ELECTRONIC SUPPLEMENTARY MATERIAL (ESM)

CALCANEAL QUANTITATIVE ULTRASOUND INDICATES REDUCED BONE STATUS AMONG PHYSICALLY ACTIVE ADULT FORAGER-HORTICULTURALISTS

Jonathan Stieglitz et al.

2. METHODS

2.2. Participants

Since 2002 the Tsimane have participated in the ongoing Tsimane Health and Life History Project (THLHP; see http://www.unm.edu/~tsimane). All Tsimane residing in study villages are eligible to participate in the THLHP, and most choose to do so at least once. Project physicians have conducted annual medical exams on Tsimane of all ages since 2002 (n~8,500 individuals). A team of physicians, biochemists, and Tsimane research assistants collects data on medical and reproductive histories, functional ability, and other aspects of lifestyle (e.g. food production and sharing), in addition to collecting biological specimens (e.g. serum, urine, feces) among a subset. To date, ~44,500 medical exams have been conducted; of those receiving a medical exam, 85% have received exams in multiple years.

All Tsimane aged 15+ residing in study villages are eligible to receive a calcaneal ultrasound, although adults aged 50+ are over-sampled given the THLHP's focus on aging. Monitoring of bone mineral status using ultrasonography began in July 2013, and to date >700 calcaneal scans have been performed (~30% of which have been performed on adults aged 50+). Since July 2013 ultrasounds were conducted among 30% of adults aged 50+ that received a medical exam (n=677), which in turn represents about 85% of the older adult population. There are no significant differences in study variables between adults who received an ultrasound and those that received a medical exam without an ultrasound.

2.3. Calcaneal quantitative ultrasonography (qUS)

qUS is commonly used for research and diagnostic purposes. In large prospective studies, diagnostic sensitivity of calcaneal qUS in the prediction of hip fracture is similar to that of hip BMD measured with dual-energy X-ray absorptiometry (DXA) [1]. Correlations between estimated BMD from qUS and DXA range from 0.28-0.86; this variance may be

attributed to differences in skeletal sites measured, ultrasound machines used, or the fact that ultrasound velocity may be dependent on aspects of bone other than mineral density. While DXA tests are preferred before administering clinical treatment for osteoporosis, qUS is used in remote settings without DXA access.

Micro-architectural properties of bone alter the shape, intensity and speed of ultrasound waves passing through bone. Wave attenuation occurs by a reduction in wave amplitude and results in loss of energy. In trabecular bone the major attenuation mechanism is scattering (i.e. redistribution of energy in one or more directions), whereas in cortical bone the major mechanism is absorption (i.e. dissipation of energy by conversion to heat).

Ultrasound transducers are mounted on a motorized caliper that enables direct contact with the heel through elastomer pads. One transducer serves as a transmitter and the other as a receiver. Foot, ankle and leg positions are fixed by a device extending from the foot to the shin. qUS measurements were taken in 2013-2014 during wet and dry season months (measurements do not vary by season).

Instrumental quality control scans of a phantom provided by the manufacturer with known qUS parameters were performed daily. Short-term reproducibility of qUS measurements was assessed by duplicate scans (morning, afternoon) of 15 THLHP research assistants (men and non-pregnant, non-lactating women) ranging in age from 23-53. For BUA, SOS and QUI the respective mean CV's were 6.3%, 0.2% and 2.3%. One sonometer was used throughout the study and measurements were taken by two operators. No differences in measurements were found across operators or over time.

2.4. Demographics, anthropometrics, immune activation and physical limitation

Birth years were assigned based on a combination of methods including using known ages from written records, relative age lists, dated events, photo comparisons of people with

known ages, and cross-validation of information from independent interviews of kin [2]. Each method provides an independent estimate of age, and when estimates yielded a date of birth within a three-year range, the average was used. Individuals for whom reliable ages could not be ascertained are not included in analyses.

Anthropometric data were obtained among all participants; in no case did severe foot calluses or other factors (e.g. unclean foot pads on the Tanita scale) obstruct the ability to generate body composition or other anthropometric data used in analyses.

Results of raw WBC count are reported since results of logged count are nearly identical. To examine whether variability in WBC count indicates immune activation primarily due to infectious burden (as opposed to other factors such as hydration status), we regressed probability of having a high WBC count (>10,000 cells/μL) at qUS measurement on whether any intestinal parasite was identified in a concurrent fecal sample (methods described in [3]) using the GEE method. Fecal samples were available for 133 individuals (n=143 observations), and an intestinal parasite was identified in 71% of observations. As expected if WBC count indicates immune activation from infectious burden, having a high WBC count is associated with intestinal parasite presence (adjusted OR=2.469, 95% CI: 1.002-6.081, p=0.049) after controlling for age (p=0.854), sex (p=0.121), village distance to San Borja (adjusted OR=1.020 per km, 95% CI: 1.002-1.039, p=0.033), seasonality (adjusted OR=2.156 for dry vs. wet season, 95% CI: 0.890-5.222, p=0.089), having a high WBC count during the previous medical exam (adjusted OR=2.142, 95% CI: 0.952-4.823, p=0.066), and time [in years] between WBC count measures (p=0.376). No anthropometric measure generated by the Tanita scale (including percent body water, i.e. the total amount of body fluid as a percentage of weight) significantly predicts WBC count (operationalized dichotomously or continuously) or affects associations between other predictors and WBC count (also see Table S2).

To assess degree of physical limitation, we coded whether adults experienced difficulty (yes=1, no=0) standing from a chair without using their arms, standing repeatedly, and balancing in the tandem position, and on each leg, without using their arms or body. We also recorded time taken (in seconds) to walk three meters, pivot, and return as quickly as possible (see [4] and [5] for additional details). Participants that started but did not complete the exercise battery (e.g. due to discomfort, 18%, n=25) were assigned the maximum value for their sex. Compared to participants who completed the battery with no missing data ("finishers"), non-finishers are, on average, older and have lower qUS parameters. Non-finishers did not differ from finishers in terms of sex, anthropometrics, WBC count, and whether any prior fracture was reported. In most cases (59%), the battery was conducted in either the same year as qUS measurement or the prior year. A random subset of participants (8%, n=12) were missing data for at least one exercise measure despite completing the battery (due to data coding error), and these participants were omitted from analyses including disability score (but not other analyses).

2.5. Data analysis

The Sahara Clinical Bone Sonometer manufacturer recommends a minimum of 50 individuals for development of young adult reference values (see section 5-12 of the User's Guide, Document #080-0631 Revision D). To develop sex-specific young adult reference values among Tsimane, we conducted qUS among 67 women and 50 men aged 20-29 residing in the same villages as study participants aged 50+. Mean±SD estimated BMD (g/cm^2) for young adult Tsimane women is 0.465±0.088, and for young adult Tsimane men 0.502±0.095. For comparative purposes, mean±SD estimated BMD for young adult Caucasian American women (see section 5-7 of the User's Guide) is 0.581±0.112, and for young adult Caucasian American men 0.606±0.107 [6].

Sample sizes for establishing Caucasian American reference values are larger than the Tsimane sample sizes, although the exact number of Caucasian American young adults sampled is not published (decade-specific reference values are based on measurements of 2,208 women and 763 men aged 19+ according to the User Guide and [5], respectively). Because of the relatively small Tsimane sample size, and because the WHO threshold of T = -2.5 for diagnosing osteoporosis requires modification when utilizing qUS to assess bone properties [7], we refrain from utilizing in analyses of Tsimane data a qUS T-score threshold equivalent to the WHO T-score threshold of -2.5 from DXA. If, however, we applied the threshold of T = -2.5 instead of -1.8 among Tsimane, the percentage of women classified as having reduced bone status would decrease from 25% (n=18) to 8% (n=6); for men the percentage would decrease from 23% (n=16) to 6% (n=4).

3. RESULTS

3.1.1. General descriptives

Table S1. Age- and sex-specific anthropometric, immune activation, physical limitation and qUS variables (mean±SD)

Age cat.	Sex	N obs.	Height	Weight	Body fat %	Fat-free mass	WBC count at qUS measurement	Disability score	BUA	SOS	QUI
50-59	F	35	151±5	54±8	25±6	40±4	9227±2511	12±5	60±12	1517±20	76±12
	M	27	160±4	61±7	17±7	50±5	10389±2465	14±7	61±12	1519±22	77±13
60-69	F	25	149±3	52±7	25±6	38±4	9518±2397	13±4	54±14	1507±19	69±12
	M	31	160±5	59±9	18±7	48±5	9629±2624	11±4	59±12	1510±21	72±13
70+	F	16	147±3	46±11	23±10	35±4	9497±1979	18±5	42±9	1490±21	57±11
	M	18	157±5	59±6	21±5	46±4	10536±3045	15±7	57±17	1511±22	72±15
P-value[a]	F	76	*	**	NS	***	NS	***	***	***	***
	M	76	NS	NS	^	*	NS	NS	NS	NS	NS

^p≤0.1 *p≤0.05 **p≤0.01 ***p≤0.001

[a]P-values are from a Kruskal-Wallis test across age categories

Table S2. Pearson correlations for demographic, anthropometric, immune activation, physical limitation and qUS variables. Results for women (n=76 observations, 72 individuals) are below the diagonal and for men (n=76 obs., 70 indiv.) above.

Variable	Age	Height	Weight	Body fat %	Fat-free mass	WBC count at qUS measurement	Disability score	BUA	SOS	QUI
Age	1	-0.23*	-0.14	0.18	-0.32**	-0.01	0.08	-0.18	-0.19^	-0.20^
Height	-0.31**	1	0.23*	-0.30**	0.55***	-0.04	-0.06	0.25*	0.13	0.19^
Weight	-0.36**	0.44***	1	0.57***	0.70***	-0.06	0.13	0.16	0.02	0.08
Body fat %	-0.11	0.08	0.72***	1	-0.17	-0.01	0.25*	-0.04	-0.07	-0.06
Fat-free mass	-0.46***	0.56***	0.81***	0.19^	1	-0.05	-0.04	0.23*	0.08	0.15
WBC count at qUS measurement	0.08	-0.10	-0.17	-0.09	-0.17	1	-0.09	-0.24*	-0.23*	-0.25*
Disability score	0.50***	-0.16	-0.23*	-0.06	-0.29**	-0.16	1	-0.13	-0.14	-0.15
BUA	-0.54***	0.24*	0.47***	0.30**	0.43***	-0.18	-0.30**	1	0.78***	0.91***
SOS	-0.53***	0.08	0.42***	0.39***	0.27*	-0.17	-0.38***	0.76***	1	0.97***
QUI	-0.57***	0.15	0.46***	0.38***	0.36**	-0.18	-0.37***	0.91***	0.96***	1

^p≤0.1 *p≤0.05 **p≤0.01 ***p≤0.001

3.1.2. Descriptives for adult fracture histories, and predicted absolute fragility fracture risk using the GARVAN Fracture Risk Calculator (GARVAN-FRC)

Of 19 adults who reported ever sustaining a skeletal fracture in adulthood, only one reported multiple fractures (n=21 fractures). Twenty-one percent of those reporting a fracture sustained trauma in the past year, and 42% in the past five years (mean=10 years since fracture, range=0.5-36 years). Fractures are more common among men (19%) than women (8%) (χ^2=3.21, p=0.073, n=142 individuals), and men report fractures at a greater diversity of skeletal sites (Figure S1A). For both sexes the majority of fractures result from falls (Figure S1B), although only men report fractures from falling while traveling in the forest (often from carrying large game and/or crossing treacherous ravines while hunting). In addition, only men report fractures from tree-chopping. Individuals who reported sustaining a fracture are not different from those reporting no fracture in terms of age, height, weight, adiposity, fat-free mass, WBC count and disability score (all p's≥0.3).

Figure S1. Self-reported sites (A) and causes (B) of skeletal fractures sustained in adulthood for Tsimane men and women aged 50+. Data on cause of fracture are missing for four fractures.

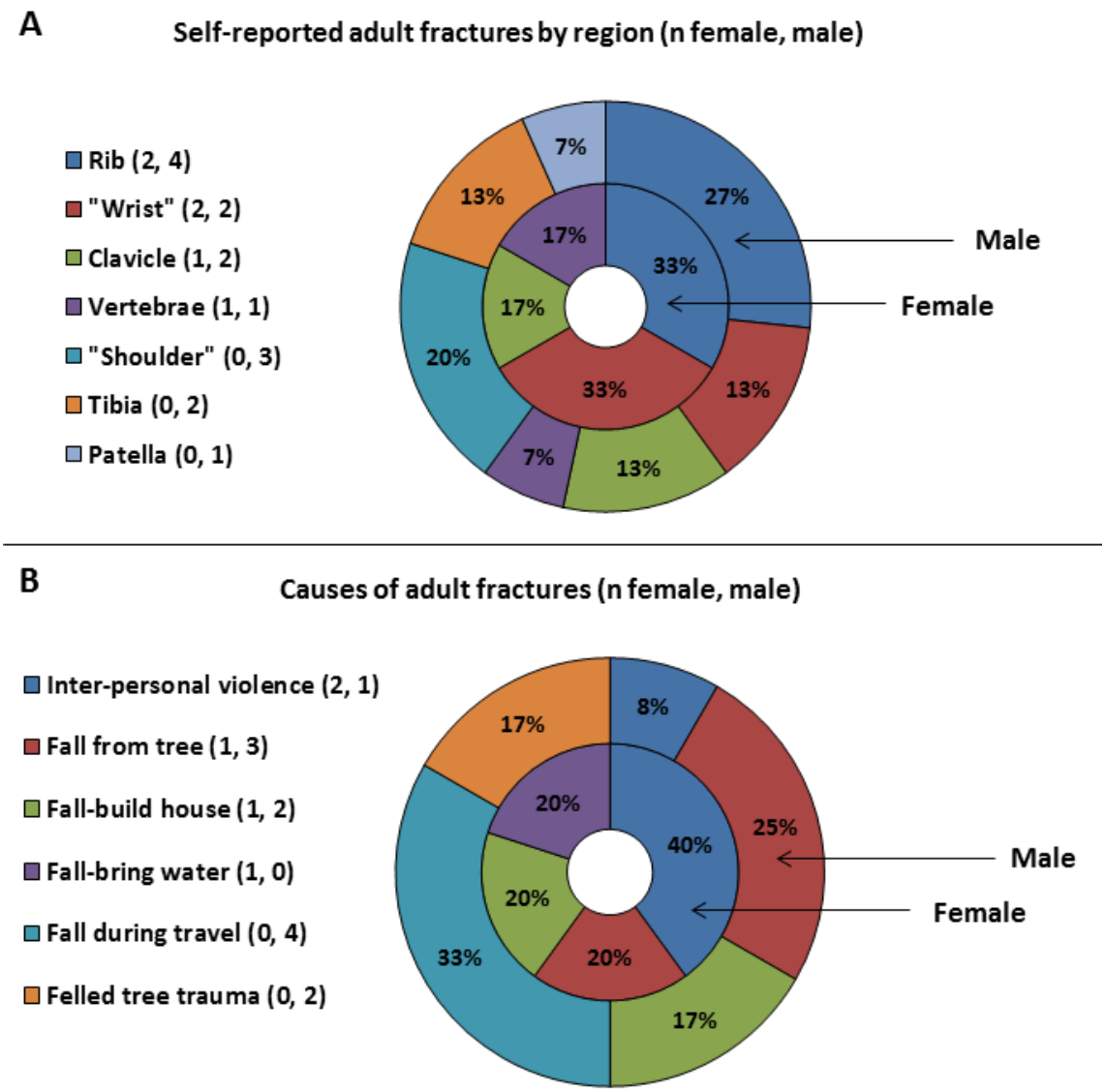


Table S3. Mean absolute risk (5- and 10-year) of any fragility fracture for Tsimane adults, using the Garvan Fracture Risk Calculator (GARVAN-FRC). This online tool (http://www.garvan.org.au/promotions/bone-fracture-risk/calculator/) uses data on age, sex, fracture history[a] (i.e. # fractures since age 50 [coded as 0, 1, 2, or 3+]), fall history (i.e. # falls in the past year [coded as 0, 1, 2, or 3+]), and BMD T-score[b] to predict absolute fracture risk for adults aged 50+. Note that the GARVAN-FRC algorithm was developed using data from developed nations, and thus may not be generalized to the Tsimane population.

Sex	Age category (years)	Bone status (T-score > -1.8 = higher; T-score ≤ -1.8 = low)	5-year absolute risk (%)				10-year absolute risk (%)			
			No prior fracture		Prior fracture		No prior fracture		Prior fracture	
			No fall	Fall	No fall	Fall	No fall	Fall	No fall	Fall
Female	50-59	Higher	3.24	4.10	8.00[d]	---[c]	6.82	8.35	17.00[d]	---[c]
		Low	5.67[d]	---[c]	---[c]	---[c]	11.67[d]	---[c]	---[c]	---[c]
	60-69	Higher	5.29	5.20	---[c]	---[c]	11.00	10.40	---[c]	---[c]
		Low	8.00	10.00[d]	12.00[d]	---[c]	16.33	20.00[d]	24.00[d]	---[c]
	70+	Higher	6.25	9.00[d]	---[c]	---[c]	12.75	18.00[d]	---[c]	---[c]
		Low	14.25	16.00	26.00	20.00[d]	27.50	30.67	47.50	38.00[d]
	All	Higher	4.34	4.75	8.00[d]	---[c]	9.04	9.59	17.00[d]	---[c]
		Low	9.80	14.50	21.33	20.00[d]	19.40	28.00	39.67	38.00[d]
Male	50-59	Higher	1.03	0.90	2.50	3.00[d]	2.00	2.00	4.00	6.00[d]
		Low	2.00	---[c]	---[c]	---[c]	3.50	---[c]	---[c]	---[c]
	60-69	Higher	2.79	3.00	---[c]	---[c]	5.26	5.67	---[c]	---[c]
		Low	4.50	6.00[d]	7.25	13.00[d]	9.00	10.00[d]	13.50	23.00[d]
	70+	Higher	7.00	---[c]	---[c]	12.50[d]	12.67	---[c]	---[c]	23.00[d]
		Low	13.88	---[c]	31.00[d]	16.00[d]	24.63	---[c]	51.00[d]	29.00[d]
	All	Higher	3.06	1.95	2.50	7.75	5.67	3.83	4.00	14.50
		Low	7.25	6.00[d]	15.17	14.50	13.05	10.00[d]	26.00	26.00

[a]Fracture histories are based mostly on self-reports; 1/6 women who reported fracturing a bone received radiographic confirmation from a San Borja physician (the remainder did not visit a physician); 6/13 men who reported fracturing a bone received radiographic confirmation.

[b]Tsimane T-scores are calculated from qUS-derived estimates of calcaneal BMD (not DXA-derived femoral neck, total hip or lumbar spine BMD). For individuals with repeated qUS measures the average estimated BMD value was used to determine T-score group. Fall histories for such individuals refer to the most recent data point.

[c]No cases were observed.

[d]Only one case was observed.

3.2. qUS parameters by age and sex

Figure S2. BUA (A), SOS (B), and QUI (C) by age for Tsimane women and men aged 50+. Predicted values are derived from GEE analyses conducted separately for each sex (n=76 observations for 72 females, 76 obs. for 70 males).

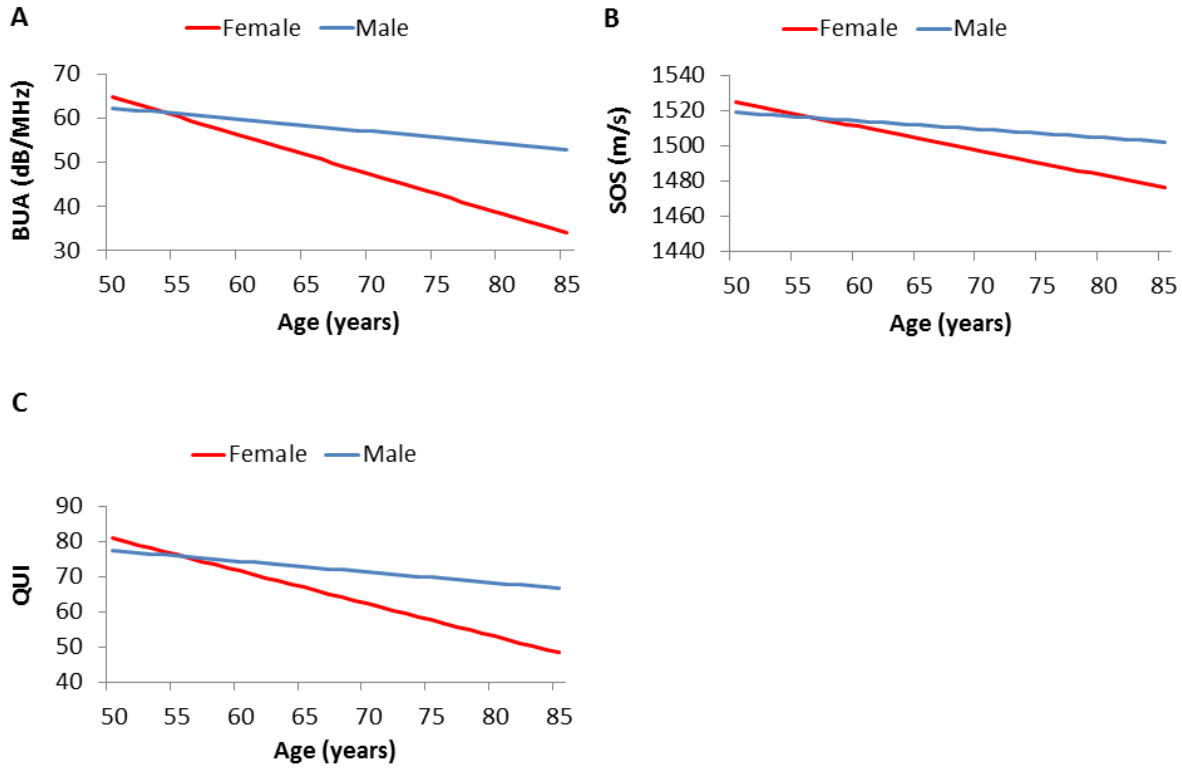


Table S4. GEE analyses of the effect of age and sex on BUA (A), SOS (B), QUI (C), and probability of having reduced bone status (D) (n=152 observations for 142 individuals).

	A) BUA		*B) SOS*		*C) QUI*		*D) Probability of having reduced bone status*	
Variable	Std. β	P	Std. β	P	Std. β	P	B (logit estimate)	P
Intercept	-0.19	0.054	-0.12	0.211	-0.16	0.100	-11.97	<0.001
Age (years)	-0.55	<0.001	-0.54	<0.001	-0.58	<0.001	0.17	<0.001
Sex (male)	0.37	0.012	0.24	0.110	0.31	0.037	6.39	0.080
Age*Sex	0.39	0.010	0.36	0.014	0.39	0.006	-0.10	0.067

3.3. qUS parameters by anthropometrics

Table S5. GEE analyses of the effect of anthropometrics on BUA (A), SOS (B), and QUI (C) for women, men, and the pooled sample. Model 1 includes as predictors age, height and weight. Model 2 includes age, percent body fat, fat-free mass and height (if height p≤0.1 from model 1). Model 3 includes significant predictors in sex-specific analyses (and is blank if all or none of the predictors in model 2 are significant). In pooled analyses, sex is additionally included as a main effect (models 1-3); age*sex (models 1-3) and sex*percent body fat (model 3) interactions are also included. Standardized betas are shown (intercepts not shown).

	A) BUA			*B) SOS*			*C) QUI*		
Variable	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
FEMALE									
Age (years)	-0.43***	-0.42***	-----	-0.48***	-0.51***	-0.50***	-0.49***	-0.50***	-0.53***
Height (cm)	-0.05	-----	-----	-0.22*	-0.14	-----	-0.16	-----	-----
Weight (kg)	0.34***	-----	-----	0.34***	-----	-----	0.36***	-----	-----
Body fat (%)	-----	0.22*	-----	-----	0.33***	0.33***	-----	0.31***	0.32***
Fat-free mass (kg)	-----	0.19*	-----	-----	0.05	-----	-----	0.07	-----
MALE									
Age (years)	-0.12	-0.12	-----	-0.18	-0.18	-----	-0.16	-0.17	-----
Height (cm)	0.20^	0.18	-----	0.10	-----	-----	0.15	-----	-----
Weight (kg)	0.10	-----	-----	-0.03	-----	-----	0.02	-----	-----
Body fat (%)	-----	0.05	-----	-----	-0.04	-----	-----	-0.02	-----
Fat-free mass (kg)	-----	0.10	-----	-----	0.02	-----	-----	0.09	-----
POOLED									
Age (years)	-0.45***	-0.45***	-0.44***	-0.50***	-0.51***	-0.51***	-0.51***	-0.52***	-0.54***
Sex (male)	-0.03	0.04	0.06	0.18	0.32	0.37*	0.10	0.23	0.43**
Height (cm)	0.13	-----	-----	-0.05	-----	-----	0.02	-----	-----
Weight (kg)	0.23**	-----	-----	0.15^	-----	-----	0.19*	-----	-----
Body fat (%)	-----	0.14^	0.24**	-----	0.17*	0.35***	-----	0.17*	0.34***
Fat-free mass (kg)	-----	0.30**	0.28**	-----	0.04	-----	-----	0.15	-----
Age*Sex	0.33*	0.32*	0.33*	0.32*	0.31*	0.33*	0.34*	0.33*	0.36**
Sex*Body fat	-----	-----	-0.22^	-----	-----	-0.39*	-----	-----	-0.37*

^p≤0.1 *p≤0.05 **p≤0.01 ***p≤0.001

Figure S3. Predicted BUA (A), SOS (B) and QUI (C) by percent body fat and age for women and men. Estimates are derived from GEE analyses including main effects of age, sex and percent body fat, and age*sex and sex*percent body fat interactions (see Table S5: Model 3 for standardized parameter estimates). Fat-free mass is additionally controlled to model BUA.

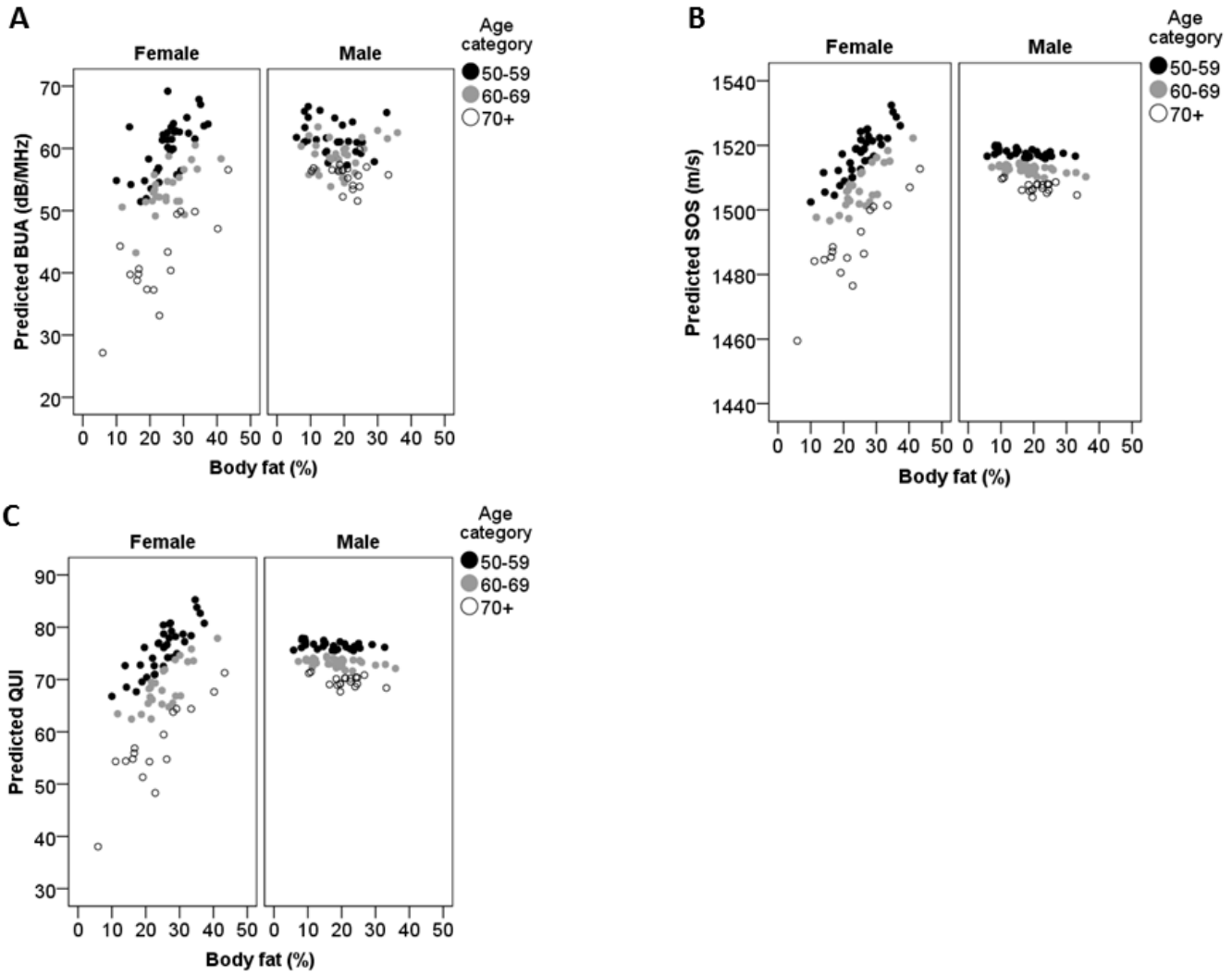
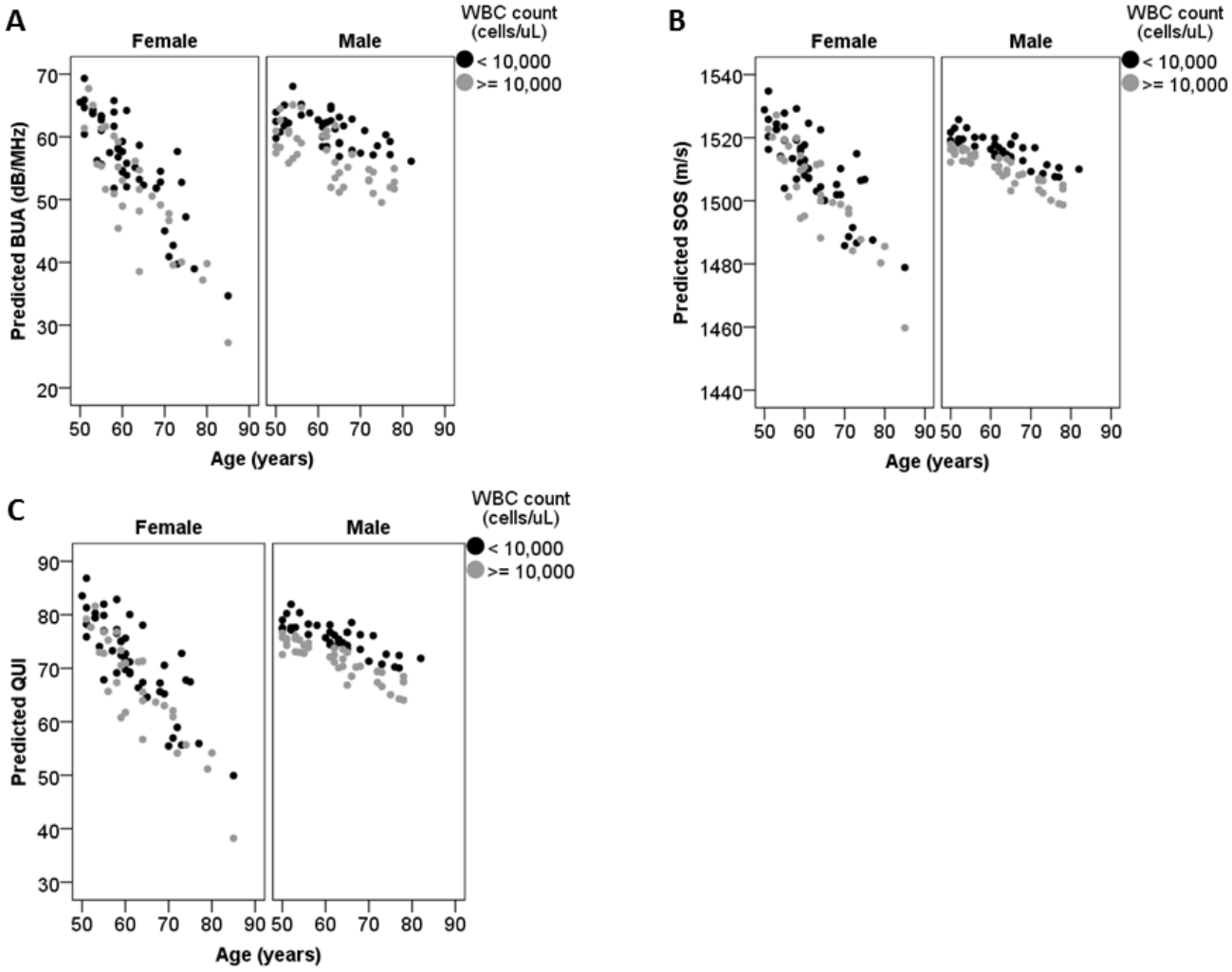


Figure S4. Predicted BUA (A), SOS (B) and QUI (C) by age and leukocyte (WBC) count for women and men. Estimates are derived from GEE analyses including main effects of age, sex, percent body fat and WBC count, and age*sex and sex*percent body fat interactions. Fat-free mass is additionally controlled to model BUA. A WBC count cut-off of >10,000 cells/µL indicates a high count (http://www.nlm.nih.gov/medlineplus/ency/article/003643.htm).



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