Supporting Information

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SI Methods

Subjects. In addition to subject inclusion information presented in the main text, young ovulating women with a history of regular menstrual cycles and no history of prior gynecological pathology were selected. Half of the women used oral contraceptives, and those not currently using oral contraceptives were required to be free from oral contraceptive use for more than 3 mo before participation and to not have irregular cycle lengths. Subjects with mood disorders, including late luteal phase dysphoric disorder (determined at clinical interview), were excluded. Menstrual phase logs indicated that women started the study in the week of menses or on the first day of the follicular phase ($n = 3$) or during the luteal phase ($n = 5$). For those women taking oral contraceptives, the study was started in the "pseudo" follicular phase ($n = 3$) and luteal phase ($n = 1$). Although we included near-equal numbers of men and women in our study, that we did not control for or determine the influence of menstrual cycle phase or oral contraceptives on our outcome measures is a limitation. Chronotype was not an inclusion/exclusion criterion. Chronotype estimates derived from the Morningness-Eveningness Questionnaire (1) were as follows: One was a definite morning type, four were moderate morning types, seven were intermediate types, and two were moderate evening types.

Meals. Diets were prepared by the CTRC Nutrition Core and contained macronutrient contents recommended by the American Heart Association (30% fat, 55% carbohydrate, and 15% protein) and no caffeine. Inpatient meals were the same each day for each individual subject and typically consisted of some variation of the following: breakfast contained eggs, toast, and juice; lunch contained a turkey sandwich, potato chips, and fruit; dinner contained a salad with meat and a roll; and the snack contained a cracker with cheese. Outpatient meals differed from inpatient meals and between subjects. Change in subjects' preferences for food provided across the study was not assessed. CTRC dieticians determined the caloric and macronutrient content of meals using ProNutra software (2).

Measures. EE is provided in units of kcal (kilocalorie) and kJ (kilojoule) per minute; 1 kcal = 4.184 kJ. Sleep recordings were obtained from C3-A2, C4-A1, O1-A2, O2-A1, F3-A2, F4-A1, right and left electrooculogram (EOG), chin electromyogram (EMG), and electrocardiogram (ECG). The sleep disorders screen tested for sleep apnea, periodic limb movements, nocturnal epilepsy, and other sleep-related movement disorders based on standardized EEG, airflow, respiratory effort, and leg EMG measurements. Sleep was manually scored in 30-s epochs according to standard guidelines from brain region C3-A2 (3) or C4-A1 if C3-A2 contained an artifact. Approximately 515 mL of blood was drawn throughout the duration of the protocol, including blood taken at screening. Blood samples were immediately centrifuged (2,000–3,000 × g) and subsequently stored at −80 °C until assayed. Serum radioimmunoassays (RIAs) for ghrelin sensitivity 93 pg/mL, within-assay coefficient of variation (CV) 4.5%; peptide-YY sensitivity 10 pg/mL, within-assay CV 5.3%; and leptin sensitivity 0.5 ng/mL, within-assay CV 5.9% (Millipore) were performed by the CTRC core laboratory; melatonin sensitivity 2.3 pg/mL, within-assay CV 13.4% (LDN Melatonin Direct RIA; Rocky Mountain Diagnostics) was performed by the Sleep and Chronobiology Laboratory. We examined total, not acetylated, ghrelin, because total ghrelin levels have been reported to respond to sleep loss and may contribute to increased hunger (4, 5). Assessment of acetylated ghrelin and of various acylghrelin isoforms should be considered in future studies, as they are more physiologically relevant in regard to ghrelin receptor activation and effects on food consumption, weight gain, and energy homeostasis compared with total ghrelin levels (6, 7). Furthermore, changes in other metabolic hormones not examined (e.g., glucagon-like-peptide, cholecystokinin, cortisol, testosterone, and catecholamines) (8, 9) could have contributed to the current findings.

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9. Leproult R, Van Cauter E (2011) Effect of 1 week of sleep restriction on testosterone levels in young healthy men. *JAMA* 305(21):2173–2174.

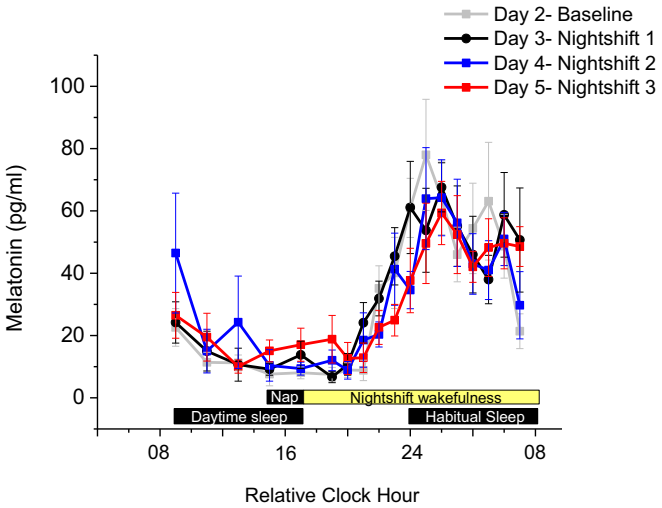

Fig. S1. Hourly melatonin levels ($n = 14$). The gray line represents day 2 (baseline), the black line is day 3 (transition to nightshift 1), the blue line is day 4 (nightshift 2), and the red line is day 5 (nightshift 3). Time of day is plotted as relative clock hour with scheduled waketime arbitrarily assigned a value of 0800 hours. Black bars represent scheduled sleep, and the yellow bar represents scheduled nighttime wakefulness during shiftwork. Error bars are SEM. There was a nonsignificant change in dim-light melatonin onset between days ($P > 0.08$).

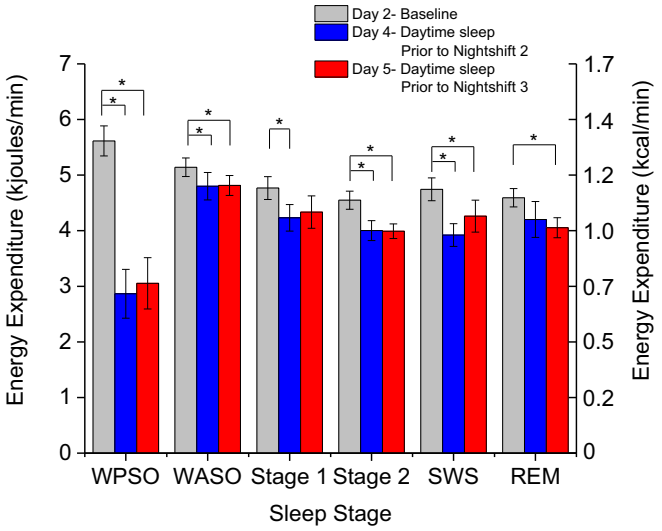

Fig. S2. Energy expenditure per sleep stage ($n = 14$; P values, planned comparisons for day, two-tailed). Solid lines and asterisks represent significant ($P < 0.05$) differences between stages at the end of each line. Error bars are SEM.

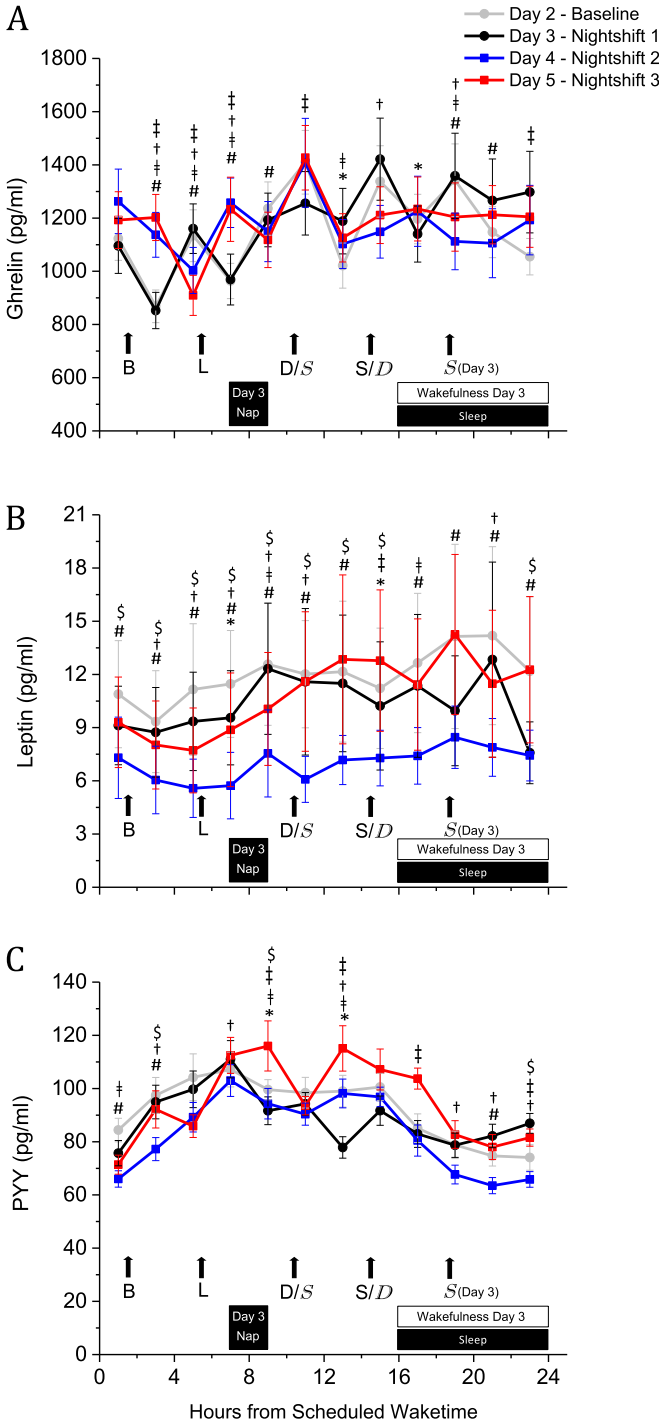

Fig. S3. Average 2-h appetitive hormones (*A*–*C*) (n = 14; two-tailed). Planned comparisons were calculated by dependent t test ($P < 0.05$). *Differences between baseline and the transition to nightshift 1; #differences between baseline and nightshift 2; ‡differences between baseline and nightshift 3; †differences between the transition to nightshift 1 and nightshift 2; ‡differences between the transition to nightshift 1 and nightshift 3; $differences between nightshift 2 and nightshift 3. Black arrows indicate the timing of meals. B, breakfast; D, dinner; L , lunch; S, snack; italicized D and S indicate different meals given at that time on transition to the first nightshift. Black bars represent sleep opportunities. Note that the nap only occurs on the transition to the first nightshift; the open bar represents sleep deprivation. Error bars are SEM. Hourly ghrelin levels fluctuated across the day. Average 2-h leptin levels were generally lower during wakefulness than during sleep. Regardless of whether sleep occurred at night or during the daytime, average 2-h PYY levels were higher during wakefulness than during sleep.

Table S1. Sleep architecture for nighttime baseline and daytime shiftwork sleep opportunities

Measure	Baseline nighttime sleep opportunity (n = 14)	Daytime sleep before nightshift 2 (n = 13)	Daytime sleep before nightshift 3 (n = 14)
Percent of recording time			
Stage 1	4.1 (0.5)	3.6 (0.4)	4.0 (0.6)
Stage 2	55.6 (1.9)	46.8 (2.2)*	47.1 (2.5)†
SWS	12.5 (1.5)	14.3 (1.9)	14.1 (1.7)
REM	18.3 (1.3)	14.3 (1.3)*	16.1 (1.0)
SE	90.4 (2.0)	79.0 (2.4)*	81.3 (2.2)†
Minutes of recording time			
Stage 1	19.6 (2.6)	17.2 (1.9)	19.2 (2.7)
Stage 2	266.6 (9.3)	224.7 (10.8)*	226.0 (12.1)†
SWS	61.4 (7.1)	70.1 (8.7)	67.4 (8.0)
REM	87.9 (6.3)	68.6 (6.3)*	77.1 (4.8)
WASO	34.8 (9.4)	95.6 (11.5)*	85.2 (10.1)†
TST	433.9 (9.7)	379.0 (11.7)*	389.6 (10.8)†
SOL	9.9 (1.2)	5.6 (0.9)*	4.7 (1.1)†
LPS	13.3 (1.7)	6.0 (1.0)*	4.9 (1.3)†
SWSL	17.9 (2.5)	20.3 (3.6)	16.9 (1.9)
REML	83.4 (8.0)	52.1 (4.7)*	54.6 (1.4)†
Duration of awakenings	1.5 (0.3)	5.2 (1.1)*	4.9 (1.0)†
No. of awakenings	22.3 (2.7)	21.6 (3.0)	23.4 (3.0)

Findings for changes in both daytime sleep opportunities are described in the main text. Daytime sleep also displayed a nonsignificant trend for increases in both percent and minutes of SWS ($P = 0.08$). Values in parentheses are SEM. LPS, latency to persistent sleep; REM, rapid eye movement; REML, latency to REM sleep; SE, sleep efficiency; SOL, sleep-onset latency; SWS, slow-wave sleep; SWSL, latency to SWS; TST, total sleep time; WASO, wakefulness after sleep onset.

*Denotes $P < 0.05$ between baseline night sleep opportunity and sleep before nightshift 2.

†Denotes $P < 0.05$ between baseline night sleep opportunity and sleep before nightshift 3.

Table S2. ANOVA *F* values for energy expenditure comparisons between sleep stages for individual sleep opportunities

Measure	Stage 1	Stage 2	SWS	REM	WPSO
Baseline nighttime sleep opportunity (n = 14)					
Stage 2	0.75	—	—	—	—
SWS	0.01	0.72	—	—	—
REM	0.89	0.09	0.53	—	—
WPSO	9.80*	12.33*	19.90*	12.99*	—
WASO	3.34†	11.64*	4.18†	8.68*	4.39†
Daytime sleep before nightshift 2 (n = 13)					
Stage 2	1.87	—	—	—	—
SWS	3.34	0.82	—	—	—
REM	0.02	1.18	3.18	—	—
WPSO	16.76*	6.99*	6.37*	8.89*	—
WASO	16.84*	25.35*	25.62*	9.20*	27.91*
Daytime sleep before nightshift 3 (n = 14)					
Stage 2	1.78	—	—	—	—
SWS	0.06	1.04	—	—	—
REM	1.97	0.26	0.96	—	—
WPSO	11.09*	5.28*	6.92*	7.17*	—
WASO	4.20†	28.49*	12.08*	38.78*	20.81*

Energy expenditure corresponding to consolidated stage 2 and slow-wave sleep epochs were binned and averaged for ≥15 min of continuous sleep for that stage, with the exception of four subjects who did not have 15 min of continuous SWS and therefore the maximum time for each subject was used (the minimum bin size was 4 min for one subject); EE bins were ≥9.5 min for rapid eye movement, ≥1 min for stage 1, and ≥30 s for wakefulness prior to sleep onset and wakefulness after sleep onset.

*Denotes $P < 0.05$ between stages.

†Denotes $0.07 < P < 0.1$ between sleep stages.