Supplementary appendix

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

SUPPLEMENTARY APPENDIX

CONTENTS

1. STROBE Statement

2. Methodological Details
   2.1. Measurement of Coronary Calcium (CAC).
   2.2. Consistency of CT criteria across studies.
   2.3. Measurement of blood lipids and inflammation
   2.4. Statistical Approach
   2.5. World Health Organization Verbal Autopsies
   2.6 Age Estimation

3. Results
   3.1 Additional Results Table
   3.2 Tsimane Parasite and Pathogen burden
   3.3 Longitudinal measures of Lipids

4. Acknowledgments

5. References

List of Tables

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

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

TABLE S3. Comparison of Log (CAC+1) with Zero-Inflated Negative Binomial.

TABLE S4. Percentiles of Coronary Calcium by Sex and Age Group.

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

TABLE S6. Longitudinal Change in Lipids from 2004-2015 for n=2339 observations from 1114 Tsimane aged 40+.

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=0.634), systolic (p=0.301) or diastolic (p=0.301) blood pressure, or body fat (p=0.942) 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.

<table>
<thead>
<tr>
<th>CAC Score</th>
<th>None: 0</th>
<th>Low: 1 - 99</th>
<th>High: ≥100</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>596</td>
<td>89</td>
<td>20</td>
<td>705</td>
<td></td>
</tr>
</tbody>
</table>

**Anthropometric**

- Mean Age± SD (years)  
  - None: 55.9±9.8  
  - Low: 64.8±11.0  
  - High: 68.2±13.2  
  - Total: 57.3±10.6  
  - P-value: 0.0001
- Male  
  - None: 0.5±0.5  
  - Low: 0.7±0.5  
  - High: 0.7±0.5  
  - Total: 0.5±0.5  
  - P-value: 0.0009
- Weight (kg)  
  - None: 58.2±9.6  
  - Low: 59.3±10.8  
  - High: 59.1±10.1  
  - Total: 58.4±9.8  
  - P-value: 0.58
- Height (cm)  
  - None: 155.6±7.4  
  - Low: 156.0±9.2  
  - High: 156.0±9.6  
  - Total: 155.6±7.7  
  - P-value: 0.86
- BMI (kg/m²)  
  - None: 24.0±3.3  
  - Low: 24.4±4.3  
  - High: 24.3±3.6  
  - Total: 24.1±3.5  
  - P-value: 0.66
- Body Fat (%)  
  - None: 22.1±8.3  
  - Low: 21.8±7.6  
  - High: 21.7±7.1  
  - Total: 22.0±8.2  
  - P-value: 0.96

**Physiology**

- Systolic Blood Pressure (mm Hg)  
  - None: 115.7±12.0  
  - Low: 116.9±14.1  
  - High: 116.9±18.8  
  - Total: 115.9±12.5  
  - P-value: 0.67
- Diastolic Blood Pressure (mm Hg)  
  - None: 73.4±10.0  
  - Low: 72.8±9.9  
  - High: 72.2±8.9  
  - Total: 73.3±9.9  
  - P-value: 0.75
- Heart rate (bpm)  
  - None: 65.7±9.0  
  - Low: 66.7±10.5  
  - High: 64.2±13.8  
  - Total: 65.8±9.3  
  - P-value: 0.47

**Lipids and Glucose**

- Total cholesterol (mg/dL)  
  - None: 3.9±29.7  
  - Low: 3.9±34.9  
  - High: 157.3±27.1  
  - Total: 150.9±30.3  
  - P-value: 0.56
- LDL-C (mg/dL)  
  - None: 91.5±26.9  
  - Low: 88.9±32.7  
  - High: 90.9±20.1  
  - Total: 91.2±27.5  
  - P-value: 0.71
- HDL-C (mg/dL)  
  - None: 39.3±7.6  
  - Low: 40.6±8.7  
  - High: 42.1±7.9  
  - Total: 42.0±10.3  
  - P-value: 0.11
- Triglycerides (mg/dL)  
  - None: 99.9±43.0  
  - Low: 112.5±43.6  
  - High: 121.2±55.2  
  - Total: 102.1±43.7  
  - P-value: 0.0058
- Glucose (mg/dL)  
  - None: 7.9±0.4  
  - Low: 7.9±0.4  
  - High: 7.8±1.0  
  - Total: 7.8±1.0  
  - P-value: 0.4459
- ApoA (mg/dL)  
  - None: 123.3±87.1  
  - Low: 161.9±103.7  
  - High: 173.6±115.4  
  - Total: 129.6±91.3  
  - P-value: 0.0001
- ApoB (mg/dL)  
  - None: 96.6±41.1  
  - Low: 106.3±49.8  
  - High: 103.5±54.0  
  - Total: 98.1±42.8  
  - P-value: 0.013
- OxLDL U/L  
  - None: 77.4±23.7  
  - Low: 75.5±23.2  
  - High: 78.9±22.8  
  - Total: 77.2±23.5  
  - P-value: 0.75

**Inflammatory markers**

- Leucocyte count (cells/μL)  
  - None: 9221±2346  
  - Low: 9203±2563  
  - High: 8663±2344  
  - Total: 9203±2370  
  - P-value: 0.60
- Lymphocyte count (cells/μL)  
  - None: 2422±731  
  - Low: 2376±669  
  - High: 2254±1079  
  - Total: 2411±736  
  - P-value: 0.56
- Eosinophil count (cells/μL)  
  - None: 1385±977  
  - Low: 1407±1093  
  - High: 1286±836  
  - Total: 1384±987  
  - P-value: 0.89
- Neutrophil count (cells/μL)  
  - None: 5323±1793  
  - Low: 5352±2071  
  - High: 4928±1898  
  - Total: 5315±1829  
  - P-value: 0.64
- Monoocyte count (cells/μL)  
  - None: 88±110  
  - Low: 82±105  
  - High: 56±57  
  - Total: 86±108  
  - P-value: 0.41
- ESR (mm/hr)  
  - None: 21.6±13.2  
  - Low: 24.8±18.1  
  - High: 24.0±14.6  
  - Total: 22.0±13.9  
  - P-value: 0.15
- hs-CRP (mg/L)  
  - None: 3.6±3.2  
  - Low: 4.0±3.3  
  - High: 4.2±1.9  
  - Total: 3.7±3.2  
  - P-value: 0.41
- IL-5 (pg/mL)  
  - None: 2.8±5.1  
  - Low: 2.3±1.8  
  - High: 2.0±1.6  
  - Total: 2.8±4.8  
  - P-value: 0.54
- IL-10 (pg/mL)  
  - None: 4.5±6.5  
  - Low: 4.7±5.2  
  - High: 3.5±1.4  
  - Total: 4.5±6.3  
  - P-value: 0.74
- Framingham Risk Score (10 Year)  
  - None: 0.04±0.04  
  - Low: 0.07±0.06  
  - High: 0.10±0.06  
  - Total: 0.04±0.05  
  - P-value: 0.0001

**Proportions above high risk cutoffs**

- Obese (BMI >30)  
  - None: 0.05  
  - Low: 0.09  
  - High: 0.10  
  - Total: 0.06  
  - P-value: 0.25
- Hypertensive  
  - None: 0.05  
  - Low: 0.06  
  - High: 0.05  
  - Total: 0.05  
  - P-value: 0.95
- Total cholesterol > 240 mg/dL  
  - None: 0.00  
  - Low: 0.01  
  - High: 0.00  
  - Total: 0.004  
  - P-value: 0.545
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

<table>
<thead>
<tr>
<th>Study Name or Scanning center</th>
<th>N participants</th>
<th>Age range or mean±SD (years)</th>
<th>Country:Ethnic Group</th>
<th>Sampling Framework</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heinz Nixdorf Recall study (HNR)¹</td>
<td>4,814</td>
<td>45-74</td>
<td>German: White</td>
<td>Population Based</td>
<td>Any CAD excluded</td>
</tr>
<tr>
<td>Multi-Ethnic Study of Atherosclerosis (MESA)²</td>
<td>6,814</td>
<td>45-84</td>
<td>USA: White, Black, Hispanic, Chinese</td>
<td>Population Based</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>Portuguese &amp; Brazilian³</td>
<td>17,563</td>
<td>50±9 - 55±12</td>
<td>USA: White; Portugal: White; Brazil: White</td>
<td>Clinical</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>Study</td>
<td>Sample Size</td>
<td>Age Range</td>
<td>Region</td>
<td>Diagnosis</td>
<td>Imaging Method</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>-------------</td>
<td>-----------</td>
<td>--------</td>
<td>-----------</td>
<td>----------------</td>
</tr>
<tr>
<td>Rotterdam Coronary Calcification Study</td>
<td>2,013</td>
<td>55-85</td>
<td>European</td>
<td>Population Based</td>
<td>Random sample</td>
</tr>
<tr>
<td>University of Illinois at Chicago (UIC)</td>
<td>35,246</td>
<td>30-90</td>
<td>USA</td>
<td>Clinical</td>
<td>Symptomatic</td>
</tr>
<tr>
<td>Seoul National University Hospital</td>
<td>5,239</td>
<td>53±9</td>
<td>Korean</td>
<td>Clinical</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>European Scanning Centre, UK</td>
<td>14,436</td>
<td>50-6±10-3 - 52-8±9-6</td>
<td>UK: South Asian, White</td>
<td>Clinical</td>
<td>Any CAD excluded</td>
</tr>
<tr>
<td>Mid-American Heart Institute (MAHI)</td>
<td>71,000</td>
<td>56±9</td>
<td>USA: Predominantly White</td>
<td>Clinical</td>
<td>Any CAD excluded</td>
</tr>
<tr>
<td>Epidemiology of Coronary Calcification (ECAC)</td>
<td>703</td>
<td>56-4±8-2</td>
<td>USA: White</td>
<td>Population Based</td>
<td>Any CAD excluded</td>
</tr>
<tr>
<td>Hospital Israelita Albert Einstein and Instituto do Coração do Hospital das Clínicas of the FMUSP</td>
<td>2,253</td>
<td>22-88</td>
<td>Brazilian: White</td>
<td>Clinical</td>
<td>Any CAD excluded</td>
</tr>
<tr>
<td>Kangbuk Samsung Hospital</td>
<td>945</td>
<td>28-82</td>
<td>Korean: South Asian</td>
<td>Clinical</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>Los Angeles Biomedical Research Institute at Harbor-UCLA</td>
<td>25,253</td>
<td>56±11</td>
<td>USA: White, Black, Hispanic, Asian, South Asian, Native American</td>
<td>Clinical</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>Toranomon Hospital, Tokyo Japan</td>
<td>1,834</td>
<td>24-89</td>
<td>Japan: Asian</td>
<td>Clinical</td>
<td>Asymptomatic</td>
</tr>
</tbody>
</table>

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 mm² for 4-detector-row CT; 1·83 mm² ) and location within an 8-mm radius of the coronary artery trajectory, whereas in other studies and ours, a CAC lesion was considered to be present with three contiguous pixels (1 mm² ) with attenuation of ≥ 130 HU. Also, MESA participants were scanned twice, and the Agatston scores obtained from the two images were averaged. In other studies and ours, participants were scanned once by 16-detector-row CT. 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.

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, 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. 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. 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 <0.0001), due to over-dispersion of the data. The Vuong test was also highly significant (p=0.0011), 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.

<table>
<thead>
<tr>
<th></th>
<th>Coef.</th>
<th>Std. Error</th>
<th>P Value</th>
<th>Coef.</th>
<th>Std. Error</th>
<th>P Value</th>
<th>Coef.</th>
<th>Std. Error</th>
<th>P_Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-2.618</td>
<td>0.455</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>2.509</td>
<td>&lt;0.0001</td>
<td>0.321</td>
<td>1.336</td>
<td>0.3765</td>
</tr>
<tr>
<td>Age</td>
<td>0.035</td>
<td>0.005</td>
<td>&lt;0.0001</td>
<td>0.037</td>
<td>0.005</td>
<td>&lt;0.0001</td>
<td>1.070</td>
<td>0.017</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male</td>
<td>0.455</td>
<td>0.111</td>
<td>&lt;0.0001</td>
<td>0.426</td>
<td>0.096</td>
<td>&lt;0.0001</td>
<td>2.860</td>
<td>0.495</td>
<td>0.0311</td>
</tr>
<tr>
<td>Body Fat</td>
<td>0.007</td>
<td>0.007</td>
<td>0.3319</td>
<td>0.426</td>
<td>0.096</td>
<td>&lt;0.0001</td>
<td>2.860</td>
<td>0.495</td>
<td>0.0001</td>
</tr>
<tr>
<td>il10</td>
<td>-0.011</td>
<td>0.009</td>
<td>--</td>
<td></td>
<td>--</td>
<td></td>
<td>0.849</td>
<td>0.066</td>
<td>0.0106</td>
</tr>
<tr>
<td>il5</td>
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<td>0.012</td>
<td>0.1730</td>
<td></td>
<td>--</td>
<td></td>
<td>0.765</td>
<td>0.111</td>
<td>0.0145</td>
</tr>
<tr>
<td>log (CRP)</td>
<td>0.107</td>
<td>0.055</td>
<td>0.0532</td>
<td>0.110</td>
<td>0.054</td>
<td>0.0399</td>
<td>1.710</td>
<td>0.203</td>
<td>0.0070</td>
</tr>
<tr>
<td>Monocyte Count (1000s)</td>
<td>-0.098</td>
<td>0.454</td>
<td>0.7236</td>
<td></td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td>0.0070</td>
</tr>
<tr>
<td>Neutrophil Count (1000s)</td>
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<td>0.028</td>
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<td>0.0192</td>
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<td>0.0282</td>
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<tr>
<td>Erythr. Sed. Rate</td>
<td>0.015</td>
<td>0.006</td>
<td>0.0188</td>
<td>0.013</td>
<td>0.006</td>
<td>0.0367</td>
<td>-0.036</td>
<td>0.018</td>
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<tr>
<td>HDL</td>
<td>0.003</td>
<td>0.001</td>
<td>0.0261</td>
<td>0.004</td>
<td>0.001</td>
<td>0.0026</td>
<td>-0.009</td>
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<td>0.0125</td>
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<td>Age</td>
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<td>&lt;0.0001</td>
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<td>Male</td>
<td>-0.073</td>
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<td>AIC</td>
<td>1702.707</td>
<td></td>
<td></td>
<td>1850.296</td>
<td></td>
<td></td>
<td>1167.6531172.456</td>
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<td>0.1970·189*</td>
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<tr>
<td>Adj. R Squared</td>
<td>0.133</td>
<td></td>
<td></td>
<td>0.141</td>
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<td></td>
</tr>
</tbody>
</table>

* 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. 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.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>45-54</th>
<th>55-64</th>
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8
3.2 Parasite and Pathogen burden. Tsimane face an elevated parasite and pathogen load compared to industrial populations. 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-occurring. 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. Immunoglobulin E (IgE) is extremely elevated (8182 IU/mL) compared to a representative US sample (52 IU/mL). 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.

The high parasite burden among the Tsimane is associated with elevated TH2 immune activation, which includes relatively high levels of IL-5 and IL-10, as well as elevated white blood cell counts, CRP, and ESR. 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.

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.

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<td>30·9</td>
<td>41·3</td>
<td>38·8</td>
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| 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


