THE LANCET

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Kaplan H, Thompson RC, Trumble BC, et al. Coronary atherosclerosis in indigenous South American Tsimane: a cross-sectional cohort study. *Lancet* 2017; published online March 17. http://dx.doi.org/10.1016/S0140-6736(17)30752-3.

SUPPLEMENTARY APPENDIX

CONTENTS

1. STROBE Statement

Since 2002 the Tsimane (population ≈16,000) have participated in the ongoing Tsimane Health and Life History Project (see http://www.unm.edu/~tsimane). All Tsimane residing in study villages are eligible to participate, and most choose to do so at least once. Project physicians have conducted annual medical exams on Tsimane of all ages since 2002. 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.

Between July 2014 to Sept 2015, men and women aged 40 years and above from 59 Tsimane communities were invited to participate in the CT scanning project. At the time, there were 1214 eligible people aged 40+ living in these communities (the only eligibility criteria was age 40+ and willing to participate). 731 individuals were present in their communities at the time and participated (see Figure 1 of the manuscript for the STROBE flow chart).

Transporting participants from their home community to the nearby market town of San Borja was logistically complicated (requiring trekking through the jungle, dug-out canoes, rafts propelled by poles pushed off the river bottom, trucks, and cars) and can require up to two days of travel time each way. From San Borja to the regional capital is an additional 6-hour car ride. Due to these logistical complications, participants not in their community at the time we arrived were not be sampled. The Tsimane are semi-mobile and often build secondary houses deep in the jungle near their horticultural plots, not returning to their community of residence for extended periods of time. Hunting and fishing trips can last days or even weeks, and some men engage in wage labor in San Borja. In an average community, approximately one-third of individuals are away hunting, fishing, working in their horticultural plots, or in the nearby market town at any given time. Additionally, a major flood in 2014 resulted in mass migration from some communities, and the creation of several new communities that were not sampled as a part of this study, further reducing the number individuals that could be sampled as a part of this study.

To address potential sources of bias, analyses comparing individuals who had CTs versus those who did not have a CT were conducted. There were no significant differences in sex ($p=0{\cdot}634$), systolic ($p=0{\cdot}301$) or diastolic ($p=0{\cdot}301$) blood pressure, or body fat ($p=0{\cdot}942$) and thus this sample is thought to be representative of all individuals over the age of 40.

Table S1. Risk Factors by CAC Score Risk Group: Tsimane.

CAC Score	None: 0	Low: 1 - 99	High: ≥100	Total	
N	596	89	20	705	P-value
Anthropometric					
Mean Age± SD (years)	55·9±9·8	64·8±11·0	68·2±13·2	57·3±10·6	0.0001
Male	0·5±0·5	0·7±0·5	0·7±0·5	0·5±0·5	0.0009
Weight (kg)	58·2±9·6	59·3±10·8	59·1±10·1	58·4±9·8	0·58
Height (cm)	155·6±7·4	156·0±9·2	156·0±9·6	155·6±7·7	0·86
BMI (kg/m^2)	24·0±3·3	24·4±4·3	24·3±3·6	24·1±3·5	0·66
Body Fat (%)	22·1±8·3	21·8±7·6	21·7±7·1	22·0±8·2	0·96
Physiology					
Systolic Blood Pressure (mm Hg)	115·7±12·0	116·9±14·1	116·9±18·8	115·9±12·5	0·67
Diastolic Blood Pressure (mm Hg)	73·4±10·0	72·8±9·9	72·2±8·9	73·3±9·9	0·75
Heart rate (bpm)	65·7±9·0	66·7±10·5	64·2±13·8	65·8±9·3	0·47
Lipids and Glucose					
Total cholesterol (mg/dL)	3·9±29·7	3·9±34·9	157·3±27·1	150·9±30·3	0·56
LDL-C (mg/dL)	91·5±26·9	88·9±32·7	90·9±20·1	91·2±27.5	0·71
HDL-C (mg/dL)	39·3±7·6	40·6±8·7	42·1±7·9	39·5±7·8	0·11
Triglycerides (mg/dL)	99·9±43·0	112·5±43·6	121·2±55·2	102·1±43·7	0·0058
Glucose (mg/dL)	79·0±10·4	79·49±10·1	80·4±13·5	78·8±10·5	0·4459
ApoA (mg/dL)	123·3±87·1	161·9±103·7	173·6±115·4	129·6±91·3	0·0001
ApoB (mg/dL)	96.6±41.1	106.3±49.8	103.5±54.0	98.1±42.8	0·013
OxLDL U/L	77.4±23.7	75.5±23.2	78.9±22.8	77.2±23.5	0.75
Inflammatory markers					
Leucocyte count (cells/μL)	9221±2346	9203±2563	8663±2344	9203±2370	0·60
Lymphocyte count (cells/μL)	2422±731	2376±669	2254±1079	2411±736	0·56
Eosinophil count (cells/μL)	1385±977	1407±1093	1286±836	1384±987	0·89
Neutrophil count (cells/μL)	5323±1793	5352±2071	4928±1898	5315±1829	0·64
Monoocyte count (cells/μL)	88±110	82±105	56±57	86±108	0·41
ESR (mm/hr)	21·6±13·2	24·8±18·1	24·0±14·6	22·0±13·9	0·15
hs-CRP (mg/L)	3·6±3·2	4·0±3·3	4·2±2·3	3·7±3·2	0·41
IL-5 (pg/mL)	2·8±5·1	2·3±1·8	2·0±1·6	2·8±4·8	0·54
IL-10 (pg/mL)	4·5±6·5	4·7±5·2	3·5±1·4	4·5±6·3	0·74
Framingham Risk Score (10 Year)	0·04±0·04	0·07±0·06	0·10±0·06	0·04±0·05	0·0001
Proportions above high risk cutoffs					
Obese (BMI >30)	0·05	0·09	0·10	0·06	0·25
Hypertensive	0.05	0·06	0·05	0·05	0·95
Total cholesterol > 240 mg/dL	0.00	0·01	0·00	0·004	0·545

LDL-C > 130 mg/dL	0·09	0·14	0·05	0·09	0·20
Triglycerides > 200 mg/dL	0.04	0·03	0·05	0·04	0·95
HDL-C < 40 mg/dL	0.57	0·49	0·39	0·55	0·17
Glucose > 125 mg/dL	0.00	0·00	0·00	0·00	0·91
Leucocytes >10,700 cells/μL	0.24	0·21	0·21	0·23	0·85
ESR Elevated	0.26	0·37	0·30	0·27	0·12
hs-CRP > 3 mg/L	0.47	0·51	0·65	0·48	0·27

To convert total, LDL or HDL cholesterol from mg/dL to mmol/L, divide mg/dL by 38.67. To convert triglycerides from mg/dL to mmol/L, divide mg/dL by 88.57. To convert glucose from mg/dL to mmol/L, divide mg/dL by 18.02.

2. Methodological Details

2.1. Measurement of Coronary Calcium (CAC). The population of Tsimane aged 40+ years is estimated to be about 1,537 individuals; the 731 adults participating in the current study thus represent about 48% of the population in this age group. CT scans were conducted at the Hospital Presidente German Busch in Trinidad, Bolivia. The technician acquired a scan on each individual consisting of 30-40 2·5 mm slices ranging from the aortic arch to the diaphragm. Fields of view of 25 cm and 50 cm were used to image the entire heart, the ascending and descending aorta. CT settings were: 250 ms exposure, 2·5 mm slice thickness, 0·5 second rotation speed, 120 kVp, and 40 mA with prospective triggering. Breath-hold instructions were given in the Tsimane language to minimize respiratory motion artifact and mis-registration. A threshold of 130 Hounsfield Units (HU) was used as the threshold for detecting coronary artery calcium. Regarding calcium scoring, a sub-sample of 100 scans were scored twice to assess intra-observer variability, which was low. The mean variance between the two scores was 1·4 Agatston Units (AU). Two patients changed categories – one from 0 to >0 and one from <100 to > 100 AU. Coefficients of variation for scan reads were 5·41%.

Table S2. Summary characteristics of CT studies that are compared to the current Tsimane study

Study Name or Scanning center	**N participants**	**Age range or mean±SD (years)**	**Country:Ethnic Group**	**Sampling Framework**	**Exclusion Criteria**
Heinz Nixdorf Recall study (HNR)[1]	4,814	45-74	German: White	Population Based	Any CAD excluded
Multi-Ethnic Study of Atherosclerosis (MESA)[2]	6,814	45-84	USA: White, Black, Hispanic, Chinese	Population Based	Asymptomatic
Portuguese & Brazilian[3]	17,563	50±9 - 55±12	USA: White; Portugal: White; Brazil: White	Clinical	Asymptomatic

Rotterdam Coronary Calcification Study[4]	2,013	55-85	European	Population Based	Random sample
University of Illinois at Chicago (UIC)[5]	35,246	30-90	USA	Clinical	Asymptomatic
Seoul National University Hospital[6]	5,239	53±9	Korean: South Asian	Clinical	Any CAD excluded
European Scanning Centre, UK[7]	14,436	50·6±10·3 - 52·8±9·6	UK: South Asian, White	Clinical	Any CAD excluded
Mid-American Heart Institute (MAHI)[8]	71,000	56±9	USA: Predominantly White	Clinical	Any CAD excluded
Epidemiology of Coronary Calcification (ECAC)[9]	703	56·4±8·2	USA: White	Population Based	Any CAD excluded
Hospital Israelita Albert Einstein and Instituto do Coração of the Hospital das Clínicas of the FMUSP[10]	2,253	22-88	Brazilian: White	Clinical	Any CAD excluded
Kangbuk Samsung Hospital[11]	945	28-82	Korean: South Asian	Clinical	Asymptomatic
Los Angeles Biomedical Research Institute at Harbor-UCLA[12]	25,253	56±11	USA: White, Black, Hispanic, Asian, South Asian, Native American	Clinical	Asymptomatic
Toranomon Hospital, Tokyo Japan[13]	1,834	24-89	Japan: Asian	Clinical	Asymptomatic

2.2. Consistency of CT criteria across studies. In MESA, three criteria needed to be met: CT attenuation of ≥ 130 HU, four contiguous pixels (1·86 mm^2 for 4-detector-row CT; 1·83 mm^2) and location within an 8-mm radius of the coronary artery trajectory[14], whereas in other studies and ours, a CAC lesion was considered to be present with three contiguous pixels (1 mm^2) with attenuation of ≥ 130 HU[14]. Also, MESA participants were scanned twice, and the Agatston scores obtained from the two images were averaged [14]. In other studies and ours, participants were scanned once by 16-detector-row CT [4]. CAC measurement in the Mid-American Heart Institute (MAHI) sample is identical to the present approach. Prior studies observed high intraclass correlation coefficients (ICCs) for correlation between both approaches[26].

2.3. Measurement of blood lipids and inflammation. Serum was separated and frozen in liquid nitrogen before transfer to the University of California-Santa Barbara where commercial immunoassays were used to measure oxidized LDL (oxLDL) (Mercodia, Winston Salem, NC), Apolipoprotein A (ApoA) (Abcam, Cambridge, MA), Apolipoprotein B (ApoB) (R&D Systems, Minneapolis, MN), and a multiplex assay including nine cytokines (GM-CSF, INF-γ, IL-10, IL-13, IL-1b, IL-2, IL-4, IL-5, IL-6) (EMD Millipore, Darmstadt, Germany). Serum high sensitivity C-Reactive Protein (hs-CRP) was assessed via immunoassay [15], and was cross-validated by the University of Washington laboratory, using the protocols utilized for the National Health and Nutrition Evaluation Survey (NHANES). Blood chemistry, including lipids and glucose, from serum samples

were measured (Stat Fax 1908, Awareness Technology, Palm City, FL) in the Tsimane Health and Life History Project's laboratory in San Borja, Beni, Bolivia.

2.4. Statistical Approach and Validations. Several methods were used to examine relationships between CAD risk factors and CAC among Tsimane [16]. These include logistic regression to model CAC presence, ordinal logistic regression of three CAC score categories (i.e. 0, 1-99, and ≥100), multiple linear regression of the logged CAC score +1, Poisson and negative binomial regressions, and zero-inflated Poisson and negative binomial regressions. Zero-inflated negative binomial models were compared against zero-inflated Poisson models[16]. The α coefficient for the test was highly significant, indicating that the zero-inflated negative binomial model has improved fit over the Poisson regression ($p < 0{\cdot}0001$), due to over-dispersion of the data. The Vuong test was also highly significant ($p = 0{\cdot}0011$), indicating that the zero-inflated negative binomial model improves the fit over the standard negative binomial model, as indicated by Bayesian Information Criteria (BIC). The regression model using log (CAC +1) is compared to the zero-inflated negative binomial in Table S2.

Table S3. Comparison of Log (CAC+1) with Zero-Inflated Negative Binomial. Note that the values for the logistic component of the zero-inflated negative binomial model are given in italics.

	Log (CAC+1): Full Model			Log (CAC+1): Best Model			Zero-Inflated Neg. Binomial		
	Coef.	Std. Error	P Value	Coef.	Std. Error	P Value	Coef.	Std. Error	P_Value
Constant	-2·618	0·455	<0·0001	-2·509	0·401	<0.0001	0.321	1·336	0·3765
Age	0·035	0·005	<0·0001	0·037	0·005	<0.0001	1·070	0·017	<0·0001
Male	0·455	0·111	<0·0001	0·426	0·096	<0.0001	2·860	0·495	0·0311
Body Fat	0·007	0·007	0·3319	--			1·110	0·028	0·0001
il10	-0·011	0·009	0·2082	--			0·849	0·066	0·0106
il5	-0·016	0·012	0·1730	--			0·765	0·111	0·0145
log (CRP)	0·107	0·055	0·0532	0·110	0·054	0.0399	1·710	0·203	0·0070
Monocyte Count (1000s)	-0·098	0·454	0·7236	--			0·005	0·009	0·0070
Neutrophil Count (1000s)	-0·038	0·028	0·2314	-0·062	0·026	0.0192	0·830	0·071	0·0282
Erythr. Sed. Rate			0·5120				0·970		0·0377
HDL	0·015	0·006	0·0188	0·013	0·006	0.0367	-0·036	0·018	0·0474
Triglycerides	0·003	0·001	0·0261	0·004	0·001	0.0026	-0·009	0·004	0·0125
Age							-1·239	0·316	<0·0001
Male							-0·073	0·014	0·0001
Constant							8·828	1·298	0·0006
AIC	1702·707			1850·296			1167.6531172·456		
Adj. R Squared	0·133			0·141			0.1970·189*		

* The R square for the zero-inflated negative binomial is the pseudo-r square using the Cragg-Uhler (Nagelkerke) formula for adjusting the Cox-Snell formula, based on log likelihoods.

2.5. World Health Organization Verbal Autopsies. In 2014, a Bolivian physician and Tsimane translator went community to community to interview family members of recently deceased

Tsimane. The physician used the World Health Organization (WHO) 2014 version of the adult (aged 12 and above) verbal autopsy paper form, translated into the Tsimane language. The data were then reviewed case-by-case by a US cardiologist (CJR) to ensure data quality; a total of 205 verbal autopsies were reviewed. Of these, 12 had unclear cause of death that was deemed non-cardiac. There was one potential case of cardiac related death where it was reported that a male aged 75 collapsed while walking, and told his family he experienced radiating chest/left arm pain. The individual succumbed while sleeping that evening.

2.6 Age Estimation

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

3. Results

3.1 Additional Result Table. Table S3 below shows additional details on the percentiles of coronary calcium by age and sex.

Table S4: Percentiles of Coronary Calcium by Sex and Age Group.

Age Group	**45-54**		**55-64**		**65-74**		**75+**	
Sex	F	M	F	M	F	M	F	M
N	140	158	112	92	61	63	26	22
Percentiles								
5	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	2
65	0	0	0	0	0	6	0	6
70	0	0	0	0	0	7	5	8
75	0	0	0	1	0	13	19	9

80	0	0	0	3	0	33	23	15
85	0	0	0	5	6	70	25	85
90	0	0	1	14	16	137	78	199
95	2	10	4	72	153	242	169	323

Table S5, Data for Figure 2, comparing Tsimane and MESA CAC[12]

Tsimane						
	40-44	45-54	55-64	65-74	75+	Total
CAC= 0	31	275	170	89	31	596
CAC=1-100	0	21	30	25	13	89
CAC>100	0	2	4	10	4	20
Total	31	298	204	124	48	705
MESA						
CAC= 0		1469	1039	727	180	3415
CAC=1-100		362	532	605	294	1793
CAC>100		116	311	683	491	1601
Total		1947	1882	2015	965	6809
t-tests for proportions comparing Tsimane and MESA CAC in each age category						
CAC= 0		p=0·0001	P<0·0001	P<0·0001	P<0·0001	P<0·0001
CAC>100		P<0·0001	P<0·0001	P<0·0001	P<0·0001	P<0·0001

3.2 Parasite and Pathogen burden. Tsimane face an elevated parasite and pathogen load compared to industrial populations [18]. The most prevalent parasitic infections are hookworm (56% of adults), Giardia lamblia (30% of adults) and Ascaris lumbricoides (15% of adults), with many of these infections co-occuring[19]. At any given time, 68% of women and 70% of men suffer from at least one species of helminth, in addition to the 30% of adults with Giardia[19]. Immunoglobulin E (IgE) is extremely elevated (8182 IU/mL) compared to a representative US sample (52 IU/mL)[20]. More than 90% of adults suffer from parasites or report symptoms of infection at any given cross sectional medical exam, and adults report being too ill to engage in normal production activities at least 10% of the time, or ≈36.5 days per year[21]. The high parasite burden among the Tsimane is associated with elevated T_H2 immune activation, which includes relatively high levels of IL-5 and IL-10, as well as elevated white blood cell counts, CRP, and ESR[22]. While elevated CRP in industrialized populations is often from metabolically driven "sterile inflammation" due to obesity, the high inflammation among the Tsimane is largely due to the extensive parasite and pathogen burden [18,20,22].

3.3 Longitudinal measures of lipids. The Tsimane live in a rapidly changing environment, with additional market integration and access to more market goods every year. For example, beginning about 2011, a number of Tsimane communities began to acquire small gasoline motors for their canoes, thus providing many of them more mobility. Between 2004 and 2011, lipid levels were relatively flat for n=2358 observations from 1114 Tsimane aged 40+. Between 2011 and 2015, total cholesterol rose an average of 4·8 mg/dL per year, LDL rose 5·9 mg/dL per year, and HDL decreased by 0·76 mg/dL per year, controlling for age, sex, and community of residence. A small subset of the CT sample (n=133) had data available from 2004. Additional zero inflated negative binomial regressions were conducted using the lipid data from 2004 to

approximate a more realistic lifetime LDL cholesterol. The results were similar, but not significant acknowledging the small sample size and inadequate statistical power.

Table S6: Longitudinal Change in Lipids from 2004-2015 for n=2339 Observations from 1114 Tsimane aged 40+. Blank cells indicate data not available.

	2004		2011	2012	2013	2014	2015
Sample Size	n=246		n=473	n=465	n=79	n=266	n=810
Total Cholesterol mg/dL	138·0		134·3	131·8	142·2	151·1	151·2
LDL-C mg/dL	70·6		69·2	72·1		89·2	91·1
HDL-C mg/dL	36·8		41·1	30·9		41·3	38·8
LDL-C >130	0·96%		2·85%	1·73%		8·17%	8·13%
LDL-C >100	12·92%		14·81%	15·10%		34·63%	35·00%
Total cholesterol >200	2·85%		4·23%	0·43%	6·33%	6·39%	5·19%
Total cholesterol >240	0·41%		0·42%	0·00%	0·00%	0·38%	0·62%
HDL-C < 40 mg/dL	66·51%		63·17%	91·37%		54·47%	67·32%

4. Further Acknowledgments

Additional contributing members of the Horus research team:
Andrew J.O. Davis, BA, Michael A. Eskander MD, Michael Miyamoto, MD, MS, Sarah R. Johnson BS, Samantha I. King BS, Laura M. McClung BA, Sallam Lotfy Mohamed BsSc, Adam M. Thompson BS.

Additional contributing members of the Tsimane Health and Life History Project:
Sarah Alami G BA, Edmund Seabright BA, Daniel Cummings, MA.

5. References

1. Schmermund A, Möhlenkamp S, Stang A, et al. Assessment of clinically silent atherosclerotic disease and established and novel risk factors for predicting myocardial

infarction and cardiac death in healthy middle-aged subjects: rationale and design of the Heinz Nixdorf RECALL Study. *American heart journal* 2002; **144**(2): 212-8.
2. Bild DE, Detrano R, Peterson D, et al. Ethnic differences in coronary calcification the Multi-Ethnic Study of Atherosclerosis (MESA). *Circulation* 2005; **111**(10): 1313-20.
3. Santos RD, Nasir K, Rumberger JA, et al. Difference in atherosclerosis burden in different nations and continents assessed by coronary artery calcium. *Atherosclerosis* 2006; **187**(2): 378-84.
4. Oei H-HS, Vliegenthart R, Hofman A, Oudkerk M, Witteman JC. Risk factors for coronary calcification in older subjects. *European heart journal* 2004; **25**(1): 48-55.
5. Hoff JAQ, L., Sevrukov,A., Lipton, R.B., Daviglus, M., Garside, D.B., Ajmere, N.K., Gandhi, S., Kondos, G. T. The Prevalence of Coronary Artery Calcium Among Diabetic Individuals Without Known Coronary Artery Disease *Journal of the American College of Cardiology* 2003; **41**(6): 1008-12.
6. Park HE, Kim M-K, Choi S-Y, et al. The prevalence and distribution of coronary artery calcium in asymptomatic Korean population. *The international journal of cardiovascular imaging* 2012; **28**(5): 1227-35.
7. Koulaouzidis G, Jenkins P, McArthur T. Comparison of coronary calcification among South Asians and Caucasians in the UK. *International journal of cardiology* 2013; **168**(2): 1647-8.
8. Thompson, RC M, AI MK, O'Keefe JH Jr, Stevens TL, House J, Fritsch N, Bateman TM. Clinical utility of coronary calcium scoring after nonischemic myocardial perfusion imaging. *J Nucl Cardiol* 2005; **12**(4): 392-400.
9. Schmermund A, Lehmann N, Bielak LF, et al. Comparison of subclinical coronary atherosclerosis and risk factors in unselected populations in Germany and US-America. *Atherosclerosis* 2007; **195**(1): e207-e16.
10. Meneghelo RS, Santos RD, Almeida B, et al. Distribution of coronary artery calcium scores determined by ultrafast computed tomography in 2.253 asymptomatic white men. *Arquivos brasileiros de cardiologia* 2003; **81**: 27-31.
11. Yu J-H, Yim SH, Yu SH, et al. The relationship of body composition and coronary artery calcification in apparently healthy Korean adults. *Endocrinology and Metabolism* 2013; **28**(1): 33-40.
12. Budoff MJ, Nasir K, Mao S, et al. Ethnic differences of the presence and severity of coronary atherosclerosis. *Atherosclerosis* 2006; **187**(2): 343-50.
13. Ohmoto-Sekine Y, Yanagibori R, Amakawa K, et al. Prevalence and distribution of coronary calcium in asymptomatic Japanese subjects in lung cancer screening computed tomography. *Journal of cardiology* 2016; **67**(5): 449-54.
14. Carr JJ, Nelson JC, Wong ND, et al. Calcified Coronary Artery Plaque Measurement with Cardiac CT in Population-based Studies: Standardized Protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Radiology* 2005; **234**(1): 35-43.
15. Brindle E, Fujita M, Shofer J, O'Connor KA. Serum, plasma, and dried blood spot high-sensitivity C-reactive protein enzyme immunoassay for population research. *Journal of Immunological Methods* 2010; **362**(1-2): 112-20.
16. Gudmundsson EF, Gudnason V, Sigurdsson S, Launer LJ, Harris TB, Aspelund T. Coronary artery calcium distributions in older persons in the AGES-Reykjavik study. *European journal of epidemiology* 2012; **27**(9): 673-87.
17. Gurven M, Kaplan H. Longevity Among Hunter- Gatherers: A Cross-Cultural Examination. *Population and Development Review* 2007; **33**(2): 321-65.
18. Blackwell AD, Trumble BC, Maldonado Suarez I, et al. Immune function in Amazonian horticulturalists. *Annals of Human Biology* 2016: 1-45.

19. Blackwell AD, Martin M, Kaplan H, Gurven M. Antagonism between two intestinal parasites in humans: the importance of co-infection for infection risk and recovery dynamics. *Proceedings of the Royal Society B: Biological Sciences* 2013; **280**(1769).
20. Blackwell A, Gurven M, Sugiyama L, et al. Evidence for a peak shift in a humoral response to helminths: Age profiles of IgE in the Shuar of Ecuador, the Tsimane of Bolivia, and the U.S. NHANES. *PLoS Neglected Tropical Diseases* 2011; **5**(6): e1218.
21. 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.
22. Gurven MD, Trumble BC, Stieglitz J, et al. Cardiovascular disease and type 2 diabetes in evolutionary perspective: a critical role for helminths? *Evolution, medicine, and public health* 2016: eow028.