Table S1—Baseline characteristics of study participants

	Mean (SEM)
Age, years	24.2 (1.3)
Sex	All male
Body mass index, kg/m^2 *	23.1 (0.4)
Blood pressure and heart rate	
Systolic blood pressure, mmHg	117.8 (3.4)
Diastolic blood pressure, mmHg	65.1 (3.2)
Heart rate, beats/min	53.2 (1.6)
Body composition	
% body fat	19.8 (1.8)
Fat mass, kg	14.1 (1.6)
Lean mass, kg	56.0 (1.5)
Lipid profile †	
Total cholesterol, mmol/l	4.03 (0.25)
Triglycerides, mmol/l	0.99 (0.15)
High-density lipoprotein cholesterol, mmol/l	1.17 (0.05)
Low-density lipoprotein cholesterol, mmol/l	2.46 (0.23)
Thyroid function	
Thyroid-stimulating hormone, mU/l (ref. range 0.35-5.5)	1.82 (0.21)
Free thyroxine, pmol/l (ref. range 10.0-19.8)	15.69 (0.59)
Oral glucose tolerance test ‡	
Fasting plasma glucose, mmol/l	4.55 (0.08)
Plasma glucose at 120 min, mmol/l	5.03 (0.45)
Self-reported sleep parameters	
Pittsburgh Sleep Quality Index §	3.8 (0.6)
Epworth Sleepiness Scale ‖	4.9 (1.0)
Sleep duration, hours	8.1 (0.2)

Mean values (standard error of the mean, SEM) for all participants. * Body mass index is weight in kilograms divided by the height in meters squared. † To convert cholesterol values to milligrams per deciliter, multiply by 38.7. To convert triglyceride values to milligrams per deciliter, multiply by 88.5. ‡ To convert glucose values to milligrams per deciliter, multiply by 18. § The Pittsburgh Sleep Quality Index[1] measures self-reported sleep quality with normal scores ≤ 5. ‖ The Epworth Sleepiness Scale[2] measures daytime sleepiness with normal values ≤ 8.

Table S2—Mood changes measured using the Profile Of Mood States (POMS) questionnaire

Mean (SEM)	09:00	18:00	Day average
Total Mood Disturbance (scale 0-200)			
Day 1 (BL)	10.44 (4.39)	6.33 (3.69)	8.10 (2.79)
Day 2 (CR)	10.25 (3.67)	7.64 (2.91)	9.00 (2.33)
Day 3 (CR)	7.09 (2.56)	11.67 (3.45)	9.48 (2.18)
Day 4 (FF)	2.83 (1.29)	3.25 (2.43)	3.04 (1.35)
Day 5 (FF)	2.08 (1.26)	2.73 (1.14)	2.39 (0.84)
Tension-Anxiety domain (scale 0-36)			
Day 1 (BL)	3.50 (0.96)	2.83 (0.85)	3.17 (0.63)
Day 2 (CR)	3.92 (0.80)	3.58 (0.71)	3.75 (0.53)
Day 3 (CR)	4.25 (0.86)	5.25 (1.09)	4.75 (0.69)
Day 4 (FF)	3.42 (0.76)	2.92 (0.73)	3.17 (0.52)
Day 5 (FF)	2.42 (0.65)	2.45 (0.58)	2.43 (0.43)
Depression domain (scale 0-60)			
Day 1 (BL)	2.33 (1.23)	1.75 (1.05)	2.04 (0.79)
Day 2 (CR)	2.83 (1.34)	3.08 (1.23)	2.96 (0.89)
Day 3 (CR)	2.00 (0.59)	2.58 (0.89)	2.30 (0.54)
Day 4 (FF)	0.83 (0.27)	1.50 (0.73)	1.17 (0.39)
Day 5 (FF)	1.17 (0.41)	1.50 (0.53)	1.33 (0.33)
Anger-Hostility domain (scale 0-48)			
Day 1 (BL)	1.17 (0.68)	1.00 (0.67)	1.08 (0.47)
Day 2 (CR)	1.33 (0.75)	1.67 (0.98)	1.50 (0.60)
Day 3 (CR)	0.58 (0.19)	1.42 (0.73)	1.00 (0.38)
Day 4 (FF)	0.25 (0.18)	0.50 (0.36)	0.38 (0.20)
Day 5 (FF)	0.17 (0.17)	0.08 (0.08)	0.12 (0.09)
Fatigue domain (scale 0-28)			
Day 1 (BL)	4.25 (0.92)	3.25 (0.84)	3.75 (0.62)
Day 2 (CR)	3.83 (1.15)	3.50 (0.87)	3.67 (0.70)
Day 3 (CR)	3.08 (0.77)	4.33 (1.04)	3.71 (0.65)
Day 4 (FF)	2.50 (0.70)	2.17 (0.76)	2.33 (0.51)
Day 5 (FF)	1.50 (0.65)	1.92 (0.56)	1.71 (0.42)
Confusion-Bewilderment domain (scale 0-28)			
Day 1 (BL)	5.78 (0.76)	5.00 (0.69)	5.33 (0.50)
Day 2 (CR)	5.08 (0.70)	4.73 (0.56)	4.91 (0.44)
Day 3 (CR)	4.83 (0.42)	5.42 (0.47)	5.12 (0.31)
Day 4 (FF)	4.00 (0.30)	3.92 (0.43)	3.96 (0.26)
Day 5 (FF)	4.17 (0.27)	4.00 (0.12)	4.08 (0.15)
Vigor-Activity domain (scale 0-32)			
Day 1 (BL)	13.09 (1.74)	11.92 (1.49)	12.48 (1.12)
Day 2 (CR)	10.50 (1.99)	9.08 (1.62)	9.79 (1.26)
Day 3 (CR)	9.75 (1.48)	9.25 (1.26)	9.50 (0.95)
Day 4 (FF)	12.42 (1.68)	12.33 (1.50)	12.38 (1.10)
Day 5 (FF)	12.83 (1.57)	10.64 (1.59)	11.78 (1.12)

Participants filled out a mood disturbance questionnaire (Profile Of Mood States, POMS)[3] twice a day across the three study phases (baseline, BL, caloric restriction, CR, and free feeding, FF). The total mood disturbance (scale 0-200) decreased after the first *ad libitum* meal and stayed low throughout FF (overall p for comparison = 0.003).

Table S3— Analysis of pulsatile cortisol secretion

Cortisol	Baseline	Caloric restriction	Free feeding	P value for comparison
Mean concentration, nmol/l	115.1 (15.7)	130.5 (11.8)	256.8 (124.5)	0.18
Area under the curve, nmol/l x min	42,151 (5877)	47,251 (4467)	44,016 (6372)	0.54
Cluster analysis				
Number of peaks	2.13 (0.23)	2.13 (0.44)	2.38 (0.38)	0.93
Interval between peaks, mins	85.0 (13.4)	69.5 (8.2)	80.0 (10.4)	0.94
Peak width, mins	71.3 (11.3)	56.8 (7.5)	55.0 (6.2)	0.56
Peak height, nmol/l	159.7 (16.3)	164.8 (30.4)	166.8 (24.9)	0.96
Peak area, nmol/l x min	5206.2 (1028.4)	4966.2 (1932.5)	3404.9 (704.0)	0.62
Valley mean, nmol/l	79.8 (11.1)	81.3 (15.3)	95.3 (12.0)	0.54
Valley nadir, nmol/l	59.5 (10.6)	67.1 (13.3)	85.3 (16.1)	0.37

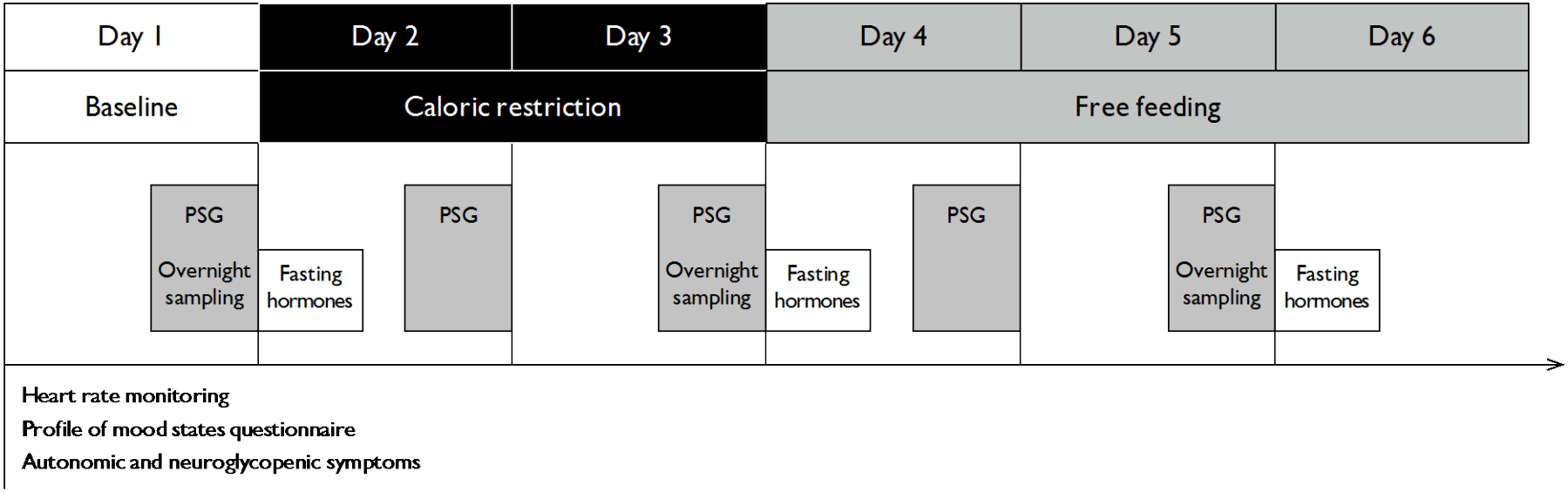
Data are reported as mean (standard error of the mean) for 8 participants. Pulsatility of cortisol was assessed by cluster analysis. Results of the three study phases (baseline, caloric restriction, and free feeding) were analyzed using analysis of variance (ANOVA) with repeated measures after log-transformation of the variables to test for within-subject changes.

Table S4—Autonomic and neuroglycopenic symptoms

Mean (SEM)	09:00	12:00	15:00	18:00	21:00	Day average
Autonomic symptoms						
Day 1 (BL)	6.08 (1.38)	6.25 (1.13)	4.83 (0.94)	4.42 (0.91)	4.75 (1.21)	5.27 (0.50)
Day 2 (CR)	5.58 (0.62)	7.58 (0.78)	8.08 (0.90)	6.58 (0.97)	6.67 (0.78)	6.90 (0.37)
Day 3 (CR)	7.50 (0.57)	9.75 (0.99)	8.17 (0.61)	6.83 (0.99)	8.25 (1.25)	8.10 (0.42)
Day 4 (FF)	2.25 (0.88)	3.00 (0.95)	2.00 (0.64)	1.67 (0.69)	2.33 (0.76)	2.25 (0.35)
Day 5 (FF)	1.75 (0.68)	3.50 (0.77)	1.67 (0.51)	1.25 (0.55)	1.83 (0.87)	2.00 (0.31)
Neuroglycopenic symptoms						
Day 1 (BL)	3.83 (1.40)	2.83 (1.07)	2.17 (1.09)	2.42 (1.03)	1.83 (0.67)	2.62 (0.47)
Day 2 (CR)	2.00 (0.49)	2.18 (0.75)	3.08 (1.24)	2.83 (0.91)	2.50 (0.94)	2.53 (0.39)
Day 3 (CR)	2.50 (0.82)	4.58 (1.28)	3.25 (1.03)	3.42 (0.89)	4.00 (1.15)	3.55 (0.46)
Day 4 (FF)	1.67 (0.72)	1.83 (0.76)	1.00 (0.60)	1.00 (0.49)	1.25 (0.58)	1.35 (0.28)
Day 5 (FF)	1.58 (0.70)	0.92 (0.67)	1.00 (0.52)	1.25 (0.59)	1.00 (0.52)	1.15 (0.26)

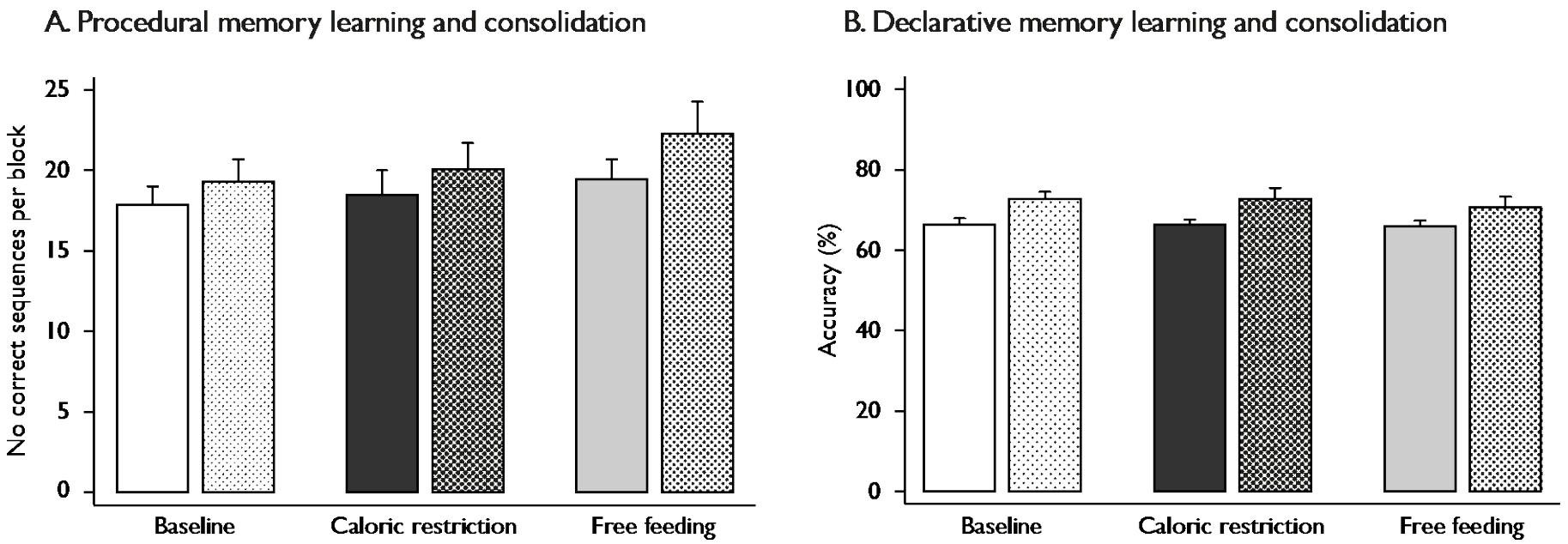
Five times a day across the three study phases (baseline, BL, caloric restriction, CR, and free feeding, FF), participants were asked to fill in a validated questionnaire on neuroglycopenia and autonomic symptoms.[4] Autonomic symptoms comprised tremor, hunger, palpitations, sweating, irritability, and anxiety, each on a 10 point-scale (0=weak, 9=strong). Results give a total score on a 54-point scale for autonomic symptoms. Neuroglycopenic symptoms of dizziness, tingling, blurred vision, difficulty in thinking, and faintness were recorded similarly and summed to give a total score of 0-45.

Figure S1— Study protocol



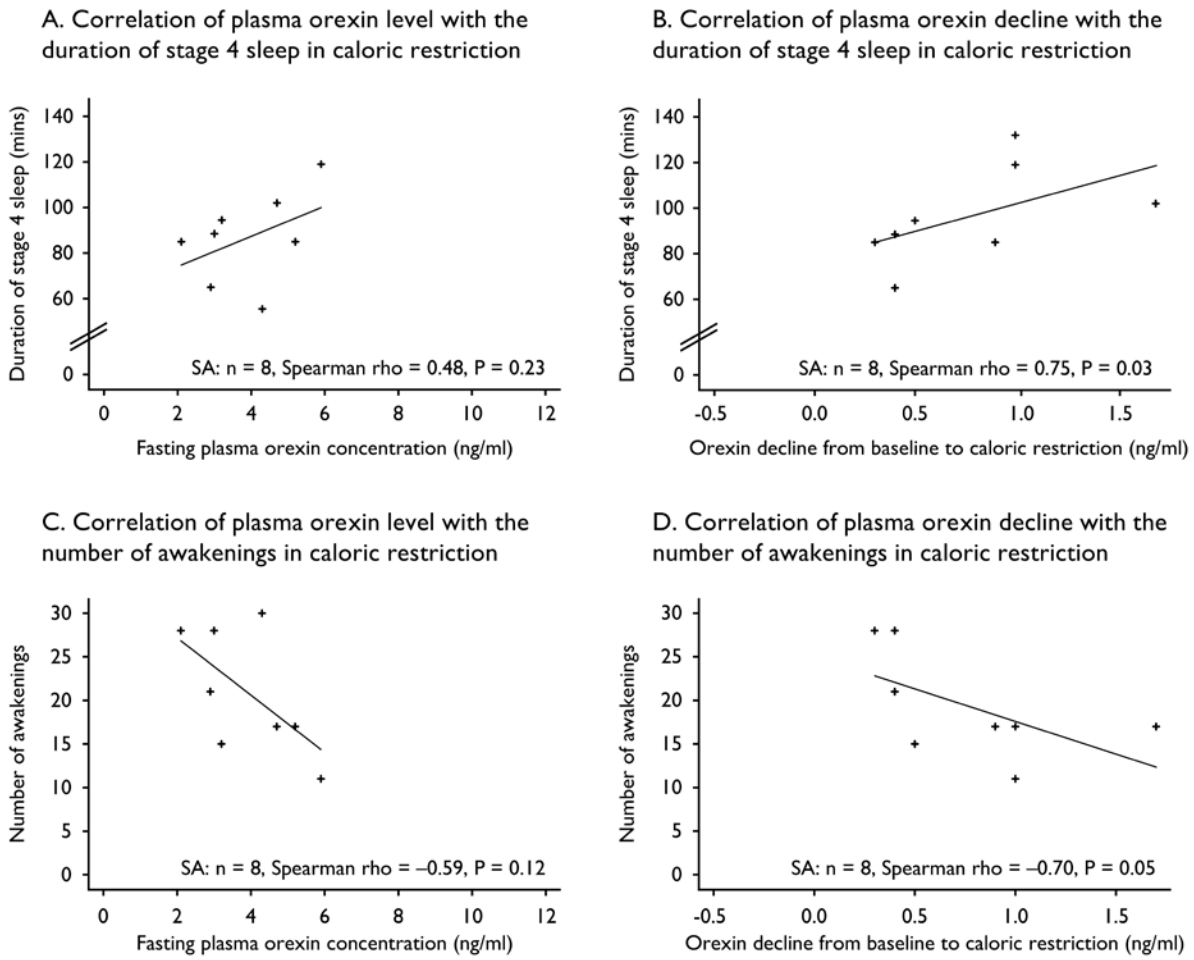
Twelve participants were studied under direct observation at baseline when food intake matched 24-hour energy requirement (day 1); after 2 days of caloric restriction to 10% of their normal energy requirement; after 2 days of free feeding to restore energy balance (a subset of individuals were studied after 3 days of free feeding). Some measurements were made continuously while others were performed at fixed periods during the course of the study as shown. Sleep architecture was recorded by polysomnography (PSG) every night from 11pm to 7am; overnight sampling for thyroid-stimulating hormone (TSH), growth hormone (GH) and cortisol was performed every 10 minutes from midnight to 6am on nights 1, 3, 5; fasting levels of hormones (leptin, insulin, total ghrelin and orexin A) were measured at 8am after completion of overnight sampling.

Figure S2—Procedural and declarative memory formation



Procedural memory formation was measured by finger tapping test[5] and declarative memory formation by associate word learning paradigm.[6] A: Sleep dependent consolidation of procedural memory consolidation, i.e. improvement of performance from learning in the evening (plain bars) to next morning recall (hashed bars, $p=0.008$), was preserved during the whole experiment and not modified by manipulation of energy balance ($p=0.66$). B: Similarly sleep dependent consolidation of declarative memory consolidation, i.e. improvement of performance from learning in the evening (plain bars) to next morning recall (hashed bars, $p<0.001$), was preserved during the whole experiment and not modified by manipulation of energy balance ($p=0.66$). Statistical analyses: Memory consolidation results from the three study phases (baseline in white, caloric restriction in black and free feeding in grey) were analyzed using analysis of variance (ANOVA) with repeated measures to test for within-subject changes among all participants. The within-subjects p-value was adjusted using the Greenhouse-Geisser correction factor for lack of sphericity. Pairwise comparisons of the three study phases were performed by two-sided Student's t-test when appropriate. A p-value of 0.05 was considered significant after Bonferroni correction for multiple comparisons.

Figure S3—Sensitivity analysis of correlations between plasma orexin levels and sleep parameters



A sensitivity analysis (SA) excluding one outlier in each panel of Figure 4 confirmed the correlation of orexin decline in 48 hours from baseline to caloric restriction (CR) with the duration of stage 4 sleep in CR (Panel B, Spearman rho=0.75, p=0.03) and the number of awakenings in CR (Panel D, Spearman rho=-0.70, p=0.05). In this SA, there was no correlation between the plasma concentration of orexin in CR and the duration of sleep stage 4 (Panel A, Spearman rho=0.48, p=0.23) or the number of awakenings in CR (Panel C, Spearman rho=-0.59, p=0.12).

REFERENCES

1. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193-213.
2. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540-5.
3. McNair DM, Lorr M, Droppleman LF. Manual for the profile of mood states. San Diego, CA: Education and Industrial Testing Service, 1971.
4. Mitrakou A, Ryan C, Veneman T, et al. Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. Am J Physiol 1991;260:E67-74.
5. Walker MP, Brakefield T, Hobson JA, Stickgold R. Dissociable stages of human memory consolidation and reconsolidation. Nature 2003;425:616-20.
6. Plihal W, Born J. Effects of early and late nocturnal sleep on declarative and procedural memory. J Cogn Neurosci 1997;9:534-47.