Appendix A. *Experimental Protocol: Tsimane, O_2 Measurement*

VO_2max was estimated in 2012 for a sub-sample of Tsimane individuals aged 36-90 (n = 284; 51 percent female). Participants were asked to step on a 20cm bench (a modified submaximal Harvard test; Astrand & Ryhming, 1954) at cadences prescribed for their age and sex (Shephard, 1980). The volume of inhaled O_2 (VO_2) was measured by a COSMED FitMate MED; VO_2 was estimated from changes in the percentage of nitrogen between inhaled and exhaled air using the Haldane equation. HR was measured by a COSMED Wireless HR monitor. FitMate software regressed HR on VO_2 for each participant and extrapolated this fit to the predicted VO_2max at the participant's age-specific maximal HR (here, 220-age; Lee et al., 2011).

Some individuals self-identified as unable to ascend the 20cm step; these individuals were asked to walk in place instead. Because it is unclear whether walking requires sufficient exertion for the FitMate's calculations, results from walking participants are excluded for the purpose of this analysis (101 excluded; remaining n = 183). Because O_2 was measured concurrently with levels of participant effort (i.e., HR), no other exclusion criteria were necessary.

Figure S1. Non-participation rates by ten year age category for Tsimane (A) females and (B) males.

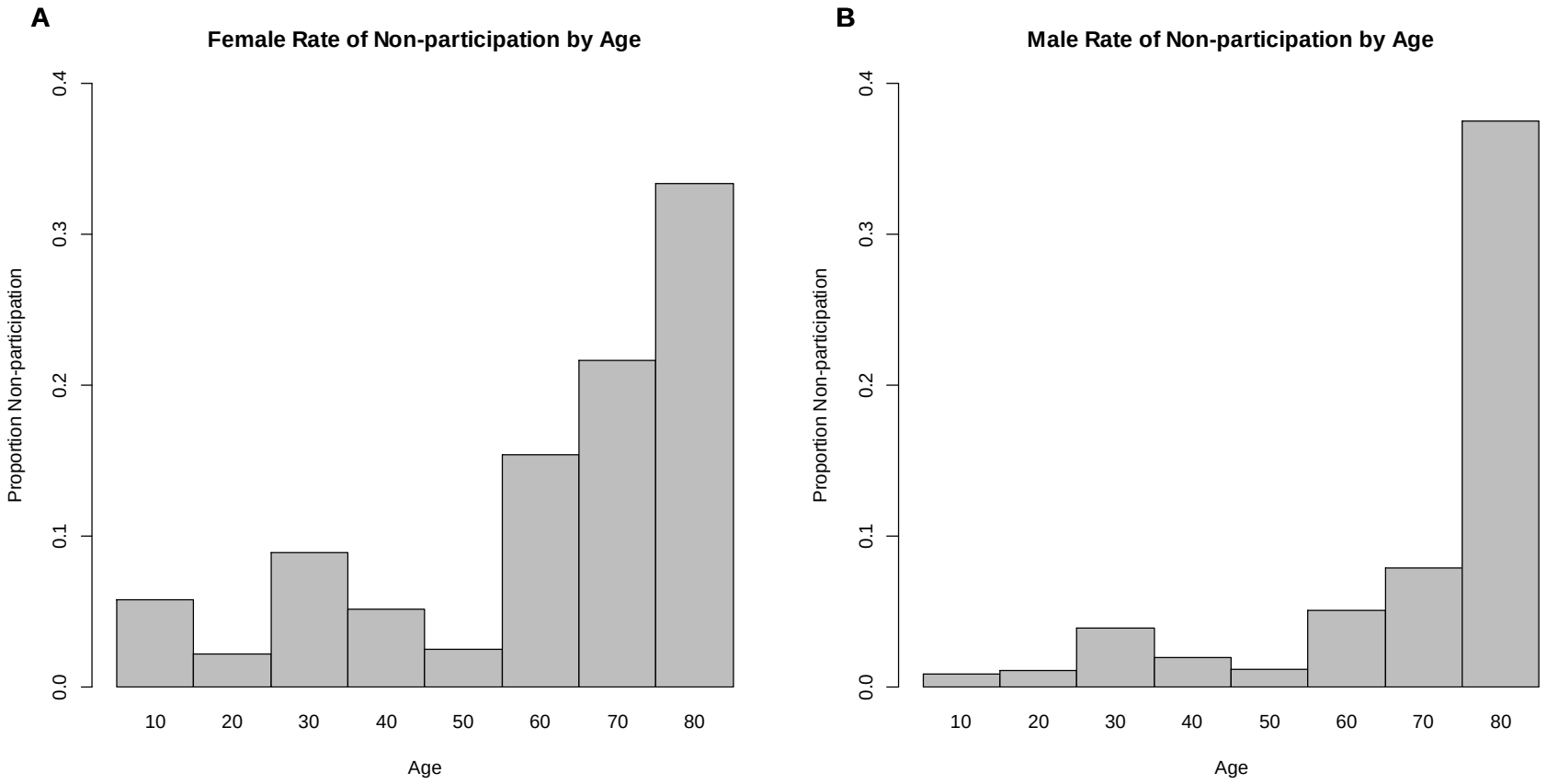

Table S1. The Tsimane sample size and percent decline per decade under different exclusion criteria, ages 20-69.

Exclusion Criteria	Female n	Female Percent Decline per Decade	Male n	Male Percent Decline per Decade
Shephard (1970)	75	6.2	48	9.2
Increase of less than 5 bpm over the course of exercise (NHANES)	205	3.6	278	6.6
Less than 65% of age-specific maximal HR (Siconolfi et al. 1985)	86	1.5	94	5.6
Less than 70% of age-specific maximal HR (ACSM)	36	-2.1	34	4.1
Less than 75% of age-specific maximal HR (NHANES)	14	-3.5	12	3.7
More than 1 SD away from age-specific (10 yr. cats.) slope of HR incr. across exercise	152	4.0	196	6.9
More than 2 SD away from age-specific (10 yr. cats.) slope of HR incr. across exercise	201	3.8	267	6.3
95% confidence intervals on age- and sex-specific (20 yr. cats.) slope of HR incr. across exercise	192	4.4	261	6.4

90% confidence intervals on age- and sex-specific (20 yr. cats.) slope of HR incr. across exercise	184	4.3	242	7.4
95th centile drawn from sex-specific GAMLSS fits (see Fig. S2 for description)	191	2.5	267	6.9

Figure S2. Medians and confidence intervals plotted on the full sample (no exclusion criteria) of Tsimane females and males using `gamlss` (Rigby & Stasinopoulos, 2005), Generalized Additive Models for Location, Scale, and Shape. By using a Box-Cox Power Equation (Rigby & Stasinopoulos, 2004) and `gamlss` to fit the data, we were able to model all four moments of the distribution; given our estimated moments, we then plotted 95th (yellow), 90th (green), 80th (light blue), and 50th (dark blue) centiles to explore the effects of outliers and potential exclusion criteria. Using 95th centiles drawn from the fitted gamlss provided very similar results to the exclusion criteria selected for analyses in the paper.

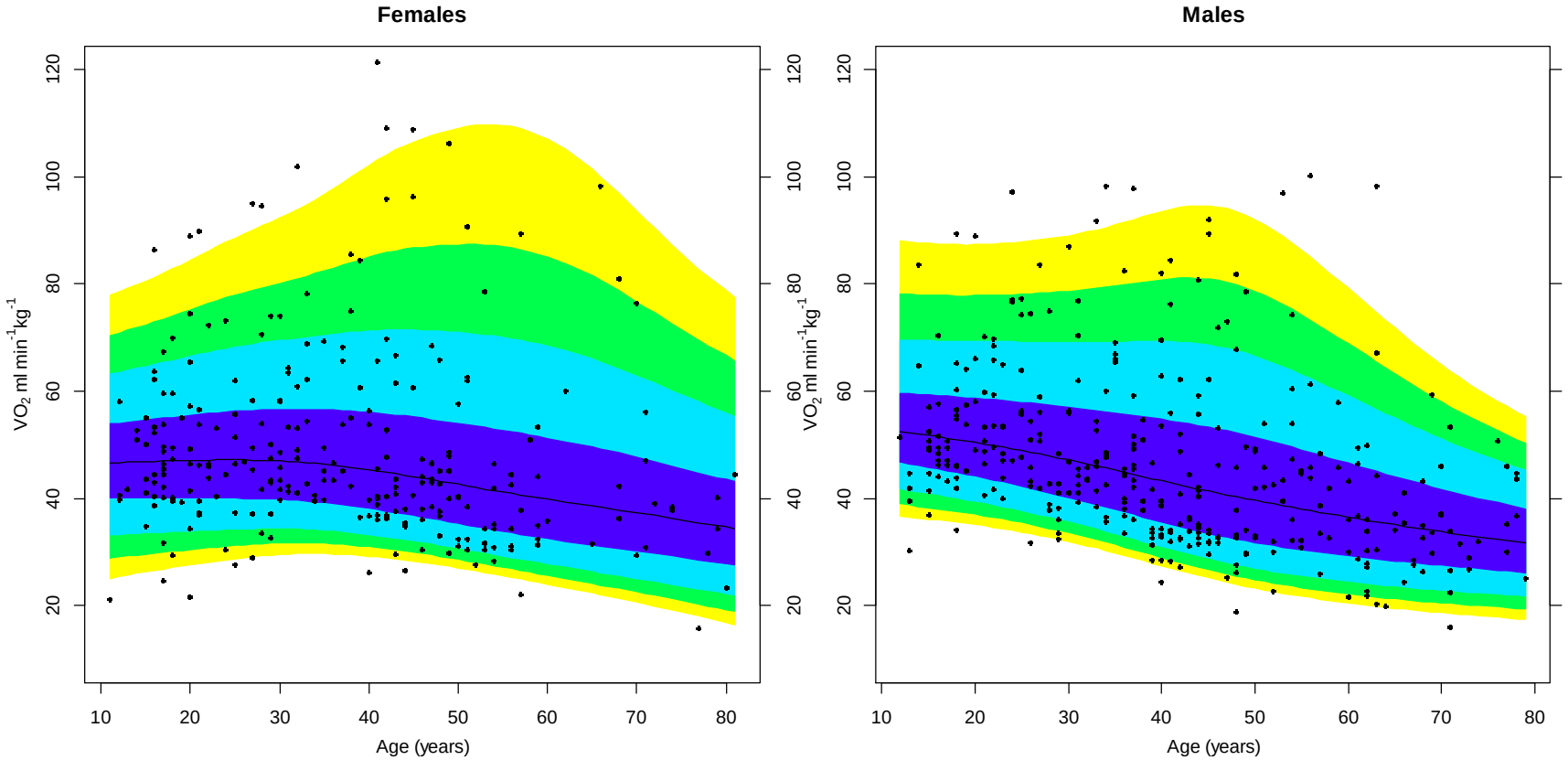

Table S2. Comparison of Canadian and Tsimane relative VO_2max by age category and sex. Percent fat-free mass was calculated by subtracting percent fat mass from one, where fat mass was calculated from skinfolds; in our other analyses, we use percent fat-free mass derived from a Tanita scale to allow a larger Tsimane sample size.

Sex	Age Group	Population	Mean ml kg^{-1} min^{-1}	Mean ml fat-free kg^{-1} min^{-1}	Percent fat-free mass
Females	12-19	Canadians	44.5	64.1	0.69
		Tsimane	45.5	63.6	0.72
	20-39	Canadians	38.4	56.6	0.68
		Tsimane	53.2	74.6	0.71
	30-59	Canadians	31.2	46.9	0.66
		Tsimane	44.2	61.8	0.72
	60-79	Canadians	24.1	36.2	0.67
		Tsimane	47.3	63.8	0.74
Males	12-19	Canadians	52.5	64.4	0.82
		Tsimane	49.9	56.1	0.89
	20-39	Canadians	44.1	56.5	0.78
		Tsimane	51.7	60.1	0.86
	30-59	Canadians	36.6	47.8	0.77
		Tsimane	42.6	49.4	0.86
	60-79	Canadians	27.6	35.5	0.78
		Tsimane	35.5	41.2	0.86

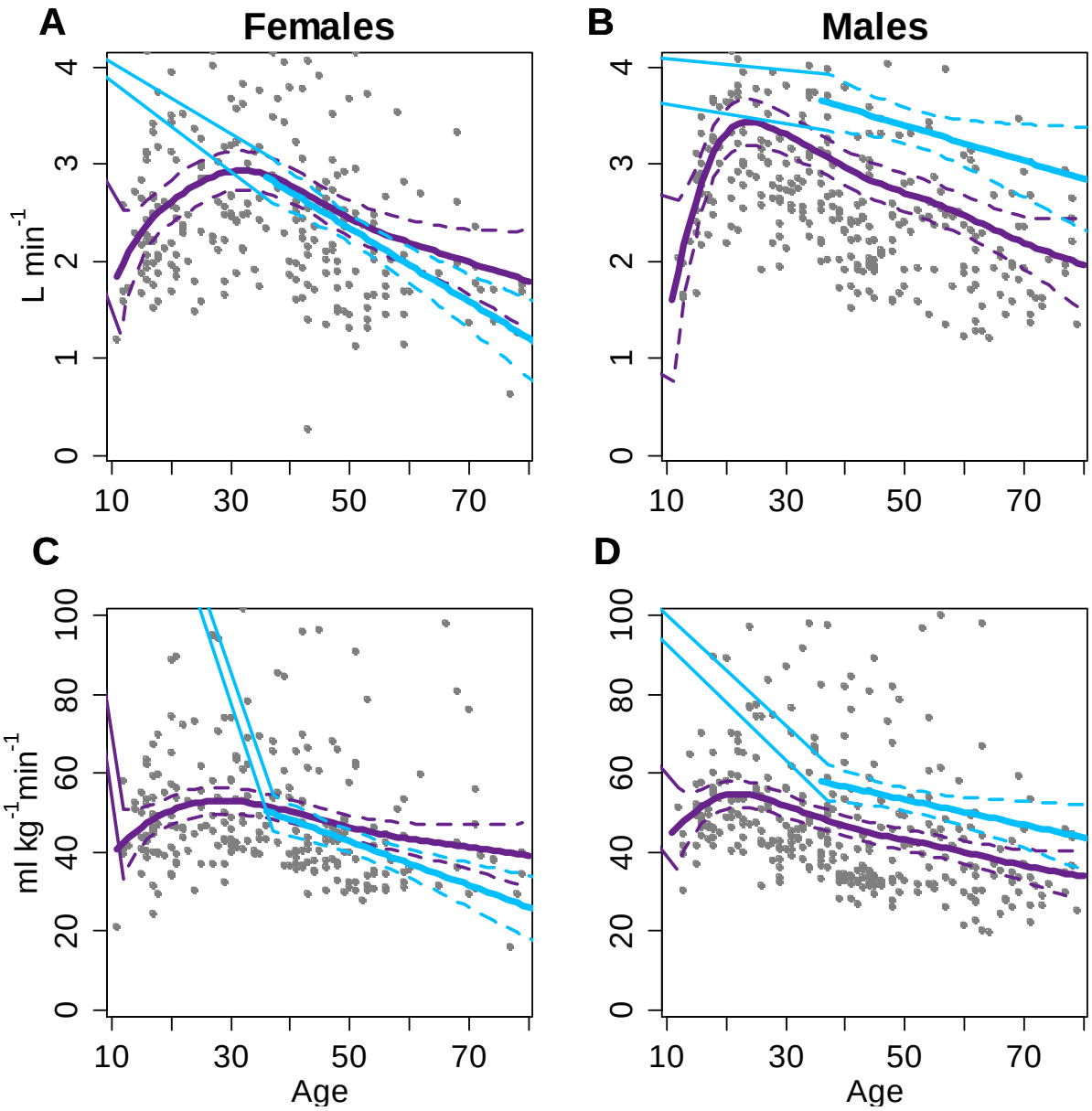

Figure S3. Predicted Tsimane VO_2max by age. Predicted values are drawn from GAM models regressing smooths of log age on VO_2max. Purple lines represent Tsimane tested under the HR method while blue lines represent Tsimane tested under the O_2 method. Intervals are 95% prediction intervals. Age was smoothed using restricted maximum likelihood (REML). Other smoothing parameters (such as Mallow's Cp and maximum likelihood) varied the age at peak absolute VO_2max (only present under the HR method) by up to 1 year for females and up to 2 years for males. Points represent the raw data for Tsimane measured under the HR method. **A.** Absolute VO_2max by age for females. **B.** Absolute VO_2max by age for males. **C.** Relative VO_2max by age for females. **D.** Relative VO_2max by age for males.

Table S3. Linear models exploring whether mediators affect the level (main effects models) and/or slope of decline (interaction effects models) of *relative* VO_2max (scaled by *absolute fat-free mass*, ml kg fat-free mass^{-1} min^{-1}) for Tsimane (A) females and (B) males. Female models control for pregnancy. P-values less than 0.10 are bolded.

A. Females

Candidate Mediator	Age Effect Est. (p-value)	Main Effect Models: Mediator Main Effect Est. (p-value)	Main Effect Models: Age Main Effect Est. (p-value)	Interaction Effect Models: Age*Mediator Interaction Est. (p-value)	Interaction Effect Models: Mediator Main Effect Est. (p-value)	Interaction Effect Models: Age Main Effect Est. (p-value)	Sample Size
Hemoglobin	-0.12 (0.22)	0.30 (**0.00**)	-0.10 (0.26)	-0.07 (0.40)	0.64 (0.12)	0.79 (0.46)	117
Helminths	-0.17 (0.11)	-0.07 (0.53)	-0.17 (0.12)	0.35 (**0.09**)	-0.73 (**0.08**)	-0.36 (**0.02**)	92
Giardia	-0.17 (0.11)	0.08 (0.45)	-0.17 (0.10)	-0.05 (0.80)	0.18 (0.66)	-0.14 (0.40)	92
Lung ailment	-0.15 (**0.09**)	-0.05 (0.55)	-0.16 (**0.07**)	-0.24 (0.35)	0.30 (0.44)	-0.13 (0.18)	133
Reported pain	-0.15 (**0.09**)	-0.03 (0.77)	-0.15 (**0.09**)	0.10 (0.57)	-0.20 (0.53)	-0.20 (0.12)	133
Arthritis	-0.15 (**0.09**)	0.03 (0.77)	-0.16 (**0.08**)	0.28 (0.25)	-0.34 (0.31)	-0.21 (**0.04**)	133
Distance to town	-0.24 (**0.00**)	0.02 (0.74)	-0.24 (**0.00**)	-0.04 (0.18)	0.28 (0.17)	-0.08 (0.53)	196
Spanish speaking	-0.24 (**0.00**)	-0.04 (0.77)	-0.25 (**0.00**)	0.07 (0.69)	-0.18 (0.63)	-0.26 (**0.00**)	197

B. Males

Candidate Mediator	Age Effect Est. (p-value)	Main Effect Models		Interaction Effect Models			Sample Size
		Mediator Main Effect Est. (p-value)	Age Main Effect Est. (p-value)	Age*Mediator Interaction Est. (p-value)	Mediator Main Effect Est. (p-value)	Age Main Effect Est. (p-value)	
Hemoglobin	-0.24 (**0.00**)	0.00 (0.97)	-0.24 (**0.00**)	0.09 (0.22)	-0.34 (0.24)	-1.49 (0.15)	157
Helminths	-0.24 (**0.01**)	0.20 (**0.02**)	-0.22 (**0.01**)	-0.02 (0.91)	0.23 (0.44)	-0.21 (0.15)	128
Giardia	-0.24 (**0.01**)	0.08 (0.34)	-0.25 (**0.00**)	-0.11 (0.53)	0.26 (0.38)	-0.21 (**0.06**)	128
Lung ailment	-0.25 (**0.00**)	-0.09 (0.21)	-0.26 (**0.00**)	-0.09 (0.60)	0.00 (0.99)	-0.24 (**0.00**)	185
Reported pain	-0.25 (**0.00**)	0.11 (0.13)	-0.26 (**0.00**)	-0.04 (0.78)	0.17 (0.44)	-0.23 (**0.08**)	185
Arthritis	-0.25 (**0.00**)	-0.07 (0.32)	-0.23 (**0.00**)	0.41 (0.44)	-0.45 (0.36)	-0.24 (**0.00**)	185
Distance to town	-0.31 (**0.00**)	-0.02 (0.68)	-0.32 (**0.00**)	0.00 (0.86)	0.01 (0.97)	-0.30 (**0.01**)	273
Spanish speaking	-0.33 (**0.00**)	0.08 (0.51)	-0.31 (**0.00**)	0.01 (0.92)	0.05 (0.90)	-0.32 (**0.00**)	271

Figure S4. Projected Tsimane relative VO_2max (ml min^{-1} kg^{-1}), assuming absolute fat mass and fat-free mass typical of the Canadian cohorts, plotted with observed Canadian and Tsimane relative VO_2max (ml min^{-1} kg^{-1}).

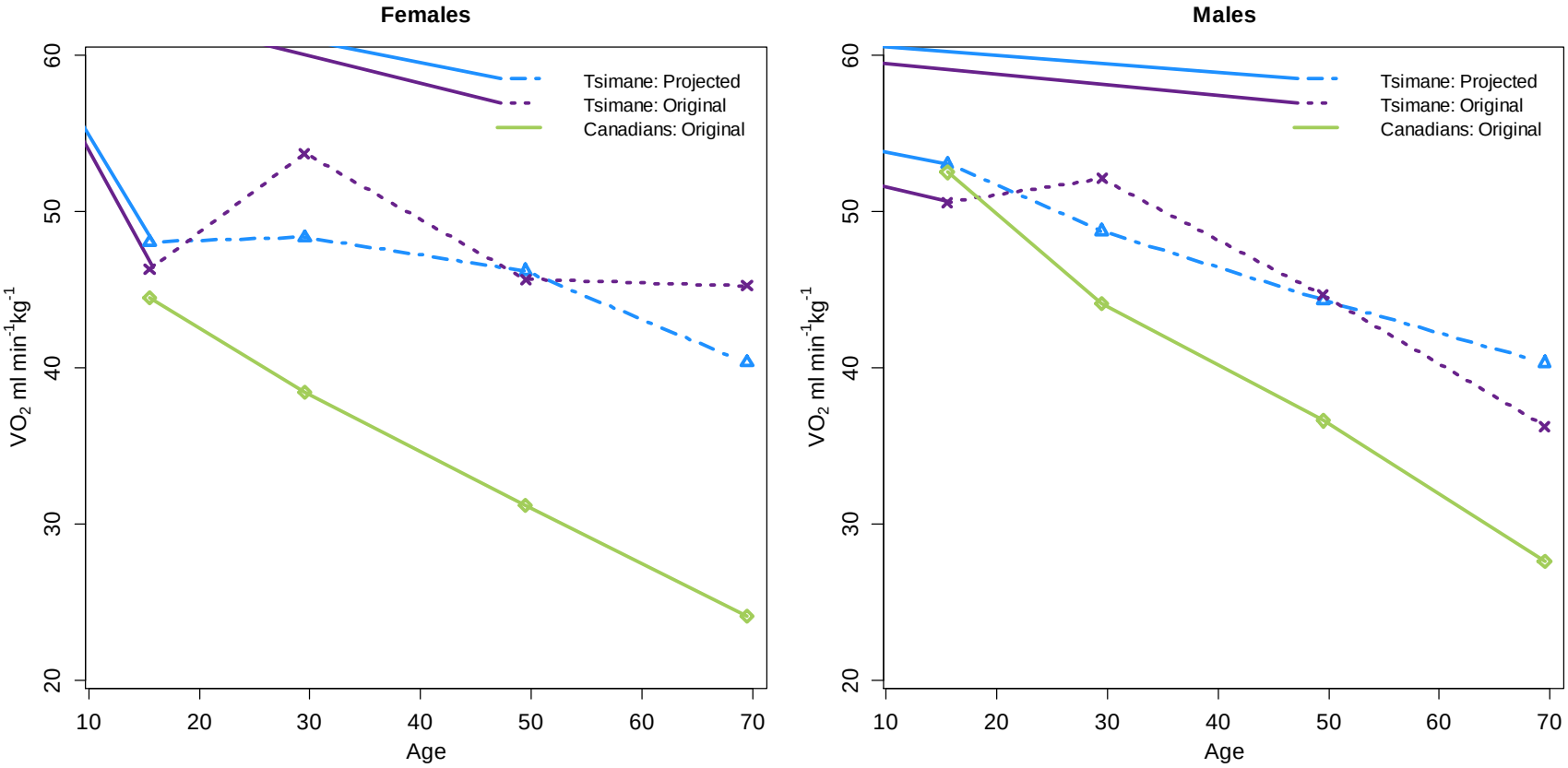

Table S4. Linear models exploring whether mediators affect the level (main effects models) and/or slope of decline (interaction effects models) of *relative* VO_2max for Tsimane (A) females and (B) males. Female models control for pregnancy. P-values less than 0.10 are bolded.

A. Females

Candidate Mediator	Age Effect Est. (p-value)	Main Effect Models: Mediator Main Effect Est. (p-value)	Main Effect Models: Age Main Effect Est. (p-value)	Interaction Effect Models: Age*Mediat or Interaction Est. (p-value)	Interaction Effect Models: Mediator Main Effect Est. (p-value)	Interaction Effect Models: Age Main Effect Est. (p-value)	Sample Size
Perc. fat-free mass	-0.23 (**0.00**)	0.00 (0.97)	-0.23 (**0.00**)	0.11 (0.89)	-0.03 (0.91)	-0.32 (0.61)	203
Hemoglobin	-0.13 (0.28)	0.24 (**0.01**)	-0.11 (0.22)	-0.04 (0.66)	0.42 (0.32)	0.36 (0.74)	117
Helminths	-0.19 (**0.07**)	0.03 (0.77)	-0.20 (**0.06**)	0.28 (0.19)	-0.49 (0.24)	-0.35 (**0.03**)	92
Giardia	-0.19 (**0.07**)	0.11 (0.28)	-0.19 (**0.07**)	-0.02 (0.93)	0.15 (0.71)	-0.18 (0.27)	92
Lung ailment	-0.17 (**0.06**)	-0.05 (0.60)	-0.18 (**0.05**)	-0.27 (0.29)	0.35 (0.36)	-0.14 (0.14)	133
Reported pain	-0.17 (**0.06**)	-0.01 (0.94)	-0.17 (**0.06**)	0.07 (0.70)	-0.13 (0.70)	-0.21 (0.12)	133
Arthritis	-0.17 (**0.06**)	-0.02 (0.83)	-0.16 (**0.07**)	0.19 (0.44)	-0.27 (0.42)	-0.19 (**0.05**)	133
Distance to town	-0.23 (**0.00**)	0.02 (0.81)	-0.23 (**0.00**)	-0.04 (0.13)	0.30 (0.14)	-0.06 (0.66)	196
Spanish speaking	-0.23 (**0.00**)	-0.08 (0.56)	-0.24 (**0.00**)	0.04 (0.81)	-0.17 (0.66)	-0.25 (**0.00**)	197

B. Males

Candidate Mediator	Age Effect Est. (p-value)	Main Effect Models		Interaction Effect Models			Sample Size
		Mediator Main Effect Est. (p-value)	Age Main Effect Est. (p-value)	Age*Mediator Interaction Est. (p-value)	Mediator Main Effect Est. (p-value)	Age Main Effect Est. (p-value)	
Perc. fat-free mass	-0.37 (**0.00**)	0.09 (0.12)	-0.34 (**0.00**)	-0.63 (0.58)	0.19 (0.31)	0.19 (0.84)	280
Hemoglobin	-0.29 (**0.00**)	-0.02 (0.76)	-0.29 (**0.00**)	0.08 (0.27)	0.33 (0.26)	-1.40 (0.17)	157
Helminths	-0.30 (**0.00**)	0.19 (**0.02**)	-0.27 (**0.00**)	-0.06 (0.72)	0.29 (0.32)	-0.23 (0.10)	128
Giardia	-0.30 (**0.00**)	0.06 (0.49)	-0.30 (**0.00**)	-0.12 (0.49)	0.25 (0.39)	-0.25 (**0.02**)	128
Lung ailment	-0.30 (**0.00**)	-0.07 (0.33)	-0.31 (**0.00**)	-0.09 (0.61)	0.02 (0.92)	-0.29 (**0.00**)	185
Reported pain	-0.30 (**0.00**)	0.11 (0.13)	-0.31 (**0.00**)	0.01 (0.94)	0.12 (0.57)	-0.30 (**0.02**)	185
Arthritis	-0.30 (**0.00**)	-0.06 (0.44)	-0.28 (**0.00**)	0.46 (0.39)	-0.47 (0.33)	-0.29 (**0.00**)	185
Distance to town	-0.37 (**0.00**)	-0.03 (0.59)	-0.37 (**0.00**)	-0.01 (0.74)	0.02 (0.89)	-0.34 (**0.00**)	273
Spanish speaking	-0.38 (**0.00**)	0.08 (0.50)	-0.37 (**0.00**)	-0.02 (0.89)	0.13 (0.72)	-0.36 (**0.00**)	271

References

Astrand, P. O., & Ryhming, I. (1954). A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during submaximal work. *Journal of Applied Physiology*, *7*(2), 218–221.

Lee, J.-M., Bassett Jr, D., Thompson, D., & Fitzhugh, E. (2011). Validation of the cosmed fitmate for predicting maximal oxygen consumption. *Journal of Strength and Conditioning Research*, *25*(9), 2573–2579.

Rigby, R., & Stasinopoulos, D. (2004). Smooth centile curves for skew and kurtotic data modelled using the Box-Cox Power Exponential distribution. *Statistics in Medicine*, *23*, 3053–3076.

Rigby, R., & Stasinopoulos, D. (2005). Generalize additive models for location, scale and shape (with discussion). *Applied Statistics*, *54*(3), 507–554.

Shephard, R. (1980). The current status of the Canadian Home Fitness Test. *British journal of sports medicine*, *14*(2 & 3), 114–125.