

Fig. S1. Data from Hadza in Tanzania. Sleep onset and offset occur in the dark. Sleep periods averaged over 60 nights, 10 participants (onset and offset SEMs are indicated). Average sleep onset is 3.4-h after sunset and offset is 1-h before sunrise. Temperature measurements are from Serengeti station near the Hadza encampment. The Hadza, at latitude -4 are the closest to the equator of the three groups examined, the San are the furthest (latitude -20) and the Tsimané fall in between (at latitude -15). In the Hadza, we can see the same temperatures excursion across the 24-h period and the same general relation of sleep and awakening with respect to this temperature cycle as in the other two groups. Supplement to figure 4.

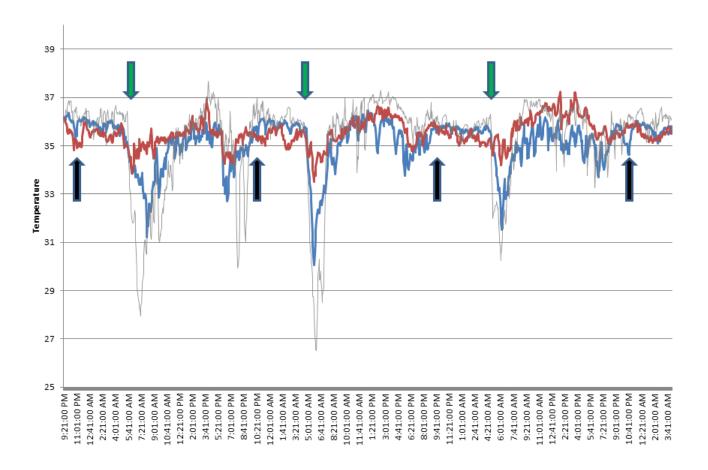


Fig. S2. Abdominal and finger temperatures from Fig 4 on an expanded scale. Sleep offset times are indicated with green arrows and onset times are indicated with black arrows. Red and blue traces illustrate group averages of abdominal and finger temperatures. The light gray trace shows representative finger temperature from an single participant. This is useful in illustrating that vasoconstriction is seen in individual, daily data. The data also shows that no vasodilation is seen in the individual raw data or in the averaged data, i.e. it has not been obscured in the averaged data by asynchronous occurrence of sleep onsets relative to sleep offset times. Smaller vasoconstrictions, not time-linked to wake onset, may be related to food preparation. Supplement to figure 4.

Table S1. Sleep duration in decimal hours (h), standard deviation (SD) of sleep duration in each subject, correlation between sleep duration and sleep onset and offset values A table is provided for each of the three groups examined that contained at least 6 days of recording. Seven subjects recorded for 4-5 days were used for mean values but not for correlation studies because samples with fewer than 6 pairs of value are not suitable for correlation studies. Sleep onset values are strongly correlated with sleep duration, such that earlier sleep onset is linked to longer sleep duration. However, sleep offset values are relatively weakly correlated with sleep duration. The San (and the other groups-see Fig. 1) do not often "sleep in" in the morning to compensate for delayed sleep onset. Significant relations are highlighted with yellow. Statistical analysis shows that the correlations between sleep onset time and sleep duration are significantly and substantially larger than those between sleep offset and sleep duration. Sleep onset and offset times are not strongly correlated. The binomial test shows that there is a significant difference in the number of significant correlations between sleep onset and sleep duration and sleep offset and duration in all 3 groups [Hadza p=0.01, Tsimane p=3.3E-10, San p=0.0006]. Values listed as 0.0000 have p values less than 0.0001 or r values of more than 0.99. See main manuscript sleep duration. [see spreadsheet for data]

Table S2. Information about participant groups

Night duration is from sunset to sunrise, clock time and times relative to solar noon are given, high and low temperatures are in °C. WASO (wake after sleep onset) amounts were comparable to those recorded in "modern" populations with the same devices (see refs. in main text), BMI=Body Mass index, Total days is the number of participant-24-h days recorded (recording duration x participants). Each group had equal number of females and males, except for groups with an odd number of participants. The Hadza were recorded from May 13-20, 2013. Overall there were 47 female and 47 male participants. ± indicates standard deviation between individual averages across recording period in each group. All groups are south of the equator, so northern seasonal timing is reversed. The Hadza are in the equatorial zone, so summer-winter designations do not apply. We label winter (W), spring (SP), summer (S). and fall (F) according to standard southern hemisphere designations. The Tsimane groups used different subjects at each time point. The San recordings used 8 of the same subjects in both the 5/14 and 1/15 recordings. Two the 10 subjects recorded during the 5/14 run had migrated out of Denui and could not be located for the summer recording. To keep the statistical treatments uniform and conservative, we used unmatched t tests in both the Tsimane and San groups in the body of the paper. See main manuscript: sleep duration, summer vs winter, BMI, light and temperature headings

GROUP/ MIDPOINT OF RECORDING	LATITUDE degrees	LONGITUDE degrees	NIGHT DURATION h	SLEEP TIME h	WASO (wake after sleep onset)	SLEEP EFFICIENCY =sleep time/time in "bed"	ENVIRONMENTAL TEMPERATURE RANGE	AGE	вмі	PARTICIPANTS	TOTAL DAYS
HADZA	-3.7315	35.1946	12.1	6.3±0.63	81±25	82±5.8	12-29	36.6±11.8	21.4±2.5	10	60
TSIMANE											
8/18/13-W	-14. <u>875</u>	-66.7282	12.4	6.6±0.93	82±17	83±6.0	14-29	38.0±10.7	26.1±3.9	9	63
9/1/13-W-SP	-14.875	-66.7282	12.2	6.4±1.03	92±16	81±4.6	15-33	35.2±14.3	22.8±3.3	9	63
10/3/13-SP	-14.875	-66.7282	11.7	6.4±1.12	72±13	85±5.54	18-28	32.2±10.7	26.2±3.5	10	70
10/18/13-SP	-14.875	-66.7282	11.5	6.5±0.32	64±17	86±4.2	22-31	44.4±23.7	25.4±4.8	7	70
10/29/13-SP	-14.875P	-66.7282	11.4	5.9±1.39	82±17	81±7.5	22-30	34.2±11.3	24.9±4.1	7	70
11/29/13-S	-14.875	-66.7282	11.1	5.7±1.15	86±8	82±5.5	23-34	37.0±15.1	24.2±3.3	12	84
SAN											
5/14-F-W	-19.803	20.6872	12.9	7.1±0.52	77±15	85±3.9	10-26	45.3±11.1	18.6±1.4	10	210
8/14-W	-19.803	20.6872	12.6	6.9±0.97	95±22	81±5.4	15-29	23.4±3.5	19.3±2.9	5	55
1/15-S	-19.803	20.6872	11.0	6.2±0.60	72±10	84±3.7	20-33	38.5±14.0	18.3±2.2	15	420
			AVERAGES→	6.4	80.3	83		36.5	TOTALS→	94	1165

Table S2: Information about participant groups (continued)

GROUP START DATE	SUNSET	ONSET CLOCK	OFFSET CLOCK	SUNRISE	SOLAR NOON	ONSET -SOLAR	ONSET - SUNSET	CENTER OF WAKE PERIOD	SOLAR NOON- OFFSET	SUNRISE - OFFSET	CENTER OF WAKE-SOLAR NOON
HADZA	18.57	21.98±0.6	5.68±0.41	6.63	12.60	9.38	3.41	16.30	6.92	0.95	3.70
TSIMANE											
8/18/13-W	18.33	21.36±0.76	5.55±0.48	6.68	12.52	8.84	3.03	15.81	6.97	1.13	3.29
9/1/13=W	18.37	21.03±0.85	5.01±0.42	6.53	12.45	8.58	2.66	16.02	7.44	1.52	3.57
10/3/13	18.40	22.13±1.8	5.68±0.46	6.13	12.27	9.86	3.73	16.45	6.59	0.45	4.18
10/18/13	18.43	21.60±1.8	5.14±0.55	5.97	12.20	9.40	3.17	16.46	7.06	0.83	4.26
10/29/13	18.48	21.16±0.4	4.93±0.85	5.87	12.17	8.99	2.68	16.23	7.24	0.94	4.06
11/29/13-S	18.72	21.26±0.97	4.39±0.72	5.78	12.25	9.01	2.54	16.87	7.86	1.39	4.62
SAN											
5/14 LF-W	18.12	21.27±0.41	5.61±0.41	6.02	11.57	9.70	4.15	15.66	5.96	0.41	4.09
8/11-W	17.42	21.38±0.61	5.85±0.51	5.97	11.70	9.68	3.96	15.52	5.85	0.11	3.82
1/15-S	18.35	22.74±0.56	6.22±0.73	5.21	11.83	10.91	4.39	16.52	5.61	-0.90	4.69

Table S3. Napping and nighttime awakening scored by Actogram program. See main manuscript: napping.

			NAPS	NIGHTIME AWAKENING			
	DAYS/		duration h	#	duration h		
SUBJECT SUBJECT			WINTE	R			
TS 11 DEN	21	0	0	0	0		
TS 12 DEN	21	0	0	2	1.5, 2		
TS 13 DEN	21	0	0	0	0		
TS 14 DEN	21	0	0	0	0		
TS 15 DEN	21	0	0	0	0		
TS 16 DEN	21	0	0	1	1.3		
TS 17 DEN	21	0	0	3	1.5, 2.5, 1.5		
TS 18 DEN	21	0	0	3	1.5, 3.5, 4.5		
TS 19 DEN	21	0	0	1	2		
TS 20 DEN	21	0	0	0	0		
		SUMMER					
DEC 13A	28	4	4, 3.5, 3.0, 3.0	1	2.5		
DEC 14A	28	0	0	0	0		
DEC 15A	28	0	0	0	0		
DEC 16A	28	0	0	0	0		
DEC 17A	28	0	0	1	0.5		
DEC 18A	28	2	2.0, 2.5	3	2.5, 1.0, 5.0		
DEC 19A	28	0	0	0	0		
DEC 20A	28	0	0	0	0		
DEC 11B	28	0	0	0	0		
DEC 12B	28	0	0	0	0		
DEC 13B	28	1	3	0	0		
DEC 14B	28	0	0	0	0		
DEC 15B	28	3	4, 3.5, 2.5	0	0		

Table S4. Higher standard deviation of sleep onset times than sleep offset (wake) times in 3 representative subjects. AVG=Average, SD=Standard Deviation. See main manuscript: sleep duration.

Subject #→	San Dec 19A	
SLEEP ONSET	DATE (+ next AM)	wake onset
24.93	Thursday 1/15/2015	5.63
22.32	Friday 1/16/2015	5.80
24.08	Saturday 1/17/2015	5.80
23.77	Sunday 1/18/2015	5.05
24.13	Monday 1/19/2015	5.97
20.98	Tuesday 1/20/2015	5.70
20.87	Wednesday 1/21/2015	5.27
20.93	Thursday 1/22/2015	6.55
25.58	Friday 1/23/2015	6.07
21.93	Saturday 1/24/2015	5.87
21.72	Sunday 1/25/2015	6.40
22.20	Monday 1/26/2015	4.83
21.67	Tuesday 1/27/2015	5.48
22.95	Wednesday 1/28/2015	5.48
23.50	Thursday 1/29/2015	6.82
22.32	Friday 1/30/2015	7.40
24.23	Saturday 1/31/2015	6.43
22.48	Sunday 2/1/2015	6.65
22.48	Monday 2/2/2015	6.60
12.30	Tuesday 2/3/2015	6.33
25.32	Wednesday 2/4/2015	5.80
21.95	Thursday 2/5/2015	6.55
24.67	Friday 2/6/2015	7.35
24.93	Saturday 2/7/2015	7.03
25.52	Sunday 2/8/2015	6.87
21.20	Monday 2/9/2015	7.78
23.12	Tuesday 2/10/2015	6.82
24.23	Wednesday 2/11/2015	5.70
22.73	AVG	6.22
2.52	SD	0.73

San Dec 20A							
SLEEP ONSET		wake onset					
23.42		5.73					
22.07		6.40					
23.88		6.47					
23.22		5.73					
24.50		5.98					
21.32		5.98					
24.87		6.63					
20.67		5.82					
25.90		6.02					
21.98		6.77					
21.53		6.68					
21.53		5.37					
24.25		5.85					
21.45		7.18					
22.32		7.47					
21.37		7.88					
20.42		7.38					
23.47		6.35					
23.33		6.63					
27.22		7.30					
24.98		5.73					
21.98		6.77					
25.45		6.93					
24.03		5.90					
23.38		6.88					
21.12		7.72					
21.73		7.63					
22.27		6.40					
22.95	AVG	6.55					
1.70	SD	0.68					

San D	ec 12	!B
SLEEP ONSET		wake onset
20.88		6.75
23.05		6.42
23.98		5.97
21.87		6.38
24.38		5.28
22.80		5.85
24.72		6.38
21.00		6.67
22.92		7.12
21.05		2.47
22.97		6.87
22.55		5.93
20.88		6.22
21.33		6.13
20.88		6.05
21.65		7.23
21.40		7.77
21.50		6.30
22.60		6.67
23.37		6.22
22.38		6.87
21.22		6.75
23.17		6.05
25.37		6.22
23.93		6.37
21.37		6.17
22.43		6.67
21.13		6.87
22.45	AVG	6.30
1.30	SD	0.88

Methods summary

Sleep was quantified with Actiwatch-2 devices worn for 4-28 days. Sleep states were scored by the Actogram program. We extracted the light and acceleration data and statistically compared these data with temperature, solar and seasonal variables. The San participants wore iButton temperature recorders on the middle fingers of both hands and on the abdomen for 4 days at the start of recording periods in both the summer and winter periods. iButton devices were also placed near the participants' sleeping sites to accurately measure environmental temperature and humidity at 4 min intervals.

Supplementary methods and discussion

<u>Participants:</u> The populations studied did not have electricity, generators or battery powered devices. All participants were interviewed with the assistance of indigenous translators. Those who were ill were excluded. Villages in which caffeine or alcohol use occurred were excluded and this was always confirmed in the individual participant interviews. Beyond this requirement, participants between 20 and 55, with equal numbers of males and females in each group, were randomly selected. Age and height were recorded using a portable stadiometer and BMI calculated. We do not see significant correlations between BMI and any other the other sleep variables. This may be because the BMI ranges were rather narrow, as were the range of sleep durations. But it is worth noting that even though a large portion of the sleep durations were at a level associated with obesity in the US populations [S1], none of the subjects were obese.

IRB approval for the San studies was obtained through the IRB of Witwatersrand University in Johannesburg, for the Hadza through Yale University and for the Tsimane through the University of New Mexico. Informed consent in the San was made with the supervision of the Nyae Nyae Development Foundation of Namibia. Each participant provided verbal consent to the research. The participants were informed of the goals of the study and were paid \$30 USD in gifts for their participation in the month long San studies. In case of Tsimane, they were given gifts for participation, with a value of 70 Bolivianos or about 10 US Dollars. The Hadza participants were provided gifts for their participation, with a value of 15,000 Tanzanian Shillings, or 10 US Dollars.

Each population eats locally acquired foods. The San eat meat from local game, baobab fruit, fish and berries. The Hadza eat game meat, baobab fruit, honey, tubers, and berries. The Tsimane eat a mix of hunted game, fish, and cultivated plant foods, primarily rice, manioc and plantains.

<u>Sleep conditions:</u> The Hadza sleep on animal skins on the ground, with Maasai style 'shuka' blankets. They sleep upon flat, circular sleeping spaces that are 2-2.5 meters in diameter. To make such a sleeping space, they clear the ground of thorns and rocks, and then place down either a kudu skin or an impala skin (both of which are prized for their soft hair), upon which they sleep. The Hadza data were collected during the wet season, and during such times, sleeping spaces are inside huts. The simple domed huts, open to the outside,

are framed with tree branches and thatched with grass. In the dry season, sleeping spaces are often open air. After sunset, a fire is usually set just at the entryway to a hut, or next to an open air sleeping area. Families sleep close together, 2-6 people often sharing a single sleeping space. They sleep either naked or with very little clothing. They cover themselves with light blankets, often made of cotton or cotton-nylon blends. San participants slept on a blanket on the ground, covered by an additional blanket, without pillows in the winter and without a blanket or much clothing in the summer. They sometime slept in grass huts with 1 meter wide openings, always completely exposed to the environment, and at other times outside the huts. The environmental iButtons were placed above ground at their sleeping sites. The Tsimane participants slept on beds above-ground made of tree-bark slats inside thatch-roofed houses with walls made of bamboo, other tree boles, or occasionally wood tablets. Most Tsimane beds have thin blankets.

Elevations:

Hadza, 1030 meters (Lake Eyasi, Tanzania)

San, 1150 meters (Tsumkwe, Namibia)

Tsimane; 197 meters (San Borja, Bolivia)

Actiwatch-2 devices: (Philips Respironics, Bend Oregon), were placed on the wrist of participants studied, in most cases remaining for at least 6 days to a maximum of 28 days (Table S2). Each record was visually inspected to identify and exclude instances of artifact. All collected data were scored by the "Actogram" program (Phillips Respironics) using the default 1-min setting that has been used in many prior studies. The sleep period analyzed was automatically selected by the software and not modified or selected by the investigators in >97% of cases. But all days of data were visually scanned for any discrepancies in the data selection of the major sleep period and corrected by defining the major sleep interval.

A Medline search for "(actigraphy or actiwatch or actigraph) and sleep" brings up 2,132 papers. Many papers have compared actigraphy to polysomnography as an indicator of sleep [S2, S5-S13]. It should be borne in mind that whereas polysomnography (PSG) is often considered the "gold standard" for identifying sleep it has long been known that when participants are in PSG defined sleep and are "awakened" they will frequently say they are not asleep [S14] This cognitive mismatch can occur in all sleep stages.

Conversely, when an actiwatch is used, and continuous movement is identified in a neurologically normal individual, it is highly likely that the participant perceives that they are awake. The main source of error in actigraphy is in somewhat overestimating sleep time. When no movement is occurring the participant need not be asleep, just immobile.

A recent comparison of the Actiwatch with simultaneous polysomnography concluded that the Actiwatch and its companion Actogram software, as used in the current study, had "a 97% sensitivity for sleep, a 96%

specificity for wakefulness and an overall accuracy of 96%." It further found that overall, "Actiwatch sleep time overestimated PSG sleep time by 26.4-min per sleep period (95% CI 18.0-34.8 min; P < 0.0001)"[S11]" consistent with some periods of inactive waking being scored as sleep. Marino et al. [S2] reported "Overall, sensitivity (0.965) and accuracy (0.863) were high. They "conclude that actigraphy is overall a useful and valid means for estimating total sleep time and wakefulness after sleep onset in field and workplace studies."

In our study of the Hadza and in all our studies of the Tsimané, conventional Actiwatch wrist bands were used. Such bands can easily be removed, although we instructed the participants not to do this. In the Tsimane participants we found a few periods of inactivity that contained an abrupt onset and zero or invariant light levels, as might be obtained when the Actiwatch was off the wrist. Therefore, in our studies of the San, which were done after the Hadza and Tsimane, we attached the Actiwatches with hospital bands (Wristband LLC, Atlanta) recommended by Respironics. They cannot be removed except by cutting the band. Repaired bands are obvious. In the few cases when bands were found to be cut, we discarded the data. The results in the San were qualitatively and quantitatively similar to those in the Hadza and Tsimane, in terms of nighttime sleep duration, sleep onset-offset and wake after sleep onset (WASO); however, somewhat fewer periods of abrupt interruptions of daytime activity were seen in the San Actiwatch data, consistent with our goal of preventing removal, so we focused our nap evaluations on the San. The number of days in each long duration recording was determined by travel and logistical constraints. All collected data are presented, with the exception described above.

Although nighttime actogram scoring has been extensively validated against polygraphic scoring, there have not been many validations studies for Actogram scored napping. A recent study concluded that Actogram scored haps were accurate, but that "Discrimination of sleep and wake during periods of waking quiescence is not as robust as during periods of mainly daytime sleep" [S3]. In our initial analysis, naps were automatically scored with a second automated analysis of the Actograms. This second analysis was done after the initial analysis of sleep parameters using the default Actogram settings that have been extensively validated using polygraphic recording. To do this second analysis, we used the Tools/Options/auto intervals/Automatically set major Rest intervals/uncheck detect only one rest interval per day/minimum minor rest interval size 15 min (the most sensitive setting for nap detection)/medium. This feature also served to identify periods of nighttime waking interrupting sleep. We find very low rates of napping (Table S3). In a second analysis, naps were scored visually by looking for periods of >15 min with activity rates at or below that in actigraphically detected sleep in the same subject. This produced somewhat higher rates of napping (see results), but this analysis also indicated very low levels of napping.

The Actogram analysis indicates that nighttime awakenings occurred on 10 of 210 recording days during the winter and 5 of the 364 days of recording during the summer.

In summary, most subjects sored visually (see Results) or by the Actogram program (see below) did not regularly nap during the winter or summer periods, although more napping occurred in the summer in the minority of subjects that showed any naps. The few subjects that did nap, did not do so regularly. Most subjects did not awaken during the nightly sleep period, with nighttime awakenings occurring on 10 of 210 recording days in summer and 5 of the 364 days of recording during the summer (Table S3). Future studies should do polygraphic recording combined with perceptual tests to more accurately identify nap intervals. However, since active, continuous movement is incompatible with napping, the current work sets an upper limit on how frequently such episodes occur. Overall we find that naps and nighttime awakenings are uncommon occurrences in all three groups.

References for actiwatch validation studies against polygraphic recording: see S2, S5-S13

iButtons: In addition to the Actiwatch, each participant in Namibia had iButton temperature recording devices taped to both middle fingers and a third such device taped to the abdomen. A description of iButtons can be found at: http://www.maximintegrated.com/. The use and interpretation of iButton data as an indication of vasoconstriction and vasodilation is reviewed in [S4]. We used Ds1821H iButton for skin temperature monitoring. We used Ds1922 for monitoring environmental temperature, with a resolution of 0.0625° C. Both types of iButtons are stainless steel disks 15 mm wide and 6 mm thick. They were attached with Fixomull medical tape to the middle finger of both hands, and to the midline of the abdomen, 2 cm above the umbilicus(28). The two iButtons placed on the middle finger were averaged for the analyses presented here. Ds1922 iButtons were placed off the ground and out of direct sunlight, adjacent to the customary sleeping sites, to measure ambient temperature. The temperature at these sites typically closely tracked the ambient temperature reported by the nearby Tsumkwe weather station, but with much higher temporal resolution. The iButtons were set at a 4-min sample interval and were removed after 4 days. Skin temperature data were analyzed according to clock hour and not sleep period, and thus any skin temperature associations with sleep was not directly determined by our analytic method. We present both average time of awakening in fig 2 and 4 and average vasoconstriction data in fig 4 and we present individual vasoconstriction data in figure S2. The iButton data was also used to exclude any data from a subject with a fever. Only one such participant was seen.

Calculation of solar variables: Precise latitude and longitude coordinates were determined with a Garmin Montana 650 device with microSD™/SD™ card TOPO Southern Africa 2013 Pro 010-11982-00, for the San recording site, and Google maps for the Hadza and Tsimané sites. Coordinates were used with the National Oceanic & Atmospheric Administration Solar Calculator at: http://www.esrl.noaa.gov/gmd/grad/solcalc/ to determine the times of sunrise, sunset and solar noon.

<u>Statistics:</u> All p values are indicated for two tailed probabilities.

Morning light: The marked dip in light exposure at midday may have implications for the control of circadian rhythms. Our results indicate that in both summer and winter in the San and Tsimane, as well as in the Hadza, who do not experience a large seasonal variation in light exposure, the light that participants are exposed to is at its peak in the morning and diminishes to approximately half this value at noon, despite the much greater intensity of sunlight at noon. In retrospect this makes sense from a thermoregulatory standpoint, preventing overheating at midday. Our findings suggest that the beneficial effect of morning light may be at least partly a result of the evolved adaptation of the melanopsin-suprachiasmatic system to morning light because this is the time our ancestors would receive their strongest light stimulus.

<u>Sex differences</u>: We tested for difference in sleep duration between males and females in the Tsimane, the group with the largest N (20 males, 26 females) of any of our groups. We did not see significant differences in sleep duration between males and female sleep durations (average sleep time in males, <u>6.20</u>±1.06h, females <u>6.54</u>±1.30h), p=0.311 2 tailed t test.

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