old female chimpanzee (Pan troglodytes schweinfurthii) from the Mahale Mountains National Park. Primates. 2003; 44(2):145–149. [PubMed: 12687478]

Gurven M, Jaeggi AV, Kaplan H, Cummings D. Physical Activity and Modernization among Bolivian Amerindians. Plos One. 2013; 8(1):e55679. [PubMed: 23383262]

Gurven M, Kaplan H, Zelada Supa A. Mortality experience of Tsimane Amerindians of Bolivia: Regional variation and temporal trends. American Journal of Human Biology. 2007; 19:376–398. [PubMed: 17421012]

Gurven M, Stieglitz J, Hooper PL, Gomes C, Kaplan H. From the womb to the tomb: The role of transfers in shaping the evolved human life history. Experimental Gerontology. 2012; 47:807–813. [PubMed: 22595699]

Gurven MD, Trumble BC, Stieglitz J, Yetish G, Cummings D, Blackwell AD, Beheim B, Kaplan HS, Pontzer H. High resting metabolic rate among Amazonian forager-horticulturalists experiencing high pathogen burden. American Journal of Physical Anthropology. 2016

Haapasalo H, Kannus P, Sievanen H, Heinonen A, Oja P, Vuori I. Long-term unilateral loading and bone mineral density and content in female squash players. Calcif Tissue Int. 1994; 54(4):249–255. [PubMed: 8062139]

Hans D, Dargent-Molina P, Schott AM, Sebert JL, Cormier C, Kotzki PO, Delmas PD, Pouilles JM, Breart G, Meunier PJ. Ultrasonographic heel measurements to predict hip fracture in elderly women: the EPIDOS prospective study. Lancet. 1996; 348(9026):511–514. [PubMed: 8757153]

Havill L, Levine S, Newman D, Mahaney M. Osteopenia and osteoporosis in adult baboons (Papio hamadryas). Journal of medical primatology. 2008; 37(3):146–153. [PubMed: 18642436]

Heinonen A, Oja P, Kannus P, Sievanen H, Haapasalo H, Manttari A, Vuori I. Bone mineral density in female athletes representing sports with different loading characteristics of the skeleton. Bone. 1995; 17(3):197–203. [PubMed: 8541131]

Ho-Pham LT, Nguyen UD, Nguyen TV. Association between lean mass, fat mass, and bone mineral density: a meta-analysis. J Clin Endocrinol Metab. 2014; 99(1):30–38. [PubMed: 24384013]

Holt BM. Mobility in Upper Paleolithic and Mesolithic Europe: evidence from the lower limb. Am J Phys Anthropol. 2003; 122(3):200–215. [PubMed: 14533179]

Huang L, Pan J, Jin H, Xiao L, Tao J, Tong P. Characteristics of distal radius speed of sound data in Chinese Mainland men and women. Journal of Clinical Densitometry. 2015; 18(2):245–251. [PubMed: 24951371]

Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. Lancet. 2002; 359(9321):1929–1936. [PubMed: 12057569]

Khaw KT, Reeve J, Luben R, Bingham S, Welch A, Wareham N, Oakes S, Day N. Prediction of total and hip fracture risk in men and women by quantitative ultrasound of the calcaneus: EPIC-Norfolk prospective population study. Lancet. 2004; 363(9404):197–202. [PubMed: 14738792]

Khosla S. Pathogenesis of age-related bone loss in humans. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2013; 68(10):1226–1235.

Knapp K, Blake G, Spector T, Fogelman I. Multisite quantitative ultrasound: precision, age-and menopause-related changes, fracture discrimination, and T-score equivalence with dual-energy X-ray absorptiometry. Osteoporosis International. 2001; 12(6):456–464. [PubMed: 11446561]

Larsen, CS., Kelly, RL., Ruff, CB., Schoeninger, MJ., Hutchinson, DL., Hemphill, BE. Living on the Margins: Biobehavioral Adaptations in the Western Great Basin. In: Reitz, EJ.Scudder, SJ., Scarry, CM., editors. Case Studies in Environmental Archaeology. New York, NY: Springer New York; 2008. p. 161-189.

Lazenby RA. Continuing periosteal apposition II: The significance of peak bone mass, strain equilibrium, and age- related activity differentials for mechanical compensation in human tubular bones. American Journal of Physical Anthropology. 1990; 82(4):473–484. [PubMed: 2399958]

Lieberman D. The Story of the Human Body: Evolution, Health and Disease: Penguin UK. 2013

Lieberman DE, Polk JD, Demes B. Predicting long bone loading from cross-sectional geometry. American journal of physical anthropology. 2004; 123(2):156–171. [PubMed: 14730649]

Lovejoy C, McCollum M, Reno P, Rosenman B. Developmental biology and human evolution. Annual Review of Anthropology. 2003:85–109.

Madimenos FC, Snodgrass JJ, Liebert MA, Cepon TJ, Sugiyama LS. Reproductive effects on skeletal health in Shuar women of Amazonian Ecuador: A life history perspective. American Journal of Human Biology. 2012; 24(6):841–852. [PubMed: 23015457]

Maga M, Kappelman J, Ryan TM, Ketcham RA. Preliminary observations on the calcaneal trabecular microarchitecture of extant large- bodied hominoids. American Journal of Physical Anthropology. 2006; 129(3):410–417. [PubMed: 16323186]

Martin MA, Lassek WD, Gaulin SJ, Evans RW, Woo JG, Geraghty SR, Davidson BS, Morrow AL, Kaplan HS, Gurven MD. Fatty acid composition in the mature milk of Bolivian forager-horticulturalists: controlled comparisons with a US sample. Maternal & child nutrition. 2012; 8(3):404–418. [PubMed: 22624983]

May H, Ruff C. Physical burden and lower limb bone structure at the origin of agriculture in the levant. American journal of physical anthropology. 2016

Morbeck, M., Galloway, A., Richman Sumner, D. Getting old at Gombe: skeletal aging in wild-ranging chimpanzees. In: Erwin, J., Hof, P., editors. Interdisciplinary Topics in Gerontology: Aging in Nonhuman Primates. Basel: Karger; 2002. p. 48-62.

Nelson DA, Sauer NJ, Agarwal SC. Evolutionary aspects of bone health. Clinical Reviews in Bone and Mineral Metabolism. 2002; 1(3-4):169–179.

Njeh C, Boivin C, Langton C. The role of ultrasound in the assessment of osteoporosis: A review. Osteoporosis International. 1997; 7:7–22. [PubMed: 9102067]

Njeh C, Hans D, Wu C, Kantorovich E, Sister M, Fuerst T, Genant H. An in vitro investigation of the dependence on sample thickness of the speed of sound along the specimen. Medical Engineering and Physics. 1999; 21:651–659. [PubMed: 10699567]

Olszynski WP, Brown JP, Adachi JD, Hanley DA, Ioannidis G, Davison KS. Multisite quantitative ultrasound for the prediction of fractures over 5 years of follow-up: the Canadian Multicentre Osteoporosis Study. Journal of Bone and Mineral Research. 2013; 28(9):2027–2034. [PubMed: 23519952]

Olszynski WP, Brown JP, Adachi JD, Hanley DA, Ioannidis G, Davison KS, Group CR. Normative data for multisite quantitative ultrasound: the Canadian Multicenter Osteoporosis Study. Journal of Clinical Densitometry. 2014; 17(4):534–540. [PubMed: 24169082]

Pearson OM, Lieberman DE. The aging of Wolff's "law": ontogeny and responses to mechanical loading in cortical bone. Am J Phys Anthropol Suppl. 2004; 39:63–99.

Perzigian AJ. Osteoporotic bone loss in two prehistoric Indian populations. Am J Phys Anthropol. 1973; 39(1):87–95. [PubMed: 4351578]

Polidoulis I, Beyene J, Cheung AM. The effect of exercise on pQCT parameters of bone structure and strength in postmenopausal women--a systematic review and meta-analysis of randomized controlled trials. Osteoporos Int. 2012; 23(1):39–51. [PubMed: 21811864]

Ross, AC., Taylor, CL., Yaktine, AL., Del Valle, HB. Dietary reference intakes for calcium and vitamin D. National Academies Press; 2011.

Rubin CT, Lanyon LE. Regulation of bone formation by applied dynamic loads. The Journal of bone and joint surgery American. 1984; 66(3):397–402.

Ruff, CB. Prehistoric lifeways in the Great Basin wetlands: bioarchaeological reconstruction and interpretation. Salt Lake City: University of Utah Press; 1999. Skeletal structure and behavioral patterns of prehistoric Great Basin populations; p. 290-320.

Ruff CB. Mechanical determinants of bone form: insights from skeletal remains. J Musculoskelet Neuronal Interact. 2005; 5(3):202–212. [PubMed: 16172511]

Ruff CB, Holt B, Niskanen M, Sladek V, Berner M, Garofalo E, Garvin HM, Hora M, Junno J-A, Schuplerova E, et al. Gradual decline in mobility with the adoption of food production in Europe. Proceedings of the National Academy of Sciences. 2015

Ruff CB, Larsen CS, Hayes WC. Structural changes in the femur with the transition to agriculture on the Georgia coast. Am J Phys Anthropol. 1984; 64(2):125–136. [PubMed: 6465303]

Ruff CB, Trinkaus E, Walker A, Larsen CS. Postcranial robusticity in Homo I: Temporal trends and mechanical interpretation. Am J Phys Anthropol. 1993; 91(1):21–53. [PubMed: 8512053]

Ryan TM, Shaw CN. Gracility of the modern Homo sapiens skeleton is the result of decreased biomechanical loading. Proc Natl Acad Sci U S A. 2015; 112(2):372–377. [PubMed: 25535352]

Saers JP, Cazorla-Bak Y, Shaw CN, Stock JT, Ryan TM. Trabecular bone structural variation throughout the human lower limb. Journal of Human Evolution. 2016; 97:97–108. [PubMed: 27457548]

Scherf H, Wahl J, Hublin JJ, Harvati K. Patterns of activity adaptation in humeral trabecular bone in Neolithic humans and present- day people. American journal of physical anthropology. 2016; 159(1):106–115. [PubMed: 26293309]

Shackelford LL. Regional variation in the postcranial robusticity of Late Upper Paleolithic humans. American journal of physical anthropology. 2007; 133(1):655–668. [PubMed: 17295298]

Shaw CN, Ryan TM. Does skeletal anatomy reflect adaptation to locomotor patterns? Cortical and trabecular architecture in human and nonhuman anthropoids. Am J Phys Anthropol. 2012; 147(2): 187–200. [PubMed: 22120605]

Shaw CN, Stock JT. Habitual throwing and swimming correspond with upper limb diaphyseal strength and shape in modern human athletes. American journal of physical anthropology. 2009; 140(1): 160–172. [PubMed: 19358297]

Shaw CN, Stock JT. Extreme mobility in the Late Pleistocene? Comparing limb biomechanics among fossil Homo, varsity athletes and Holocene foragers. Journal of human evolution. 2013; 64(4): 242–249. [PubMed: 23453436]

Sievanen H, Cheng S, Ollikainen S, Uusi-Rasi K. Ultrasound velocity and cortical bone characteristics in vivo. Osteoporos Int. 2001; 12(5):399–405. [PubMed: 11444089]

Stieglitz J, Beheim BA, Trumble BC, Madimenos FC, Kaplan H, Gurven M. Low mineral density of a weight-bearing bone among adult women in a high fertility population. American Journal of Physical Anthropology. 2015; 156:637–648. [PubMed: 25488367]

Stieglitz J, Madimenos F, Kaplan H, Gurven M. Calcaneal Quantitative Ultrasound Indicates Reduced Bone Status Among Physically Active Adult Forager- Horticulturalists. Journal of Bone and Mineral Research. 2016; 31(3):663–671. [PubMed: 26460548]

Stock J, Pfeiffer S. Linking structural variability in long bone diaphyses to habitual behaviors: foragers from the southern African Later Stone Age and the Andaman Islands. Am J Phys Anthropol. 2001; 115(4):337–348. [PubMed: 11471132]

Straub RH, Cutolo M, Pacifici R. Evolutionary medicine and bone loss in chronic inflammatory diseases—A theory of inflammation-related osteopenia. Seminars in arthritis and rheumatism: Elsevier. 2015:220–228.

Trinkaus E, Churchill SE, Ruff CB. Postcranial robusticity in Homo. II: Humeral bilateral asymmetry and bone plasticity. American journal of physical anthropology. 1994; 93(1):1–34. [PubMed: 8141238]

Trinkaus E, Ruff CB. Diaphyseal cross-sectional geometry of Near Eastern Middle Palaeolithic humans: the tibia. Journal of Archaeological Science. 1999; 26(10):1289–1300.

Tsegai ZJ, Kivell TL, Gross T, Nguyen NH, Pahr DH, Smaers JB, Skinner MM. Trabecular bone structure correlates with hand posture and use in hominoids. PLoS One. 2013; 8(11):e78781. [PubMed: 24244359]

Wachter N, Krischak G, Mentzel M, Sarkar M, Ebinger T, Kinzl L, Claes L, Augat P. Correlation of bone mineral density with strength and microstructural parameters of cortical bone in vitro. Bone. 2002; 31(1):90–95. [PubMed: 12110418]

Wallace IJ, Nesbitt A, Mongle C, Gould ES, Grine FE. Age-related variation in limb bone diaphyseal structure among Inuit foragers from Point Hope, northern Alaska. Arch Osteoporos. 2014; 9(1): 202. [PubMed: 25491658]

Weiss M, Ben-Shlomo A, Hagag P, Rapoport M. Reference database for bone speed of sound measurement by a novel quantitative multi-site ultrasound device. Osteoporosis International. 2000; 11(8):688–696. [PubMed: 11095172]

Wood, S. Generalized additive models: an introduction with R. CRC press; 2006.
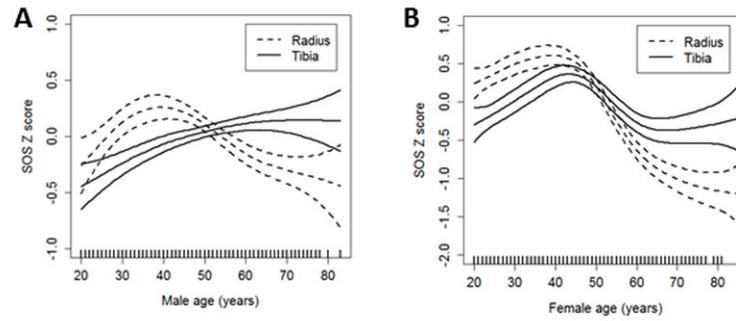
Figure 1.
Generalized additive models for men (A) and women (B) of the effect of age on radial and tibial SOS (radius [men]: edf=3.82, p<0.001, adj. R^2=0.10; tibia [men]: edf=2.19, p<0.001, adj. R^2=0.05; radius [women]: edf=5.13, p<0.001, adj. R^2=0.45; tibia [women]: edf=4.65, p<0.001, adj. R^2=0.12). Upper and lower lines of each curve represent local 95% confidence intervals for the thin plate spline. X-axis marks indicate unique ages in the sample (range: 20-85).
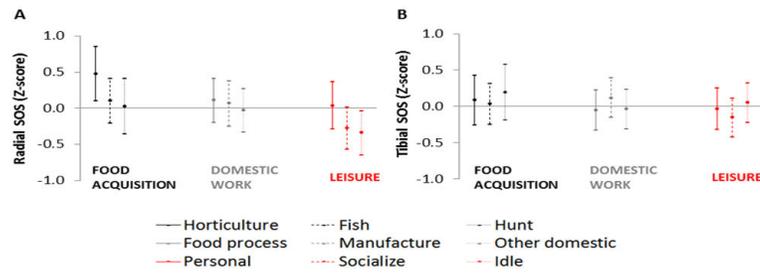
Figure 2.
Effect of earlier-life time allocation to work (in black or gray) and leisure (red) on later-life radial (A) and tibial (B) SOS (n=116 individuals). Effect sizes (95% CIs) represent the difference in SOS for individuals in the upper quartile of task-specific time allocation compared to all others. Least-squares regressions are weighted by number of behavioural scans/person. For models of radial SOS, controls include age and time lag between behavioural observation and ultrasound (mean±SD=12±1 years); additional controls for models combining sexes (all except hunt, where n=59 men) include age^2, sex and sex*age^2. For models of tibial SOS, controls include age, height, weight and time lag; additional controls for models combining sexes include sex, sex*age and sex*height.

Table 1

Descriptive statistics for study variables.

Variable	MEN					WOMEN				
	N	Mean	SD	Min	Max.	N	Mean	SD	Min.	Max.
Demographics and anthropometrics										
Age at behavioural observation (yrs)	59	40.6^	14.4	10.0	70.0	57	35.1	17.2	8.0	76.0
Age at ultrasound (yrs)	442	50.1**	14.0	20.0	83.0	480	47.6	15.8	20.0	85.0
Height at ultrasound (cm)	442	161.5***	5.0	146.0	178.0	480	150.5	4.8	135.3	167.6
Non-dominant upper limb length^a (cm)	416	23.0***	1.0	17.0	26.5	455	21.1	0.9	14.5	25.0
Lower left limb length^b	416	24.8***	1.1	18.5	28.5	453	22.9	1.2	14.0	28.5
Weight at ultrasound (kg)	442	61.9***	8.2	40.3	98.1	480	53.4	9.5	34.9	92.8
Body fat at ultrasound (%)	442	15.4***	6.3	5.0	41.4	480	23.1	7.6	5.0	47.0
Fat-free mass at ultrasound (kg)	442	52.1***	5.9	33.4	72.6	480	40.5	5.0	28.9	60.5
Bone status from qUS										
Radius speed of sound (m/s)	442	3897***	104	3583	4148	478	3854	170	2941	4180
Tibia speed of sound (m/s)	440	3832***	111	3389	4150	478	3773	131	2996	4122
Time allocation (proportion of all scans)										
Hunt	59	0.10	0.09	0.00	0.48	---	---	---	---	---
Fish	59	0.07**	0.08	0.00	0.28	57	0.04	0.06	0.00	0.25
Horticulture	59	0.09	0.11	0.00	0.41	57	0.07	0.08	0.00	0.31
Other food acquisition	59	0.03	0.04	0.00	0.17	57	0.04	0.05	0.00	0.23
Food process	59	0.04***	0.04	0.00	0.18	57	0.17	0.11	0.01	0.46
Manufacture	59	0.07	0.07	0.00	0.31	57	0.08	0.07	0.00	0.30
Other domestic work	59	0.04***	0.04	0.00	0.18	57	0.09	0.04	0.00	0.20
Idle	59	0.09	0.07	0.00	0.32	57	0.08	0.07	0.00	0.33
Personal	59	0.20	0.09	0.07	0.42	57	0.23	0.09	0.07	0.55
Socialize	59	0.19^	0.11	0.02	0.53	57	0.16	0.08	0.02	0.41

***
p 0.001 (Mann-Whitney U test across sexes)

**

p 0.01

^

p 0.1

[a] From the olecranon process of the ulna to the tip of the distal phalanx of the third digit (measured at ultrasound).

[b] From the plantar surface of the foot to the soft tissue above the distal portion of the femur (measured at ultrasound).

Abbreviations: qUS=quantitative ultrasonography.

Table 2

Segmented linear regressions estimating age at breakpoint in bone status, and rates of pre- and post-breakpoint change. SOS age slopes are reported as standardized βs. Post-breakpoint change/decade is reported in SD units.

Sex	Bone	Age (yrs) at SOS breakpoint (SE)	Pre-breakpoint age slope (95% CI)	Post-breakpoint age slope (95% CI)	Post-breakpoint change per decade (95% CI)
Male	Radius	34.95 (2.39)	0.73 (0.24 - 1.22)	-0.29 (-0.38 - -0.20)	-0.20 (-0.26 - -0.14)
	Tibia	34.03 (4.53)	0.62 (0.06 - 1.17)	0.07 (-0.02 - 0.17)	0.05 (-0.02 - 0.11)
Female	Radius	40.35 (1.81)	0.31 (0.04 - 0.58)	-0.84 (-0.94 - -0.73)	-0.56 (-0.63 - -0.49)
	Tibia	41.42 (2.67)	0.49 (0.21 - 0.78)	-0.38 (-0.50 - -0.26)	-0.25 (-0.33 - -0.17)