Habitual Sleep/Wake Patterns in the Old Order Amish: Heritability and Association with Non-Genetic Factors

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Study Objectives: We sought to evaluate the contribution of genetic and non-genetic factors on habitual sleep/wake patterns in a community-dwelling agrarian population using a physical activity monitoring device, the Actical.

Design: Cross-sectional population-based study of healthy Old Order Amish enrolled in the Heredity and Phenotype Intervention (HAPI) Heart Study.

Setting: Lancaster County, PA, USA.

Participants: 723 healthy adults (54% men) with a mean age of 43.3 ± 13.8 years (range: 20-80). 96% of the subjects were connected into one 5-generation pedigree.

Interventions: N/A.

Measurements: Participants wore Actical accelerometers 24 hours/day for 7 days to determine physical activity level, as well as habitual wake time, bedtime, and sleep duration. Participants completed the Horne-Östberg Morningness-Eveningness Questionnaire (MEQ), a modified Epworth Sleepiness Scale (ESS), and a lifestyle questionnaire. A sub-study of 164 participants kept sleep diaries.

Results: Habitual wake time and bedtime determined by Actical were highly correlated with results from sleep diaries (r = 0.82 for wake time and 0.72 for bedtime, both P < 0.0001). After adjustment for age, sex, occupation, and season, higher activity level was associated with earlier wake time but not with bedtime, and correspondingly with shorter sleep duration. After adjustment for the aforementioned factors and the effects of a shared household, habitual wake time, MEQ score, and ESS score showed significant heritability (wake time h^2 = 0.20, MEQ h^2 = 0.21, and ESS h^2 = 0.17).

Conclusions: Objectively measured wake time, self-reported morningness-eveningness preference, and daytime sleepiness appear heritable and wake time may be associated with physical activity level.

Keywords: Actical, physical activity, sleep phase, circadian, heritability, Old Order Amish, Horne-Östberg, MEQ, Morningness-Eveningness, Epworth, ESS, daytime sleepiness

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INTRODUCTION

The preferred wake time and bedtime, also referred to as the preferred sleep phase or the diurnal preference, is regulated by at least two factors: the endogenous circadian pacemaker and environmental stimuli.[1] The endogenous circadian pacemaker, commonly named the biological clock, resides in the suprachiasmatic nucleus (SCN) of the hypothalamus.[2] Environmental stimuli, such as light, exercise, social contact, and strict schedules, entrain the endogenous pacemaker to the environment.[3] Light, the most potent entraining factor, regulates the SCN by exciting retinal photoreceptors that connect to the SCN via the retinohypothalamic tract.[2]

Many diseases display a circadian pattern of risk and have been shown to be associated with sleep disturbance.[1] Epidemiologic studies have shown that both long and short sleep duration is highly prevalent in the United States[4] and is associated with many common diseases, such as hypertension,[5] obesity,[6] diabetes,[7] and coronary heart disease.[8] Shift workers who experience a misalignment between their endogenous circadian pacemaker and behavioral cycles face an increased risk for cardiovascular disease and cancer.[9] Recently, circadian misalignment has been directly manipulated in a small sample of people in a laboratory setting, resulting in adverse cardiometabolic effects.[10] Therefore, identifying biological and environmental factors that affect sleep/wake patterns may lead to interventions that reduce the incidence of many common disorders.

Much of what is known about the molecular mechanisms of the circadian pacemaker comes from studies of model organisms and Mendelian human sleep disorders. Two functionally conserved gene families, *Period* and *Cryptochrome*, play a central role in the control of the mammalian circadian pacemaker by engaging in an autoregulatory negative feedback loop.[2] In humans, mutations in components of the well-known circadian rhythm pathway have been found to result in rare Mendelian sleep disorders such as advanced or delayed sleep phase syndrome, which are estimated to affect less than 1% of the adult population.[2,11] Much less is known, however, about the genetic regulation of less extreme variation in sleep phase among community-dwelling individuals. The few studies that have been conducted rely on self-administered questionnaires or sleep diaries to determine sleep phase. Based on these instruments, a substantial degree of heritability (h^2) is suggested (h^2 estimates for diurnal preference, sleep length, and daytime sleepiness are 0.23–0.52,[12-17] 0.4–0.44,[12,18] and 0.38–0.48,[19-21] respectively). To date, a single genome-wide association study of daytime sleepiness, usual bedtime and usual sleep duration assessed by

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self-administered questionnaires in a community-dwelling population has been performed and evidence for significant heritability and genetic association for all three traits was found.[22]

In order to study the association between genetic/non-genetic factors and habitual sleep patterns, it is important to objectively measure sleep phase for multiple days in a large number of community-dwelling individuals. This poses the formidable challenge of accurately measuring a person's true sleep phase efficiently without being so invasive as to disrupt normal sleep habits. To this end, we explored the usefulness of Actical physical activity monitors (formerly Mini Mitter, Bend, OR, now Philips Respironics) to determine habitual bedtime and wake time. While the Actical device is mechanically and electronically similar to actigraphy devices used to measure sleep,[23] its software is optimized for the measurement of daytime physical activity level.[24,25] Moreover, our study participants wore the Actical on the hip, the recommended placement for physical activity measurement, as opposed to the wrist, the standard placement for sleep assessment.[23] Accelerometers worn on the hip to measure physical activity level are increasingly being used in large epidemiological studies, including the National Health and Nutrition Examination Survey (NHANES) for the 2003-2004 cycle and later cycles. Demonstrating the utility of such measurements for the assessment of sleep could potentially lead to the development of valuable resources for sleep researchers.

Our use of the Actical accelerometer to determine habitual bedtime, wake time, and sleep duration, a use for which it was not designed, compelled us to validate these sleep parameters using sleep diaries. Sleep diaries have been extensively used in sleep research since the 1960s and have been shown to provide reliable estimates of bedtime and wake time.[26-29] Upon observing strong correlations between Actical-based and diary-based sleep parameters, we sought to determine the extent to which Actical-based sleep parameters are heritable and associated with non-genetic factors. Study participants also completed the Horne-Östberg Morningness-Eveningness Questionnaire (MEQ)[30] to estimate diurnal preference and a modified version of the Epworth Sleepiness Scale (ESS)[31] to estimate daytime sleepiness. Both the categorical diurnal types and the continuous scores from the MEQ have been shown to be correlated with the endogenous circadian period.[32-35] We examined the contribution of genetic and non-genetic factors on these questionnaire scores as well as the relationship between questionnaire scores and Actical-based sleep parameters.

Factors that accompany urbanization and industrialization are likely to affect sleep.[3,36-39] We attempted to avoid much of the influence from these factors by conducting our study in the Old Order Amish (OOA), a conservative Christian sect residing in rural areas of Lancaster County, Pennsylvania. While the avoidance of modern electrical conveniences by the OOA likely reduces non-genetic influences on sleep, the OOA are still bound to schedules by occupational and social obligations. Little is known about sleep in rural populations,[40,41] and even less in populations that avoid modern technology.[14] This community-based study of the OOA offers a rare examination of sleep without the influence of industrialization. In addition to the traditional agrarian lifestyle of the OOA, the homogeneity of socioeconomic status and lifestyle is expected to facilitate the identification of genetic factors contributing to variation in habitual sleep by reducing sources of non-genetic influences. The heritability estimation of habitual sleep parameters was made possible by the use of data collected from extended pedigrees.[42]

METHODS

Study Population

The Heredity and Phenotype Intervention (HAPI) Heart Study has been previously described.[43] Briefly, the study population consists of 868 healthy adults (460 men and 408 women) from the Old Order Amish community in Lancaster County, Pennsylvania. Of the 868 study participants, 723 adults had complete measurements for Actical-based sleep parameters and covariates used in statistical analysis. The majority (96%) of the participants could be connected into a single 5-generation pedigree using information from AGDB.[44] The remaining 4% were either singletons (n = 14) or belonging to one of 5 small pedigrees, consisting of between 2 to 6 members. Institutional review board approvals were obtained from the University of Maryland, Baltimore for data collection and from the University of California, San Francisco for data analysis.

Sleep Measurements

Actical

The time of day when participants go to sleep (bedtime), the time of day when they wake up (wake time), and sleep duration were measured using Actical physical activity monitors. Actical detects motion using an omnidirectional accelerometer. Similar to most accelerometers used for sleep studies,[23] Actical has a detection bandwidth of 0.35-3.5 Hz. The Actical accelerometer detects 0.05-2 *g* gravitational forces in multiple directions.[45] With the recording interval set for 15 sec, study participants wore the Actical instrument on their hip for 7 consecutive days, 24 h/day. Data were uploaded from the activity monitor to a personal computer and Actical software version 2.04 was used to produce a visual representation of each participant's activity levels, called an actogram. To determine daily bedtime and wake time, 2 independent readers who were blinded to the sleep questionnaires and sleep diaries examined the actograms and estimated these sleep parameters to the nearest 30 min time point. Bedtime and wake time were determined from the pattern of activity using the guideline that these times are at the boundary between periods of inactivity lasting > 1 h and periods of activity lasting > 1 h. A representative example of a 7-day actogram with sleep/wake boundaries is shown in Figure 1. Daily values that were > 1 h apart between the 2 readers were excluded. Similar to previous approaches to estimate habitual sleep times,[46] daily Actical time points that were more than 2 h different from the participant-specific weekly average were excluded. Data from subjects with ≥ 4 days of valid sleep values were used to calculate the mean of these phenotypes. Data cleaning procedures only removed weekly habitual sleep parameters in 11 of the 777 (1%) subjects with Actical physical activity data. Among the 723 study participants with complete measurements from Actical-based sleep parameters and covariates, 61 (8%) had at least one discrepancy in bedtime or wake time between the 2 independent readers.

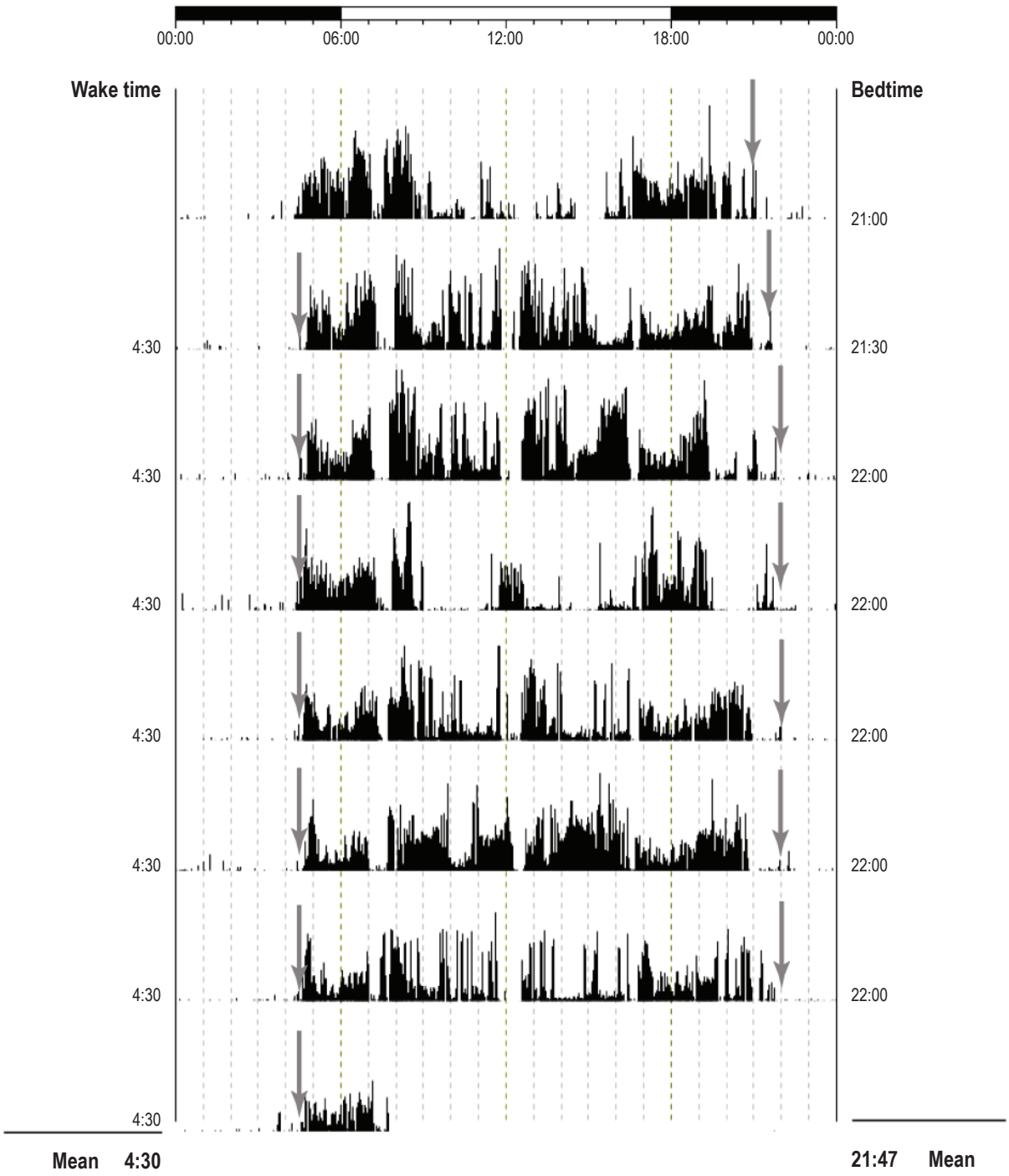

Figure 1—Representative 7-day actogram. Daily and mean wake time and bedtime are shown (h:min). Arrows indicate sleep/wake boundaries.

In addition to sleep parameters, total activity counts were determined for each participant during wake and sleep hours combined and during sleep hours only. Actical devices were version 8.2, 8.3, or 8.4, as identified by a serial number beginning with V82, V83, or V84, respectively. As we have found in separate methodological studies (S. Snitker, unpublished observations) that Version 8.4 records only 0.55 times as many total counts as the previous models, activity counts (awake and sleeping) from participants (n = 66) obtained by Version 8.4 were divided by 0.55. Actical device version was not associated with any of the Actical-based sleep parameters.

Sleep diaries

Sleep diaries were also administered to a subset of the study participants (260 participants). The bedtime from the previous night and the wake time were recorded by the participant in the morning. The wake time from the sleep diary came from the question, "What time did you wake up in the morning?" and the bedtime from the question, "What time did you begin to sleep?" referring to the previous night. Daily sleep diary time points that were > 2 h different from the participant-specific weekly average were excluded. The weekly average of a sleep outcome measured by sleep diaries required ≥ 4 days of recorded values.

Diurnal preference and daytime sleepiness

The Horne-Östberg Morningness-Eveningness questionnaire (MEQ) was administered using established scoring criteria.[30] Daytime sleepiness was evaluated using the Epworth Sleepiness Scale (ESS),[31] modified for Amish use. The original ESS asks the participant to rate their chance of dozing from 0-3 (3 being the highest chance of dozing) in 8 different situations. The ESS score, the sum of these 8 scores, ranges from 0 to 24. Two of these situations, "Watching TV" and "In a car, while stopped for a few minutes in traffic," did not apply to the Amish population, and were removed. As a consequence, the modified Amish ESS ranges from 0 to 18. In addition, the situations "Sitting, inactive in a public place (e.g., a theater or a meeting)," "As a passenger in a car for an hour without a break," and "Sitting quietly after a lunch without alcohol" were modified to "Sitting inactive in a public place (e.g., during church service)," "As a passenger in a vehicle for an hour without a break," and "Sitting quietly after lunch," respectively.

Statistical Analysis

Of the 260 participants who kept sleep diaries, 164 participants had Actical-based sleep parameters for the same days. The validation study of these 164 participants compared average wake time and bedtime from sleep diaries and Actical using Pearson correlation coefficients, paired Student 2-sided *t*-tests, and Bland-Altman plots.[47]

In order to estimate heritability and to identify non-genetic factors associated with habitual sleep quantitative traits, linear multivariate regression with variance component analysis was performed using the software SOLAR (Southwest Foundation for Biomedical Research, San Antonio, TX).[48] Relatedness was taken into account with a matrix of kinship coefficients. The non-independence of individuals within common households was taken into account using a variable assigning an identification number based on the street address of the study participants. Occupation was modeled using the occupation with the earliest habitual wake time, farming, as the reference. Season of the year when the Actical device was worn was modeled using winter as the reference and three indicator variables for other seasons. Winter was defined as months December through February, spring as March through May, summer as June through August, and fall as September through November. The napping variable from a lifestyle questionnaire indicates whether a participant made time for a nap never or rarely (less than once per week), or at least once per week. The amount of physical movement during sleep (sleep activity) was determined by quantifying the activity counts between bedtime and wake time. Accounting for only 1% of total activity levels, sleep activity levels were included in measures of total activity. In an attempt to reduce the potential confounding effect of more waking hours leading to an accumulation of higher total activity levels, daily and sleep activity levels were standardized by dividing by the number of waking and sleeping hours, respectively. The score on the shortened Epworth Sleepiness Scale was analyzed as is with no attempt to compensate for the fewer number of questions compared to the original scale. In regression analysis, total activity and sleep activity were transformed by taking the natural log, then outliers > 3 standard deviations from the mean were excluded. Residuals for all traits appeared to be normal based on skewness, kurtosis, QQ plots, and histograms.

RESULTS

There were slightly more males (388) than females (335) in the study population of 723 community-dwelling adults (P = 0.05) (Table 1). Combining both sexes, the mean age was 43.3 ± 13.8 years, and the range was from 20 to 80 years. Male subjects were significantly younger than female subjects (men: 41.9 ± 13.2; women: 44.9 ± 14.3 years; P = 0.003) (Table 1). Very few participants classified themselves as retired; 87% of men were farmers, carpenters, or mechanics, while 79% of women were housewives (Table 1). Napping was common in the study population, with 54% of men and 62% of women reporting that they made time in the day to take a nap at least once a week (Table 1). Total activity level was significantly higher in men than in women (men: $525.2 \times 10^3 \pm 269.6 \times 10^3$; women: $377.5 \times 10^3 \pm 210.2 \times 10^3$ counts/day; P < 0.0001), but sleep activity level did not differ by sex (Table 1).

Sleep parameters measured by Actical and sleep diaries were compared in 164 people from whom both sets of data were available. Wake time obtained from the 2 instruments was highly correlated, as was bedtime (wake time: $r = 0.82$; bedtime: $r = 0.72$; both P < 0.0001; Figure 2 A, B). On average, the diary wake times and bedtimes were only 6 and 11 minutes earlier, respectively, than those measured by Actical, but these differences were significant (P = 0.0004 and P < 0.0001, respectively). Bland-Altman plots indicate that there is no obvious relationship between the difference and the mean of the 2 measurements (Figure 2 C, D).

The mean Actical-based habitual wake time, bedtime, and sleep duration when both sexes were combined was 5:06 ± 00:44, 22:00 ± 00:44, and 7:05 ± 00:51, respectively. The Actical-based habitual wake time was significantly earlier for men (4:59 ± 45 min) than for women (5:14 ± 43 min, P < 0.0001) (Table 1). Actical-based habitual bedtime was not significantly different by sex, and consequently, habitual sleep duration was significantly shorter in men (7:01 ± 52 min) than in women (7:11 ± 49 min, P = 0.01) (Table 1). Based on the MEQ where lower scores indicate evening diurnal types, only 1% of the participants were classified as moderately evening types, and none were classified as definitely evening types (Table 1). Given the narrow range of diurnal types in the study population, continuous MEQ scores, which were not significantly different by sex (Table 1), were used in regression analysis. Based on the ESS where higher scores indicate higher levels of daytime sleepiness, men had higher levels of daytime sleepiness than women (men: 9.1 ± 3.4; women: 7.6 ± 3.2; P < 0.0001) (Table 1).

The association between sleep parameters and factors known to be associated with sleep phase and duration, including age, sex, season of the year, occupation, and activity level, was evaluated in regression models conditioned on the relatedness of the study participants (Table 2).[3,49] In general, factors associated with an unequal shift in wake time and bedtime were associated with a shift in sleep duration. The observation that sleep duration was significantly different by sex in univariate analysis (Table 1) but not multivariate analysis (Table 2) most likely reflects the high degree of collinearity between sex and the housewife occupation. Indeed, in multivariate analysis, the housewife

Table 1—Characteristics of the study population

	Males		Females	
Trait (unit)	**n**	**Mean ± SD (range) or %**	**n**	**Mean ± SD (range) or %**
Age (years)	388	41.9 ± 13.2 (20 – 77)	335	44.9 ± 14.3* (20 – 80)
BMI (kg/m^2)	388	25.6 ± 3.2 (18.4 – 37.9)	335	27.7 ± 5.4* (18.1 – 46.8)
Occupation				
Housewife	0	0%	266	79%
Farmer	159	41%	25	8%
Carpenter/mechanic	177	46%	0	0%
Shopkeeper	33	8%	30	9%
Craftmaker/teacher	8	2%	8	2%
Retired	11	3%	6	2%
Nap ≥ once/week[a]	208	54%	207	62%*
Total activity (counts·day^{-1}·1000^{-1})	388	525.2 ± 269.6 (88.2 – 1891.0)	335	377.5 ± 210.2* (89.7 – 1384.8)
Sleep activity (counts·day^{-1}·1000^{-1})	388	5.0 ± 3.9 (0.8 – 33.4)	335	5.6 ± 4.6 (0.6 – 32.6)
Wake time	388	04:59 ± 00:45 (03:04 – 07:34)	335	05:14 ± 00:43* (02:50 – 08:35)
Bedtime	388	21:58 ± 00:45 (19:38 – 24:43)	335	22:02 ± 00:43 (19:35 – 24:24)
Sleep duration	388	07:01 ± 00:52 (04:20 – 09:50)	335	07:11 ± 00:49* (05:08 – 09:24)
MEQ score[b]	332	63.4 ± 6.9 (37 – 79)	293	63.7 ± 7.5 (37 – 80)
Definitely Morning	65	19%	62	21%
Moderately Morning	192	58%	168	57%
Neither	73	22%	59	20%
Moderately Evening	2	1%	4	1%
Definitely Evening	0	0%	0	0%
ESS score[c]	388	9.1 ± 3.4 (1 – 18)	335	7.6 ± 3.2* (0 – 16)

*Men ≠ women (P < 0.05) unpaired 2-tailed *t*-test or χ^2 test. [a]385 men and 334 women had non-missing answers. [b]Morningness-Eveningness Questionnaire. [c]Epworth Sleepiness Scale (ESS) scores come from the modified Amish ESS, which ranges from 0 to 18 (see Methods).

occupation was associated with longer sleep duration (18.0 ± 7.2 min, P = 0.02), indicating that the sex-associated variance was attributed to occupation in some of the models (Table 2). Summer, the season with the latest sunset, also had the latest bedtime compared with winter, the baseline season (43.8 ± 4.2 min, P < 0.0001, Table 2).

Higher total activity level was significantly associated with earlier wake time (−18.6 ± 4.2 min, P < 0.0001), but not bedtime (−3.0 ± 3.6 min), and accordingly, was significantly associated with shorter sleep duration (−13.8 ± 4.8 min, P = 0.004) (Table 2). Although sleep activity level was not significantly associated with wake time or bedtime, it was significantly associated with longer sleep duration (6.0 ± 3.0 min, P = 0.04, Table 2). The effects of BMI and napping frequency were not significantly associated with any of the outcomes in multivariate analyses, and were not included in final models.

From the analysis of outcomes obtained from questionnaires, increasing age was associated with a higher MEQ score (1.7 ± 0.2, P < 0.0001), corresponding to an increasing tendency towards the morning diurnal type, but no other factor was significantly associated (Table 2). ESS score was significantly associated with age, sex, and occupation, but not with season of the year or activity level (Table 2).

It was investigated whether Actical-based sleep parameters were associated with MEQ or ESS scores by individually adding these Actical-based parameters to the final regression models analyzing MEQ and ESS scores adjusted for age, sex, season, occupation, and activity level (Table 2). As expected, Actical-based wake time and bedtime later in the day were significantly associated with lower MEQ scores, corresponding to more evening diurnal types (wake time: −2.0 ± 0.4, P < 0.0001; bedtime: −1.2 ± 0.4, P = 0.004; Table 2). Actical-based sleep duration was not associated with diurnal preference measured by MEQ scores (−0.3 ± 0.4, Table 2). Consistently, Actical-based wake time and bedtime showed significant, negative correlation with MEQ scores (r = −0.18 and −0.15, respectively, both P < 0.0001), but Actical-based sleep duration did not. Actical-based wake time later in the day was significantly associated with lower ESS scores (−0.6 ± 0.2, P = 0.001), corresponding to less daytime sleepiness, and later Actical-based bedtime was significantly associated with higher ESS scores (0.4 ± 0.2, P = 0.01), corresponding to more daytime sleepiness (Table 2). As expected, longer sleep duration was significantly associated with lower ESS scores (−0.7 ± 0.2, P < 0.0001), corresponding to less daytime sleepiness (Table 2).

After adjustment for non-genetic factors and the effects of a shared household, variance component analysis revealed that wake time, MEQ score, and ESS score were significantly heritable (all P < 0.01), but not bedtime or sleep duration (Table 3). Additive genetic influences explained 20%, 21%, and 17% of the residual variance in Actical-based wake time, MEQ score, and ESS score, respectively (Table 3). There was a significant genetic correlation between Actical-based wake time and MEQ score (ρ_G = −0.60 ± 0.26, P = 0.05), but not between Actical-

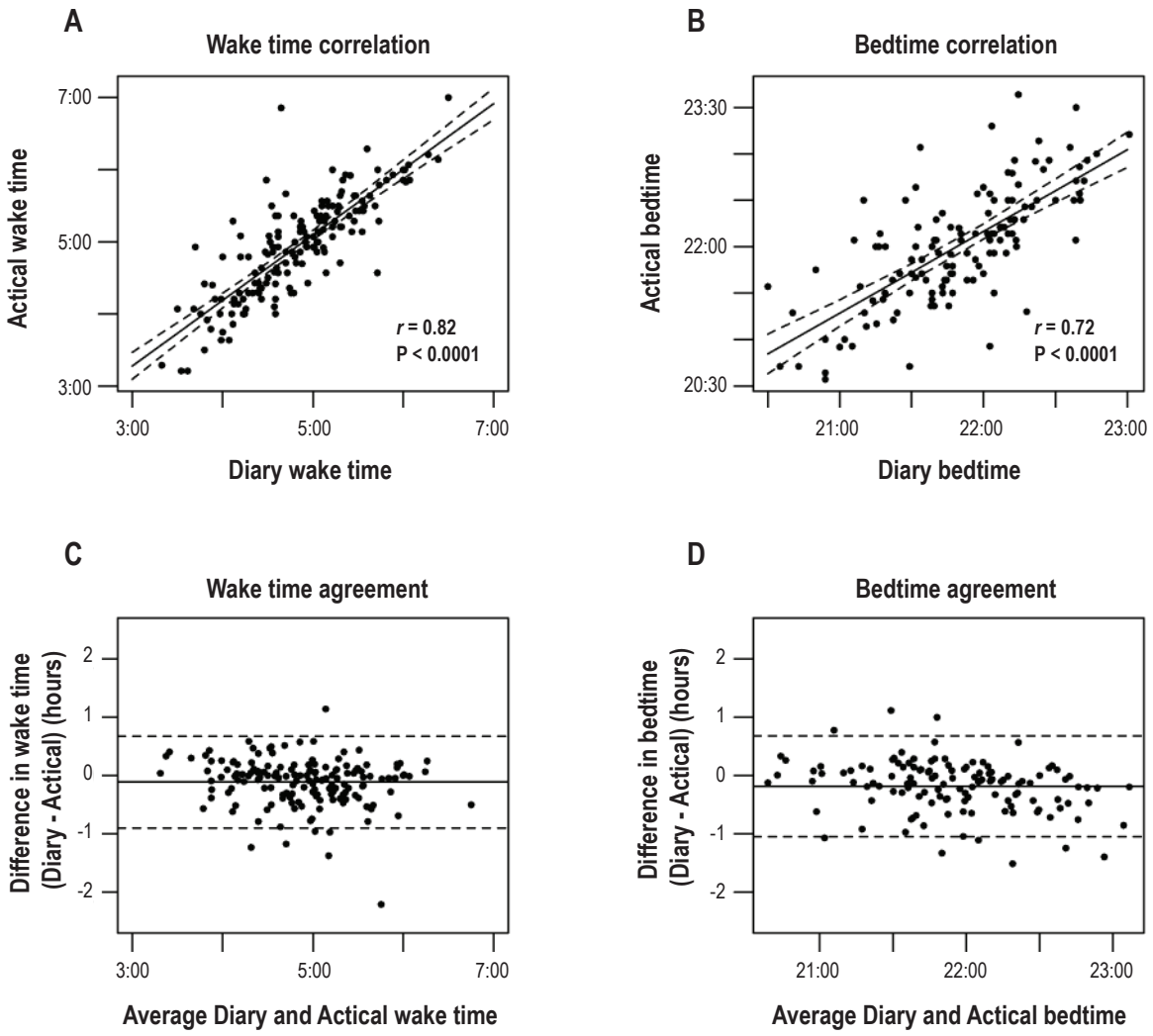

Figure 2—Correlation and agreement between diary-based and Actical-based wake time and bedtime. **(A, B)**. Scatter plots with solid lines that represent the fitted linear regression between the 2 variables, and the dashed lines represent 95% point-wise confidence bands of the fitted regression line. Pearson's correlation coefficient (*r*) and its P-value are shown. **(C, D)**. Bland-Altman agreement plots. Difference between measurements is plotted against the average of the measurements. The solid line marks the mean of the differences, and the dashed lines mark the 95% confidence intervals of the differences.

based wake time and ESS score, or between MEQ and ESS score. The effects of a shared household were significantly associated with all three Actical-based sleep parameters, but not with MEQ score or ESS score (Table 3). There was no evidence for sex-specific effects on heritability.

DISCUSSION

In this study, we used a physical activity monitor, the Actical, to measure habitual wake time, bedtime, and sleep duration. Upon observing that sleep parameters from Actical and sleep diaries were highly correlated and in agreement, we evaluated the contribution of genetic and non-genetic factors on Actical-based sleep parameters, as well as on questionnaire-based diurnal preference and daytime sleepiness. As would be expected from an informative measure of sleep phase and duration, we found Actical-based sleep parameters to be associated with factors known to be associated with sleep phase, such as age, occupation, and season of the year. Also, we found Actical-based wake time and bedtime to be associated with diurnal preference and shortened Actical-based sleep duration to be associated with increased daytime sleepiness. The measurement of physical activity level and sleep parameters with the Actical revealed an association between greater amounts of total physical activity with earlier wake time and shorter sleep duration in this study population. After adjusting for the effect of non-genetic factors and shared households, Actical-based wake time, diurnal preference, and daytime sleepiness showed significant heritability. Furthermore, diurnal preference had a significant genetic correlation with Actical-based wake time, suggesting a shared genetic influence or a similarity in these traits.

To study habitual sleep phase in large community-dwelling populations, actigraphy provides a reasonable alternative to the gold-standard method of polysomnography.[23] The Actical device used in this study is similar in design to sleep actigraphy devices, but instead of being worn on the wrist as is standard for sleep studies, the Actical was worn on the hip to optimize the detection of physical activity. In addition, a validated computer algorithm was not used to determine sleep parameters from Actical data. Despite these limitations, we observed Actical-based wake time and bedtime to be highly correlated with sleep di-

Table 2—Association between sleep parameters and non-genetic factors

Parameter	Wake time[a] (n = 723) β ± SE	Bedtime[a] (n = 723) β ± SE	Sleep duration[a] (n = 723) β ± SE	MEQ score[b] (n = 625) β ± SE	ESS score[c] (n = 723) β ± SE
Age (10 years)	-3.6 ± 1.2*	-2.4 ± 1.2	-0.5 ± 1.2	1.7 ± 0.2**	0.4 ± 0.1**
Sex (ref. male)	16.2 ± 5.4*	4.8 ± 4.8	9.0 ± 6.6	-1.3 ± 1.1	-1.3 ± 0.5*
Season (ref. Winter)	-**	-**	-**	-	-
Spring	13.2 ± 4.2*	20.4 ± 4.8**	-5.4 ± 5.4	0.5 ± 0.8	0.1 ± 0.4
Summer	25.2 ± 4.2**	43.8 ± 4.2**	-14.4 ± 5.4*	-0.6 ± 0.8	0.3 ± 0.3
Fall	21.6 ± 4.2**	10.8 ± 4.8*	13.8 ± 5.4*	-0.2 ± 0.8	0.3 ± 0.4
Occupation (ref. Farmer)	-**	-	-**	-	-**
Housewife	10.2 ± 6.0	-4.8 ± 5.4	18.0 ± 7.2*	2.0 ± 1.2	-1.2 ± 0.5*
Shopkeeper	30.6 ± 6.0**	-1.8 ± 5.4	30.0 ± 7.2**	-0.5 ± 1.2	-1.3 ± 0.5*
Craftmaker/teacher	25.8 ± 9.6*	-6.0 ± 8.4	31.8 ± 12.0*	2.6 ± 2.0	-2.8 ± 0.9*
Carpenter/mechanic	34.8 ± 4.2**	-4.8 ± 3.6	38.4 ± 5.4**	0.5 ± 0.9	-1.8 ± 0.4**
Retired	52.2 ± 9.6**	0.6 ± 8.4	52.2 ± 11.4**	-3.3 ± 2.0	-1.1 ± 0.8
Total activity	-18.6 ± 4.2**	-3.0 ± 3.6	-13.8 ± 4.8*	0.8 ± 0.8	0.6 ± 0.3
Sleep activity	3.6 ± 2.4	-1.8 ± 2.4	6.0 ± 3.0*	-0.2 ± 0.5	0.0 ± 0.2
Wake time	-	-	-	-2.0 ± 0.4**	-0.6 ± 0.2**
Bedtime	-	-	-	-1.2 ± 0.4*	0.4 ± 0.2*
Sleep duration	-	-	-	-0.3 ± 0.4	-0.7 ± 0.2**

*P < 0.05, **P < 0.001. [a]Units of effect size in minutes. [b]Morningness-Eveningness Questionnaire. [c]Epworth Sleepiness Scale.

Table 3—Variance component analysis of sleep parameters

	Wake time (n = 723)		Bedtime (n = 723)		Sleep duration (n = 723)		MEQ score[c] (n = 625)		ESS score[d] (n = 723)	
Parameter	% Variance ± SE	P	% Variance ± SE	P	% Variance ± SE	P	% Variance ± SE	P	% Variance ± SE	P
Fixed effects[a]	0.30	< 0.0001	0.16	< 0.0001	0.19	< 0.0001	0.10	< 0.0001	0.13	< 0.0001
Heritability[b]	0.20 ± 0.09	0.007	0	NS	0	NS	0.21 ± 0.09	0.002	0.17 ± 0.08	0.008
Shared household[b]	0.27 ± 0.07	< 0.0001	0.72 ± 0.03	< 0.0001	0.38 ± 0.06	< 0.0001	0	NS	0	NS

[a]Age, sex, season, occupation, total activity, and sleep activity. Percent variance shown. [b]Adjusted for fixed effects listed above. [c]Morningness-Eveningness Questionnaire. [d]Epworth Sleepiness Scale.

ary measures. Consistent with a previous comparison between actigraphy and sleep diaries,[29] we observed weaker agreement between sleep diary and Actical-based bedtime than for wake time, possibly reflecting reduced accuracy for diary-based bedtime. A previous study determined wake time and bedtime using the ActiGraph (formerly CSA) accelerometer model 7164 worn on the hip without using a validated computer algorithm.[50] Taken together, these data suggest that activity monitors worn on the hip can provide useful information on habitual wake time and bedtime.

It has been posited that urbanization has contributed to the lack of sleep that is so common in modern industrialized societies, and that time spent watching television before bedtime can possibly be exchanged for sleep time.[38,39] Thus, the freedom from modern technology among the OOA might be expected to result in longer habitual sleep duration, but in fact, habitual sleep duration in our OOA study population was similar to that found in other study populations with similar age distributions.[22,38,51,52] In addition to sleep duration, it was of interest to compare sleep phase in our OOA study population with other study populations. While sleep phase is generally early in middle-aged study populations such as ours, we found habitual sleep phase in our study population to be slightly earlier than that found in other study populations with similar age distributions.[22,33,51,53] Consistent with this finding, the diurnal preference was similar, if not slightly earlier, to other middle-aged study populations.[33,53] Even after applying revised MEQ score cutoffs designed for middle-aged populations, we only classified 7% of the OOA study participants as evening diurnal types, potentially indicating our study population has a slightly earlier average diurnal preference relative to other middle-aged populations.[53] Consistent with our findings, previous studies have found rural populations to have earlier chronotypes compared to urban populations.[36,37] ESS scores that measure daytime sleepiness also appear to be higher in our study population compared with other populations.[22,54] Considering the omission of two items from the modified Amish ESS (see Methods), the level of daytime sleepiness in our study population might be underestimated. This high level of daytime sleepiness is unlikely to be a result of short sleep duration, as sleep duration in the OOA is

similar to what has been reported for other populations. Perhaps other factors associated with high ESS scores are prevalent in our OOA study population.

The early sleep phase of the OOA apparently did not mask known associations with sleep phase. Actical-based wake time was earlier with increasing age, consistent with previous findings.[33,49] It has been suggested that the influence of the solar light/dark cycle on sleep timing[3,49] is stronger in smaller towns compared with larger cities.[36] Consistently, Actical-based sleep parameters in this agrarian OOA study population were significantly associated with the season of the year. Other seasonal factors, such as temperature and/or harvest activities, could also explain this observed association. Consistent with previous findings that diurnal preference is more strongly associated with sleep phase than sleep duration,[49,55] we observed MEQ score to be associated with Actical-based wake time and bedtime but not with sleep duration.

Our use of Actical accelerometers provided us with the opportunity to explore the complex relationship between sleep patterns and total physical activity level. This relationship has been shown to be potentially modulated by many factors, such as age, sex, and the type and timing of the exercise.[56,57] Perhaps not surprisingly, various studies have found inconsistent associations between chronic physical activity and sleep duration, but in general, increased levels of chronic physical activity are associated with more sleep.[56,57] In contrast, we observed a negative correlation between activity level per waking hour and sleep duration in the OOA. Perhaps the direction of the association between total physical activity and sleep duration changes at different levels of physical activity. In fact, it has been postulated that strenuous exercise might stimulate high concentrations of pro-inflammatory cytokines that can increase wakefulness.[57] The OOA appear to be a very physically active population; Actical physical activity counts are much higher in our study population compared with a previous study of non-Amish participants fitted with the Actical.[58] A pedometer-based study of an OOA population in Canada also concluded that physical activity levels in the Amish are much higher than in other populations.[59] Further work will be needed to determine if total physical activity and sleep duration are negatively correlated in other highly active populations, or if this finding is specific to the OOA.

Our heritability estimates of various sleep-related traits in the Amish are similar to estimates obtained from other populations. We found Actical-based wake time, but not Actical-based bedtime or sleep duration, to be moderately heritable ($h^2 = 0.20 \pm 0.09$). Our heritability estimate for diurnal preference based on MEQ score ($h^2 = 0.21 \pm 0.09$) was similar to previous estimates in other populations.[12-16] A study of the Hutterites,[14] a rural population with a traditional lifestyle similar to the OOA, estimated the heritability of diurnal preference using a modified version of the MEQ and the heritability of wake time and bedtime using the Sleep Timing Questionnaire. Their heritability estimates for sleep parameters were very similar to our estimates, but they did not adjust for shared household effects (wake time: $h^2 = 0.12 \pm 0.06$; bedtime: $h^2 = 0.10 \pm 0.10$; diurnal preference: $h^2 = 0.24 \pm 0.09$).[14] Individuals within a household not only have a shared environment, but are also more likely to be related. Thus, our heritability estimates should be considered conservative as the effects of a shared household were taken into account. Without adjusting for the effects of a shared household, our heritability estimate for Actical-based wake time increases to 0.32 ± 0.09. The absence of heritability for Actical-based bedtime was primarily due to the strong influence from household effects (72% variance explained). Daytime sleepiness measured by the ESS was also significantly heritable, but the genetic influence on ESS appeared to be distinct from the genetic influence on diurnal preference or Actical-based wake time based on our genetic correlation analyses.

In conclusion, findings from our study suggest that Actical could be a promising alternative for the ascertainment of information on habitual sleep patterns. Further validation studies are needed to compare sleep measurements recorded by Actical with measurements from established methods such as polysomnography. In addition to information on sleep, Actical also measures the level of total physical activity. As physical activity, a modifiable factor, is observed to be strongly associated with sleep patterns in the Amish, a better understanding of its relationship with sleep in other populations may lead to effective preventive measures for sleep-related disorders. In addition, the observation that wake time, diurnal preference, and daytime sleepiness are heritable provides justification for further genetic studies of these traits in the OOA.

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