The indigenous South American Tsimane exhibit relatively modest decrease in brain volume with age despite high systemic inflammation

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Abstract

Brain atrophy is correlated with risk of cognitive impairment, functional decline, and dementia. Despite a high infectious disease burden, Tsimane forager-horticulturists of Bolivia have the lowest prevalence of coronary atherosclerosis of any studied population and present few cardiovascular disease (CVD) risk factors despite a high burden of infections and therefore inflammation. This study (A) examines the statistical association between brain volume and age for Tsimane, and (B) compares this association to that of three industrialized populations in the U.S. and Europe. This cohort-based panel study enrolled 746 participants aged 40 to 94 (396 males), from whom computed tomography (CT) head scans were acquired. Brain volume (BV) and intracranial volume (ICV) were calculated from automatic head CT segmentations.

The linear regression coefficient estimate $\hat{\beta}_T$ of the Tsimane (T), describing the relationship between age (predictor) and BV (response, as a percentage of ICV), was calculated for the pooled sample (including both sexes) and for each sex. $\hat{\beta}_T$ was compared to the corresponding regression coefficient estimate $\hat{\beta}_R$ of samples from the industrialized reference (R) countries. For all comparisons, the null hypothesis $\beta_T = \beta_R$ was rejected both for the combined samples of males and females, as well as separately for each sex. Our results indicate that the Tsimane exhibit a significantly slower decrease in brain volume with age than populations in the U.S. and Europe. Such reduced rates of brain volume decrease, together with a subsistence lifestyle and low cardiovascular disease risk, may protect brain health despite considerable chronic inflammation related to infectious burden.

Keywords

Brain aging, cardiovascular disease, neurodegeneration.

Introduction

Ageing-related brain atrophy is strongly associated with elevated risk of cognitive impairment, functional decline, and dementia, and is moderated by factors like diet, smoking and physical activity. Brain atrophy trajectories are positively associated with cardiovascular disease (CVD) risk factors (1) like high cholesterol, diabetes, and hypertension, which are widespread in modern, industrialized populations (2). Thus, populations with a lower prevalence of CVD risk factors are hypothesized to exhibit *slower* brain volume (*BV*) loss and lower risk for associated cognitive decline. On the other hand, populations with high infectious and inflammatory burdens are hypothesized to exhibit *faster BV* loss (3, 4) because systemic inflammation predicts greater brain atrophy (5-7).

Indigenous Tsimane forager-horticulturists of lowland Bolivia have a lifestyle similar to that of subsistence populations prior to mechanized agriculture, urbanization and industrialization. The ~16,000 members of this population live by farming, hunting, gathering, and fishing in the Amazon basin, and have a documented high burden of infections and inflammation (8), including increased systemic inflammation (9). This is reflected by their biomarkers of chronic immune activation, including higher leukocytes counts, faster erythrocyte sedimentation rates, and higher levels of C-reactive protein (9), interleukin-6 and immunoglobulin-E (10) than in Americans of all ages (9, 11, 12). The Tsimane feature endemic polyparasitism involving helminths (13) and frequent gastrointestinal illness; most morbidity and mortality in this population being due to infections (9, 14). The higher inflammatory burden among the Tsimane suggests the hypothesis that they exhibit *steeper* negative associations between *BV* and age compared to populations with relatively lower systemic inflammation. Despite their inflammatory load, however, Tsimane have the lowest prevalence of coronary atherosclerosis of any studied population (8) and present few CVD

risk factors like hypertension (15), obesity, type 2 diabetes and hyperlipidemia (16, 17). Additionally, Tsimane have CVD protective factors like high levels of physical activity starting early in life (18), and a diet that is rich in fiber, omega-3 fatty acids and polyunsaturated fatty acids (19), but low in saturated fats and preservatives (20). Thus, to the extent that CVD and cognitive decline risk factors are shared (21), one might hypothesize a shallower negative association between brain volume and age for Tsimane compared to industrialized populations. These divergent hypotheses were tested in this cohort-based panel study.

Methods

Study design and participants. The Tsimane Health and Life History Project (THLHP) team has been studying the Tsimane since 2002, covering 85 villages and sampling persons aged 40 or older in a cohort-based panel design. The team visits Tsimane villages regularly and undertakes clinical examinations to study within- and cross-population variations in aging, including studies of infections and inflammation, coronary atherosclerosis, diet, and physical activity, as described above. The cross-sectional sample of this study includes individuals who self-identified as Tsimane and who were aged between 40 and 94. The Tsimane exhibit a pyramidal population structure, with relatively fewer persons aged over 60. For this reason, all individuals over 60 were screened for enrollment. Potential participants aged 40-59 were identified for screening at the community—rather than individual—level. This approach was adopted for reasons of cultural appropriateness, so that no potential volunteer would feel either targeted or excluded. All Tsimane over 60 participated except for 81 individuals; 9 declined to participate, 17 were not in their home communities at the time of the study, 9 were busy with subsistence activities, one person was hospitalized, and 49 individuals wanted to participate in the future but had prior obligations at the time of scanning. There were no

significant differences in age or sex for participants and non-participants over age 60 (age: t_{103} = 1.98, Cohen's d = 0.27, p = 0.95; sex: t_{114} = -0.81, Cohen's d = -0.10, p = 0.58). A total of 746 participants (396 males) participated in the study. Included were 143 participants under age 50, 239 between 50 and 59, 226 between 60 and 69, 105 between 70 and 79, 29 between 80 and 89 and 4 aged 90 or above. Participants were compensated for their time.

CT acquisition. Participants were bussed to the city of Trinidad, Bolivia, whose CT imaging facility is closest to the Tsimane villages. Although MRI would have been preferable to CT, providing participants with transportation to an MRI scanner facility was not logistically feasible. A licensed radiological technician acquired head CT scans using a 16-detector row scanner (GE BrightSpeed, Milwaukee, WI, USA). At least three team clinicians supervised and reviewed the scans. Images were acquired clockwise, in helical mode, with a standard convolution kernel, and with two reconstructions: one with a voxel size of 1.25 mm × 1.25 mm × 1.25 mm, and another with a voxel size of 0.625 mm × 0.625 mm × 0.625 mm. Additional parameters included a kilovoltage peak (kVp) of 120 kV, a data collection diameter of 250 mm, a mean exposure time of 1417 ms, an X-ray tube current of 140 mA, and a focal spot of 0.7 mm.

CT segmentation. CT volumes were segmented as described extensively elsewhere (22). Briefly, a probabilistic classification method (23) was used to perform tissue classification; voxel intensity values were used to assign their probabilities of belonging to one of several tissue classes by estimating the intensity distributions parameters of each class. The segmentation approach assigns each CT voxel in the cranium to either BV, or $CSFV$, such that $ICV = BV + CSFV$. The distribution of image intensities was modeled by a mixture of clusters, each consisting of Gaussian random variables (24). By estimating the parameters of

the intensity distributions for each class, voxel intensity information can be used to assign each voxel a probability of belonging to each tissue class. First, an objective function derived from a mixture of Gaussian random variable models is derived, and the value of this function is minimized using a parameter optimization process. *A priori* tissue probability maps specified by an atlas are leveraged to assist this classification. This probabilistic atlas is used to specify the prior probability for each voxel to belong to any tissue class in the Gaussian mixture model. The objective function weighs the probability maps of the atlas using Bayesian inference and then deforms the maps so that they match the volumes to be segmented. At this stage, the atlas template is warped to each subject's brain volume (25), the latter is segmented and the spatial classifications are smoothed (26). Model parameters are optimized using an expectation maximization (EM) algorithm (24), where the Gaussian mixture and deformations are reassessed iteratively and deformations are optimized using a Gauss-Newton scheme (27). When integrated with *a priori* information provided by the template, Bayesian inference facilitates the calculation of posterior probabilities based on each subject's voxel intensities.

CT-to-MRI validation. We validated our CT segmentation approach using a sample of 24 US adults aged 54 to 75 who had both MRI and CT scans of the head. In this validation study, CT scan parameters and spatial resolution were like those of the CTs acquired from the Tsimane. T_1-weighted MRI volumes were acquired at 3 T in a Prisma MAGNETOM Trio TIM scanner (Siemens Corp., Erlangen, Germany) using a magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence with the following parameters: repetition time (T_R) = 1,950 ms; echo time (T_E) = 3 ms; inversion time (T_I) = 900 ms; flip angle (FA) = 9 degrees; percentage sampling = 100; pixel bandwidth (BW) = 240 Hz/pixel; matrix size = 256 × 256; voxel size = 1 mm × 1 mm × 1 mm. In the CT-to-MRI comparison, CT

segmentations were validated against MRI segmentations which had been implemented using Freesurfer 7.0 software, as detailed elsewhere(22). Three quantitative measures were calculated: (A) the Sørensen-Dice coefficient (conveying the overlap between CT and MRI tissue labeling maps), (B) the Hausdorff distance (a measure of how far the CT- and MR-based boundaries are between tissues), (C) the intraclass correlation coefficient (a metric of measurement reproducibility which is useful when distinct techniques are used to measure the same empirical quantity). The Sørensen-Dice coefficient was found to have a Gaussian distribution with mean μ = 89.3% and standard deviation σ = 0.85% for the brain; for ventricular CSF, μ = 92.2% and σ = 0.175%. The average Hausdorff distance and its $\pm 2\sigma$ interval were 2.1 ± 0.9 mm for the brain, and 2.5 ± 1.3 mm for ventricular CSF. The intraclass correlation coefficient was 0.78 for the brain and 0.74 for CSF. Together, these metrics were found to indicate segmentation quality which was adequate for the purpose of BV calculations. Across the entire validation sample, the mean percentage difference between CT- and MRI-based volumetrics and its $\pm 2\sigma$ interval were found to be ~0% ± 3.2% for BV and ~0% ± 2.6% for $CSFV$. Thus, on average in this small sample, our CT- and MRI-derived measures differ by a negligible amount, confirming that the CT-based volumetrics can be compared to the MRI-based measures with little average inter-modality error.

Tsimane comparison to other cohorts. We compared the Tsimane regression coefficients β_T to those calculated from two MRI studies of aging. The first study, by Schippling *et al.* (28), involved 564 participants scanned in either Hamburg, Germany (N = 248) or St. Louis, USA (N = 316). This study was included partly because it utilized a segmentation technique for MRI scans similar to ours used for CT scans, which adds to the comparability of β_T and β_R. The second study, by Vinke *et al.* (29), investigated 5,286 participants in the Rotterdam Study, a population-based study of age-related diseases. Both Schippling *et al.* and Vinke *et*

al. analyzed MRIs with voxel sizes equal or less than 1 mm^3, thus satisfying the typical spatial resolution requirements of today's MRI studies.

For statistical comparison of our BV trajectories to those of Schippling *et al.* and Vinke *et al.*, we compared their slopes using linear models. For Schippling *et al.*, who had provided regression coefficients for five-year age intervals, β_R was computed as a weighted average over the age interval from 40 to 70 years (yr). Weights equaled the values of the empirical probability density function associated with the ages of Tsimane participants, thereby allowing us to eliminate the potential confounding effect of differences in age distributions across cohorts. For Vinke *et al.*, β_R was calculated based on the volumetric trajectories and data reported in **Figure 1A** and in **Table 3** of this publication. The volumetric trajectories of Vinke *et al.* are approximately linear within the age intervals [45, 70] yr and [70, 95] yr. Within each of these intervals, β_R is therefore well approximated by $\Delta V/\Delta t = [V(a_2) - V(a_1)]/(a_2 - a_1)$, i.e., by the difference in volume V divided by the difference in age a across the interval from a_1 to a_2. The value of β_R within the full interval [45, 95] yr was calculated as the average value of $\Delta V/\Delta t$ over the intervals [45, 70] yr and [70, 95] yr. Although neither reference study reports their model parameters, both report nonlinear relationships with age. For this reason, we explore nonlinear (quadratic) models for illustration (**Figure 1B**); the reader is referred to the study of Irimia (30) for further quantitation, comparison and meta-analysis of both linear and nonlinear models for BV, WMV, GMV and $CSFV$.

To gain insight into potential neuroanatomic differences between the Tsimane and the reference populations as a function of age, three representative Tsimane female subjects (ages: 40, 60 and 80 yr, respectively) were selected. Three CT scan slices (axial, sagittal, and

coronal) from each subject were collated for illustration of typical age effects upon brain features within the sample (**Figure 2**). To highlight how such features can reflect discrepancies between Tsimane and the reference populations, we also selected two Tsimane subjects aged 80, one whose BV was closest to the regression line for the Tsimane (see continuous red trace in **Figure 1A**), and another whose BV was closest to the regression line for the reference population (see continuous black trace in **Figure 1A**). Although both subjects are Tsimane, the latter subject's brain CT features are representative of the neuroanatomic profile of an 80-yr-old subject in the reference population. The comparison between the two subjects is illustrated in **Figure 3**.

Statistical analysis. Ages were centered on their means prior to statistical analysis. We implemented univariate linear regressions to test the null hypothesis that the Tsimane linear regression coefficient β_T describing the association between age (predictor variable) and ICV-adjusted BV is equal to zero. Two-tailed Welch's t tests were used. The significance of sex as a predictor was explored, the age association was tested both across sexes and within each sex group, and the significance of the sex-by-age interaction was investigated. Statistical analyses of Tsimane data were implemented using IBM SPSS software, version 27.

The linear regression coefficients β_T of the Tsimane (describing associations between age and volumetrics) were compared against regression coefficients β_R reported elsewhere (28, 29) for samples from three industrialized countries (Germany, USA, and the Netherlands, see previous subsection). The null hypothesis $\beta_T = \beta_R$ was tested both within the full age interval [40, 95] yr and within each of the intervals [40, 70] yr and [70, 95] yr. For BV, the null hypothesis $\beta_T = \beta_R$ was tested at a significance threshold α of 0.05 using Welch's two-

tailed t test for independent samples with unequal variances. Regression coefficients were assumed to be normally distributed.

The variance estimates of the regression coefficients β_T and β_R were obtained via bootstrapping using 10,000 simulations. In each simulation, a random subsample S of size 300 was drawn from the set of Tsimane participants' ages, thus allowing $\sigma(\beta_R)$ to be estimated without the potentially confounding effect of age distribution differences between the Tsimane and the simulated samples. Then, for a simulated set of Western subjects with ages specified by S, ICV-normalized BV values were simulated, assuming that these volumes were normally distributed with parameters μ and σ equal to those reported by each of the two reference studies (i.e., Schippling *et al.* and Vinke *et al.*). In other words, for each reference study, each sample of simulated volumes had the same distribution as those in the reference sample, and the ages of the subjects whose volumes were simulated were those in the subset S of 300 subjects selected at random from the Tsimane sample. The regression coefficient estimate $\hat{\beta}_R$ was then calculated for each such realization. After 10,000 realizations we then computed the estimates $\hat{\mu}(\beta_R)$ and $\hat{\sigma}(\beta_R)$, i.e., the estimated parameters of the normally distributed, ICV-normalized BV values reported by the reference study in question.

Once $\mu(\beta_R) \simeq \beta_R$ and $\sigma(\beta_R)$ had been estimated, the null hypothesis $\beta_R = \beta_T$ was tested using Welch's t test. For all simulations, the error of the Tsimane participants' recorded ages was assumed to be normally distributed with zero mean and a worst-case-scenario value of σ equal to 1.5 yr. This assumption was made because Tsimane birth records are occasionally inaccurate by up to 3 yr (31). Similarly, to reflect uncertainties in the accuracy of CT-derived brain segmentations, the measurement error associated with BV was assumed to be normally distributed ($\hat{\mu}$ = 0%, $\hat{\sigma}$ = 0.8%), corresponding to a measurement error interval [-2$\hat{\sigma}$, 2$\hat{\sigma}$]

equal to [-3.2, 3.2] %, as observed empirically during validation (see also *CT-to-MRI validation*). The approach described above was used to estimate $\mu(\beta_I)$ and $\sigma(\beta_I)$ for each reference cohort. Bootstrapping simulations and statistical tests comparing the Tsimane to reference cohorts were implemented in MATLAB 2020a. *Post hoc* statistical power was calculated using G*Power 3.1.9.4 software. No hierarchical or complex design implementation was applicable to this analysis.

To evaluate the potential effect of mortality selection on the observed relationship between age and BV in the Tsimane, a Cox proportional hazards model was fit to the Tsimane brain CT sample data, including terms accounting for sex, age, and BV as a percentage of intracranial volume (ICV). To estimate the effect of differential mortality, we simulated hypothetical trajectories of brain aging with and without mortality selection. This begins with the cohort of Tsimane under age 60 at scan time and simulates aging and mortality in each subsequent year of life. During each year included in the simulation, each participant's BV atrophies by some specified amount. The expected mortality is then estimated directly from the Cox model and depends on each simulated BV and age in each year. Mortality selection occurs as individuals with smaller brains are more likely to be removed from the sample, thereby increasing the average BV of remaining participants.

Results

All Tsimane adults aged over 60 yr were invited to enroll, whereas those aged 40-59 were sampled from a subset of representative communities. The study was approved by the University of New Mexico Health Sciences Institutional Review Board (HRRC 07-157; 15-133). After explaining the procedure and risks in the Tsimane language, informed consent was obtained from the Tsimane government, village leadership, and participants. Because

magnetic resonance imaging (MRI) was logistically unfeasible, low radiation-dose computed tomography (CT) head scans were acquired from 746 participants aged 40 to 94 (396 males) who had accepted our invitation for transportation to the nearest CT imaging facility. BV and ICV were calculated from automatic head CT segmentations (22). Volumetrics were normalized by ICV (i.e., BV as percent of ICV was calculated) to alleviate the confounding effect of head size.

The linear regression coefficient estimate $\hat{\beta}_T$ of the Tsimane (T), describing the relationship between age (predictor) and BV (response, as a percentage of ICV), was calculated for the pooled sample (including both sexes) and for each sex. $\hat{\beta}_T$ was compared to the corresponding regression coefficient estimate $\hat{\beta}_R$ of samples from three industrialized reference (R) countries. Specifically, $\hat{\beta}_R$ was extracted from two reference MRI studies (28, 29) that were the most methodologically comparable to our CT study, based on criteria related to image processing and quality. Schippling *et al.* (28) had a combined sample (N = 564) of healthy volunteers aged 35 to 75 from Hamburg, Germany (N = 248) and St. Louis, USA (N = 316). Vinke *et al.* (29) studied 5,286 neurologically healthy participants from Rotterdam, the Netherlands. The null hypothesis $\beta_T = \beta_R$ was tested at a significance threshold α of 0.05 across each of two distinct age intervals (40 – 70 yr and 70 – 95 yr), and across the combined age interval 40 – 95 yr. Across a variety of functional and cognitive aging indicators, change accelerates around 70 yr (32), which was selected to define the boundary between the former two intervals.

Values of $\hat{\beta}_T$ are -0.228%/yr for males and -0.200%/yr for females (**Table 1**). The model including both sexes shows a small significant main effect of male sex reducing BV; however, the age-by-sex interaction is not significant, indicating that slopes do not differ

significantly by sex. **Figure 1A** shows ICV-normalized BV and its age-dependent trajectories for Tsimane and for reference samples. On the interval 40 – 70 yr, $\hat{\beta}_T$ = -0.171%/yr is significantly shallower than $\hat{\beta}_R$, which equals -0.307%/yr for Schippling et *al.* and -0.225%/yr for Vinke *et al*. (**Table 2**). Strikingly, on the interval 70 – 95 yr, the Rotterdam estimate $\hat{\beta}_R$ = -0.507%/yr is almost twice the value of $\hat{\beta}_T$ = -0.271%/yr. The null hypothesis $\beta_T = \beta_R$ was rejected for the entire age interval 40 – 95 yr both for the combined samples of males and females in the two reference MRI studies, as well as separately for each sex (**Table 3**).

Figure 2 displays axial, sagittal and coronal CT slices from three representative Tsimane female subjects of ages 40, 60 and 80, respectively. Visual comparison of these CT slices reveals progressive aging of the gray matter and white matter (GM and WM; white and yellow arrows, respectively). Neuroanatomical features of GM aging include GM volume loss reflected by gyral shrinking and sulcal widening in regions like the medial frontal lobes (*cf.*, inferior frontal sulci), lateral and medial temporal lobes (*cf*. superior temporal sulci, temporal opercula), parietal lobes (*cf*. parietal opercula), insulae (*cf*. circular sulci), and hippocampal formations. WM aging features include enlargement of the lateral and third ventricles, as well as brain stem atrophy (e.g., at the boundary between the pons and the medulla oblongata, anterosuperior to the olivary nuclei). Cerebellar atrophy reflects both GM and WM loss in this structure.

Figure 3 highlights typical neuroanatomic differences between Tsimane and the reference populations of Vinke *et al*. This figure displays CT slices from two Tsimane subjects of age 80; one subject's BV is closest to the Tsimane regression line (see continuous red trace in **Figure 1A**), whereas the BV of the other is closest to the reference regression line (see

continuous black trace in **Figure 1A**). The BV of the second subject (who is representative of the reference population) is ~6.5% larger than that of the first subject (who is representative of Tsimane). The comparison highlights the likely typical extent and features of neuroanatomy differences in old age between the Tsimane and the reference population.

The results of the Cox proportional hazards model are summarized in **Table 4**, which implies that, when BV increases by 1%, the mortality hazard decreases by about 5% (HR = 0.944). Although the effect is not statistically significant (p = 0.196), it is large enough to have potential biological significance, thus justifying the simulation already described to estimate differential mortality effects. The results of this simulation suggest that, when accounting for mortality effects, the observed regression coefficient $\hat{\beta}_T$ = -0.2163%/yr for individuals with ages in the interval [40, 95] yr (**Table 2**) corresponds to a mortality-adjusted coefficient of $\hat{\beta}_T$ = -0.2240%/yr, which is 3.5% more negative than that observed. Similarly, for individuals with ages in the interval [70, 95] yr, accounting for mortality selection implies that the observed regression coefficient $\hat{\beta}_T$ = -0.2706%/yr (**Table 2**) should be ~4% more negative, i.e., that the actual BV decrease rate $\hat{\beta}_T$ is about -0.281%/yr.

Discussion

Although our CT segmentation approach (22) was tested, validated, and compared thoroughly to MRI, this study has limitations. The statistical comparison between Tsimane and the studies of European and US samples relies on a comparison between CT-derived and MRI-derived volumes, respectively, rather than on random sampling and matching of participants scanned using the same neuroimaging modality. While different survival biases across Tsimane and reference samples are potential confounds, the small magnitude of the estimated mortality effect on the slope of BV as a function of age suggests that mortality

selection alone is unlikely to account for the large differences in brain volume trajectory between Tsimane and reference samples. Nevertheless, because this study is cross-sectional, inferences on within-individual change in brain atrophy with age await longitudinal data. A total of 567 participants (76% of our sample) constituted 80% of the sample studied by Kaplan *et al* (8), and 685 participants (92% of our sample) constituted 52% of the sample of Rowan *et al.* (33). Thus, although our sample is not identical to those of Kaplan *et al.* and Rowan *et al.*, the biometric profiles of the three samples are likely similar insofar as inflammatory, ccardiovascular, and metabolic parameters are concerned.

In conclusion, the Tsimane exhibit smaller age-related *BV* declines relative to industrialized populations, suggesting that their low CVD burden (8) outweighs their high, infection-driven inflammatory risk. If (A) the cross-sectional data (which we believe are population-representative of Tsimane adults aged 40 and over) represent well the average life course of individuals and if (B) the Tsimane are representative of the baseline case prior to urbanization, these results suggest a ~70% increase in the rates of age-dependent *BV* decrease accompanying industrialized lifestyles. Despite its limitations, this study suggests that brain atrophy may be slowed substantially by lifestyles associated with very low CVD risk, and that there is ample scope for interventions to improve brain health, even in the presence of chronically high systemic inflammation. Lastly, the slow rate of age-dependent *BV* decrease in the Tsimane raises new questions about dementia, given the role of both infections and vascular factors in dementia risk.

Statement of competing interests

The authors state that they have no perceived or actual competing interest.

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Author contributions

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Tables

Table 1. Regressions of age and sex (predictor variables) against ICV-normalized BV (response variable) in the Tsimane. Intercepts, linear regression coefficient estimates ($\hat{\beta}_T$), standard errors of the mean (SEMs), Student's t statistics, 95% confidence intervals (CIs), effect sizes (partial η^2) and observed power are reported. BV and age are significantly associated both when each sex (M, F) is considered separately, and when the sexes are considered together (M&F). Ages were centered on their mean prior to statistical analysis. For all statistical tests, p-values are smaller than 1.0×10^{-3}. The age-by-sex interaction was not found to be significant (p = 0.234) but the test is underpowered (power = 0.221).

group	variable	$\hat{\beta}_T$	SEM	t	CI		partial η^2	power
M	intercept	84.801	0.170	499.798	[84.467,	85.134]	0.998	1.000
	age	-0.228	0.016	-13.893	[-0.260,	-0.196]	0.327	1.000
F	intercept	85.698	0.170	503.324	[85.363,	86.032]	0.999	1.000
	age	-0.200	0.016	-12.340	[-0.232,	-0.168]	0.304	1.000
M&F	intercept	85.698	0.176	486.484	[85.352,	86.043]	0.997	1.000
	age	-0.200	0.017	-11.927	[-0.233,	-0.167]	0.160	1.000
	male sex	-0.897	0.241	-3.716	[-1.371,	-0.423]	0.018	0.960
	age × male sex	-0.028	0.023	-1.191	[-0.073,	0.018]	0.002	0.221

Table 2. The relationship between age in years (yr, predictor) and ICV-normalized BV (percentage of ICV, response) on the age intervals [40, 70] yr, [70, 95] yr and [40, 95] yr, as described by linear regression coefficient estimates $\hat{\beta}$ and their confidence intervals (CIs). Aside from values for the Tsimané on the age intervals [40, 70] yr, [70, 95] yr and [40, 95] yr, results are also provided for the reference studies of Schippling *et al.* (28) (on the age interval [40, 70] yr) and Vinke *et al.* (29) (on age intervals [45, 70] yr, [70, 95] yr and [45, 95] yr). Age intervals are in yr; values of β and their CIs are in %/yr. On the age interval [70, 95] yr, the latter two quantities are not provided for the sample of Schippling *et al.* because this study did not include subjects older than 75 yr.

cohort	*N*	ages	$\hat{\beta}$	CI ($\hat{\beta}$)	ages	$\hat{\beta}$	CI ($\hat{\beta}$)	ages	$\hat{\beta}$	CI ($\hat{\beta}$)
Tsimane	746	[40, 70]	-0.1710	[-0.1731, -0.1689]	[70, 95]	-0.2706	[-0.2779, -0.2633]	[40, 95]	-0.2163	[-0.2192, -0.2135]
Schippling *et al.*	564	[40, 70]	-0.3069	[-0.3124, -0.3014]	[70, 95]	—	—	[40, 95]	—	—
Vinke *et al.*	5286	[45, 70]	-0.2246	[-0.2281, -0.2211]	[70, 95]	-0.5071	[-0.5106, -0.5036]	[45, 95]	-0.3649	[-0.3683, -0.3635]

Table 3. Results of testing the statistical null hypothesis $\beta_T = \beta_R$ using Welch's t test for samples with unequal variances. The number of degrees of freedom was approximated using the Welch-Satterthwaite equation. Results are reported separately for males (M), females (F) and for combined samples including both males and females (M&F). The null hypothesis was tested for the Tsimane against the samples of both Schippling *et al.* as well as Vinke *et al.* In the latter case, the null hypothesis could only be tested for the combined samples of males and females (M&F) because Vinke *et al.* did not state the numbers of males and females in their sample. Furthermore, because the trajectories of Vinke *et al.* differ substantially across the age intervals [45, 70] and [70, 95], the null hypothesis was tested separately within each of these intervals. In all cases, the threshold for statistical significance is α = 0.05. All tests have p-values smaller than 1.0×10^{-200} and power exceeding 99%.

study	Schippling *et al.*		Vinke *et al.*					
ages	[40, 70]		[45, 70]		[70, 95]		[45, 95]	
sex	t	df	t	df	t	df	t	df
M	-56.99	3961						
F	-81.90	4489						
M&F	-75.47	3388	-60.89	1477	-424.05	1516	-159.05	1469

Table 4. Cox proportional hazards model for the Tsimane, including terms for male sex, age, and BV as a percentage of ICV. The estimated coefficients $\hat{\beta}$ of the models are reported in addition to hazards ratios (HR) and to *p*-values, for a total of 676 Tsimane (37 deaths).

parameter	$\hat{\beta}$	HR	*p*
male sex	0.0406	1.501	0.237
age [yr]	0.0995	1.105	<0.001
BV [%]	-0.0577	0.944	0.196

Figure Captions

Figure 1. (A) Cross-sectional brain volume (BV) as a percentage of intracranial volume (ICV) and its age-dependent linear trajectories in the Tsimane (red), in the reference sample of Schippling *et al.* (blue) and in that of Vinke *et al.* (black). Dots represent Tsimane participants' BVs. Dash-dotted lines mark the range of trajectories within the confidence interval (CI) of the bootstrapping-estimated regression coefficient estimate $\hat{\beta}$ (*not* within the CI of the BV measurements themselves). The Tsimane and reference samples are assumed to have the same mean BV at ages 40 years (yr), to facilitate comparison of their slopes and to obviate the divergence of trajectories as a function of age. Note the small CIs of $\hat{\beta}$ across the MRI reference samples and the substantial divergence of the Tsimane CI (red) from it, particularly after age ~70 yr. The null hypothesis $\beta_T = \beta_R$ fails to be accepted both on the interval 40 – 70 yr (Tsimane vs. Schippling *et al.*: t_{3388} = -75.47, $p \simeq 1.0 \times 10^{-250}$, power ≃ 99%; Tsimane vs. Vinke *et al.*: t_{1477} = -60.89, $p \simeq 1.0 \times 10^{-250}$, power ≃ 99%) and on the interval 70 – 95 yr (Tsimane vs. Vinke *et al.*: t_{1516} = -424.05, $p \simeq 1.0 \times 10^{-250}$, power ≃ 99%). **(B)** Like (A), comparing quadratic models of BV trajectories as a function of age in the Tsimane (red) against quadratic models of the trajectories in Schippling *et al.* (blue) and in Vinke *et al.* (black). CIs and data points for individual Tsimane BV values are omitted to facilitate comparison. The curvilinear fit to the Tsimane data confirms and reproduces a slower rate of BV decrease in the Tsimane after age 70, in contrast to the reference samples, where faster rates of cross-sectional BV decline are observed.

Figure 2. Axial, sagittal and coronal CT slices for three representative Tsimane subjects (three females of ages 40, 60 and 80, respectively). Typical aging effects upon the gray matter (GM) and white matter (WM) are highlighted, with WM depicted by semi-transparent

beige overlays superimposed onto the CT scan images. GM aging features include overal shrinking of the gyri and widening of the sulci, particularly in the medial frontal lobes (axial inset arrows: cingulate sulci), lateral and medial temporal lobes (e.g., Sylvian fissures and temporal opercula), parietal lobes (e.g., parietal opercula), insulae (coronal inset arrows: circular sulci), and hippocampal formations (e.g., choroid fissures and ambient cisterns). WM aging is highlighted by enlargement of CSF structures (lateral and third ventricles) and by brain stem atrophy (sagittal inset arrows: boundary between the pons and medulla oblongata). Cerebellar atrophy (sagittal inset) reflects both GM and WM loss.

Figure 3. Neuroanatomic differences between a subject representative of the Tsimane trajectory at age 80 (first row, *cf*. continuous red trace in **Figure 1A**) and a subject representative of the reference trajectory at the same age (second row, *cf*. continuous black trace in **Figure 1A**). Axial, sagittal, and coronal CT slices are shown for each subject. Key neuroanatomic distinctions between the two subjects are highlighed by arrows within each inset and include the extent of atrophy along (A) the circular sulcus of the insula, (B) the precentral and central sulci, and (C) the hippocampal formation.

Figure 1

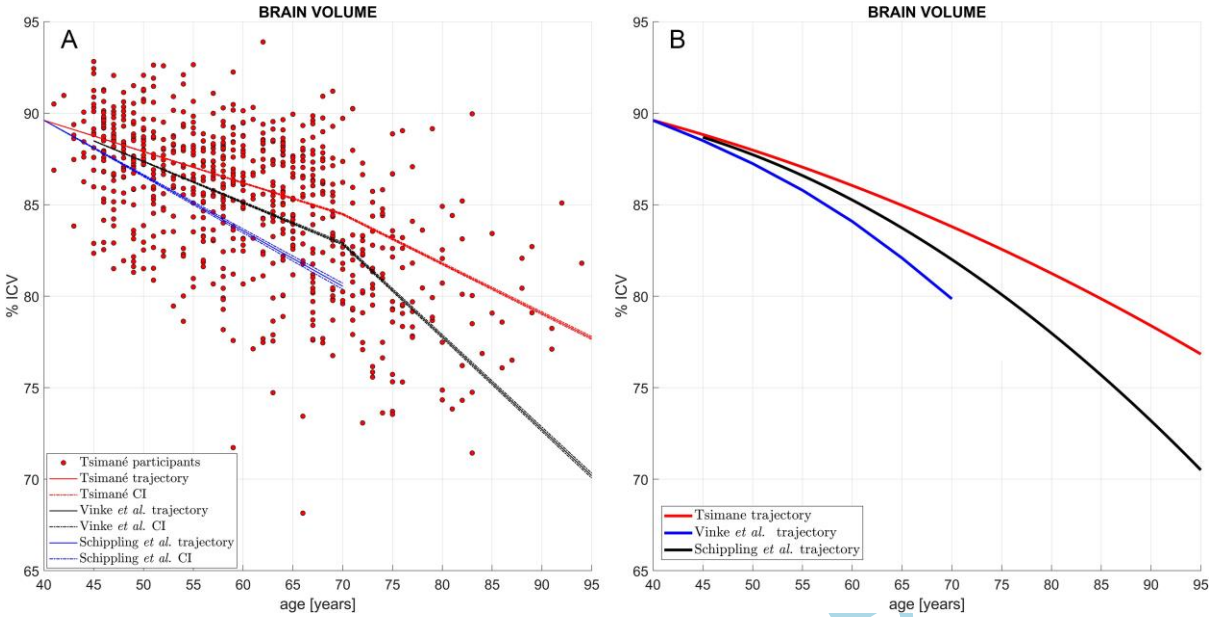

Figure 2

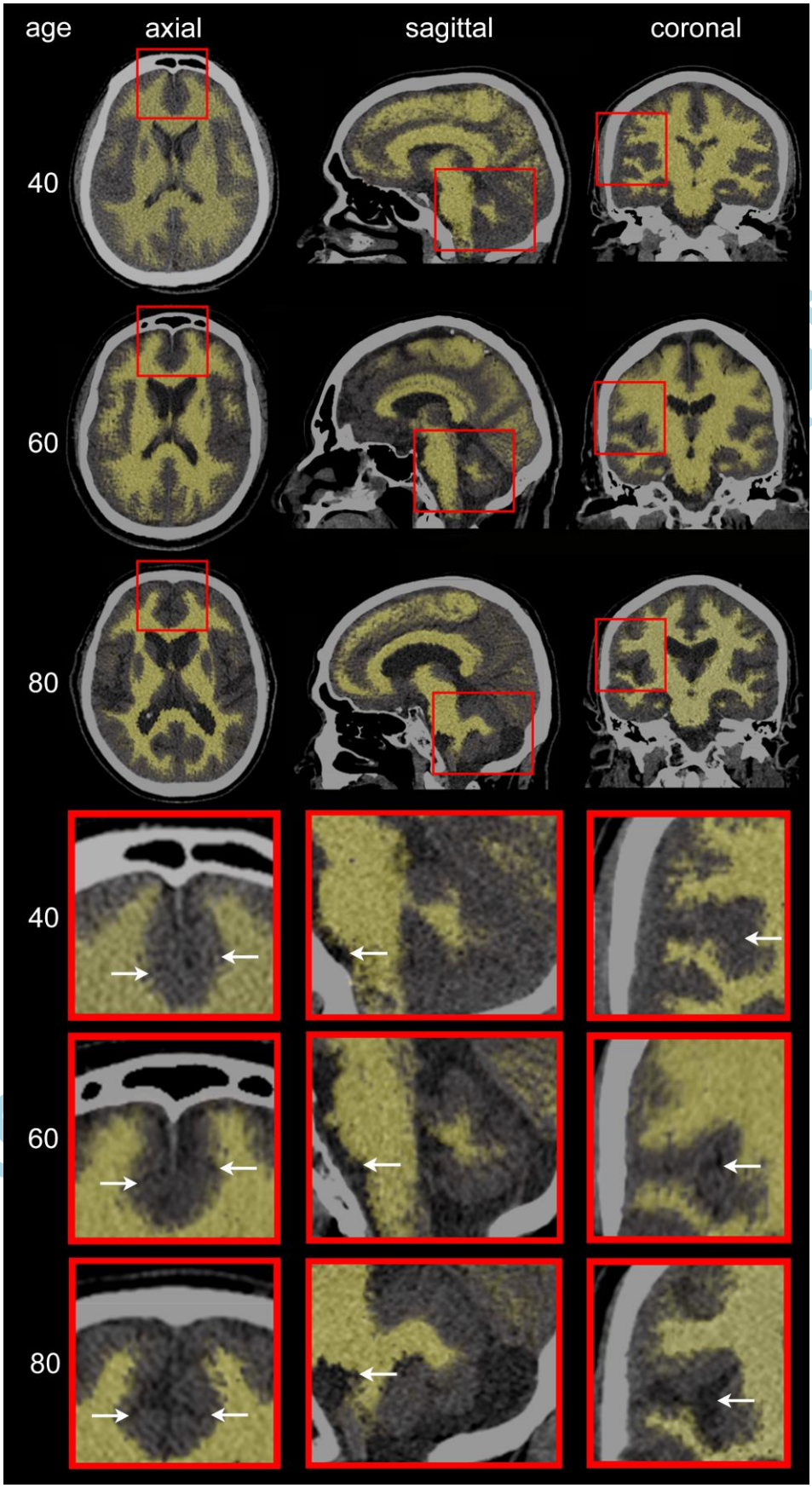

Figure 3