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Supplementary Materials for

Daytime eating prevents internal circadian misalignment and glucose intolerance in night work

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This PDF file includes:

Supplementary results

Effects of meal timing intervention on the 3-h postprandial glucose, early-phase and late-phase postprandial insulin profiles averaged across both Test meals

To isolate the effect of circadian misalignment on postprandial glucose and insulin profiles (while accounting for the effect of circadian phase), we averaged the 3-h postprandial glucose, early-phase and late-phase postprandial insulin profiles during the Breakfast and Dinner Test meals in the simulated night vs. simulated day work conditions.

Glucose profile. The meal timing intervention significantly modified the impact of simulated night work on the 3-h postprandial glucose profile (mixed-model analyses of variance, interaction of meal timing group and simulated day/night work: *p*FDR=0.002; **Figure S3**). In the NMC Group, simulated night work significantly increased postprandial glucose profile (average of the two Test meals) by 10.9% , as compared to baseline (95% CI, 4.1% to 17.8% [11mg/dl, 95% CI, 4.3mg/dl to 17.6mg/dl]; Tukey´s post-hoc test adjusted for multiple comparisons, *P*=0.002; **Figure S3A**). Conversely, in the DMI Group, no significant changes occurred, as compared to baseline (95% CI, -9.5% to 4.5% [-10.2mg/dl to 7.2mg/dl]; Tukey´s post-hoc test adjusted for multiple comparisons, *P*=n.s.; **Figure S3B**).

Early-phase insulin profile. The meal timing intervention significantly modified the impact of simulated night work on the early-phase postprandial insulin profile (mixed-model analyses of variance, interaction of meal timing group and simulated day/night work: *p*FDR=0.01). In the NMC Group, simulated night work significantly decreased early-phase insulin profile by -41.7% relative to baseline (95% CI, -70.1% to -12.4% [-15.8µU/ml, 95% CI, -26.4µU/ml to -5.1µU/ml]; Tukey´s post-hoc test adjusted for multiple comparisons, *P*=0.015; grey bar in **Figure S3C**). Conversely, in the DMI Group, no significant changes occurred relative to baseline (95% CI, -29.4% to 3% [-16.5µU/ml to 5.7µU/ml]; Tukey´s post-hoc test adjusted for multiple comparisons, *P*=n.s.; grey bar in **Figure S3D**).

Late-phase insulin profile. The meal timing intervention did not significantly modify the impact of simulated night work on the late-phase postprandial insulin profile (mixed-model analyses of variance, interaction of meal timing group and simulated day/night work: *p*FDR=n.s.). Furthermore, simulated night work did not significantly affect late-phase postprandial insulin profile, as compared to baseline, in either group (Tukey´s post-hoc test adjusted for multiple comparisons, all *P*= n.s.; **Figures 3C, D**).

Effects of meal timing intervention on postprandial glucose and insulin area under the curve (AUC)

Breakfast, Glucose AUC. The meal timing intervention significantly affected the change from baseline to simulated night work on the 3-h postprandial glucose AUC during the Breakfast Test meal (two-sided, unpaired *t*-test for meal timing group effect: *P*=0.008). The change from baseline to simulated night work in glucose AUC significantly differed between groups (NMC Group: 95% CI, 9% to 30% [95% CI, 1785.2mg/dl*min to 5950.8mg/dl*min]; DMI Group: 95% CI, -5.1% to 8.4% [95% CI, -949.9mg/dl*min to 1564.5mg/dl*min]) (two-sided, unpaired *t*-test for meal timing group effect: *P*=0.008; **Figure S4A**).

Dinner, Glucose AUC. The meal timing intervention did not significantly affect the change from baseline to simulated night work in the 3-h postprandial glucose AUC during the Dinner Test meal (two-sided, unpaired *t*-test for meal timing group effect: P=n.s.) (**Figure S5A**).

Breakfast, Insulin AUC. The meal timing intervention did not significantly affect the change from baseline to simulated night work in the 3-h postprandial insulin AUC during the Breakfast Test meal (two-sided, unpaired *t*-test for meal timing group effect: P=n.s.) (**Figure S4B**).

Dinner, Insulin AUC. The meal timing intervention did not significantly affect the change from baseline to simulated night work in the 3-h postprandial insulin AUC during the Dinner Test meal (two-sided, unpaired *t*-test for meal timing group effect: P=n.s.; **Figure S5B**).

Averaged across Test meals, Glucose AUC. The meal timing intervention significantly affected the change from baseline to simulated night work in the 3-h postprandial glucose AUC averaged across both Test meals (two-sided, unpaired *T*-test for meal timing group effect: *P*=0.006; **Figure S6**). The change from baseline to simulated night work in glucose AUC significantly differed between groups (NMC Group: 95% CI, 7.4% to 18.3% [95% CI, 1908.5mg/dl*min to 3109.5mg/dl*min]; DMI Group (95% CI, -8.1% to 4.1% [95% CI, -528.5mg/dl*min to 105.7mg/dl*min]) (two-sided, unpaired *t*-test for meal timing group effect: *P*=0.006; **Figure S6A**).

Averaged across Test meals, Insulin AUC. The meal timing intervention did not significantly affect the change from baseline to simulated night work in the 3-h postprandial insulin AUC averaged across both Test meals (two-sided, unpaired *t*-test for meal timing group effect: *P*=n.s.) (**Figure S6B**).

Supplementary Figures

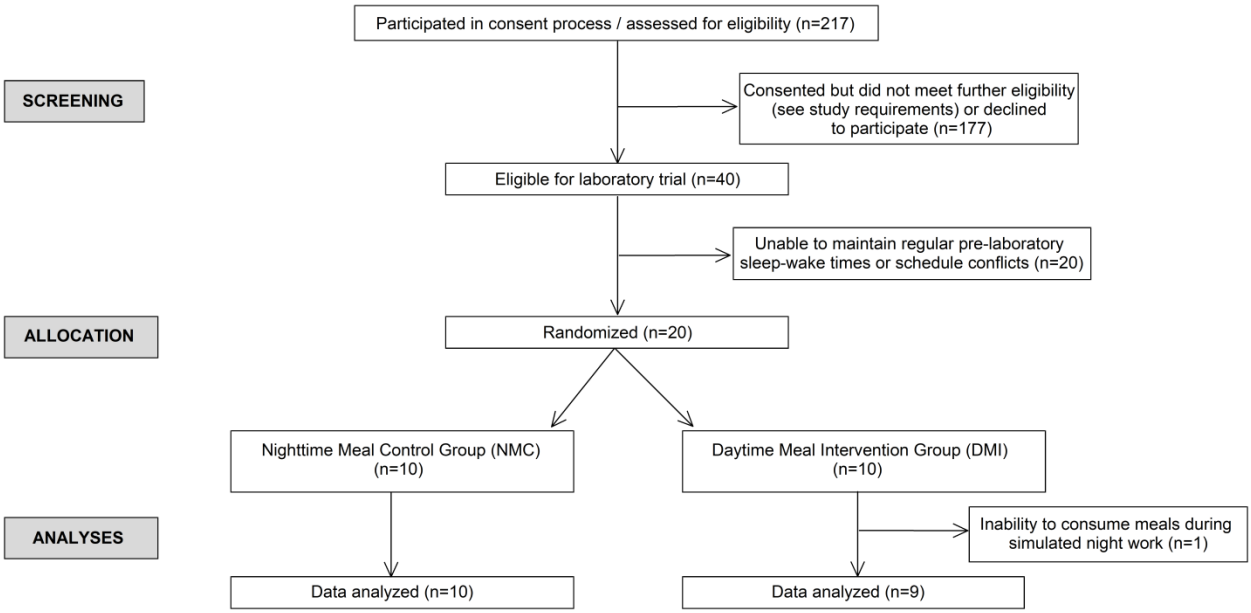

Figure S1. CONSORT Participant flow diagram.

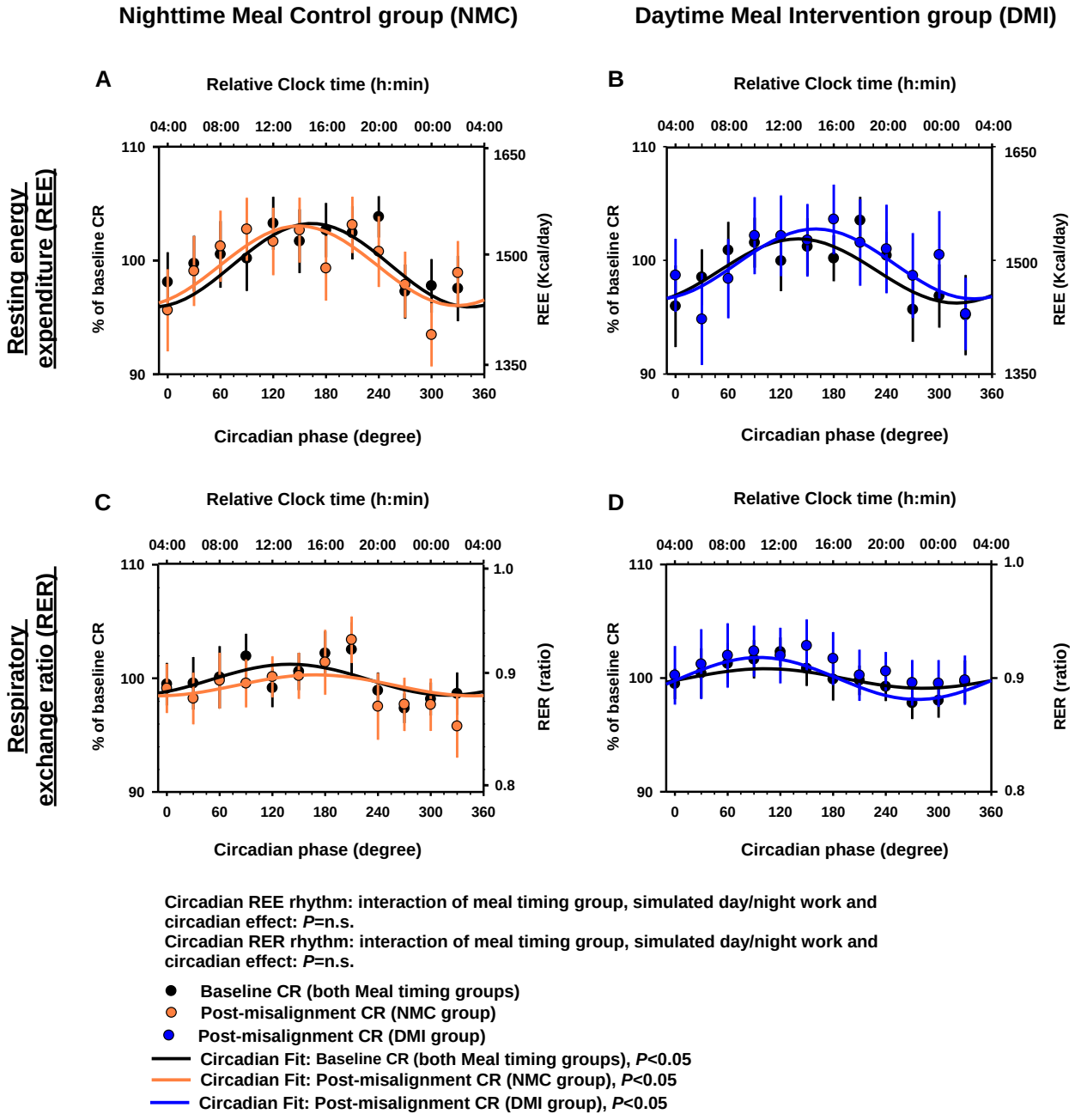

Figure S2. Effects of meal timing intervention on circadian energy expenditure rhythms following simulated night work. (**A-B**) The meal timing intervention did not significantly modify the impact of simulated night work on the circadian resting energy expenditure rhythms. **(C-D).** Likewise, the meal timing intervention did not significantly modify the impact of simulated night work on the circadian resting respiratory exchange ratio rhythms. Bottom X axes: Data grouped into 15-circadian degree windows (~1h resolution) with SEM error bars. Top X axes: scaled to the time of CBT minimum. Data in A-D are the mean ± SEM across participants per simulated day/night work condition and per meal timing group (n=10 in the NMC Group and n=9 in the DMI Group).

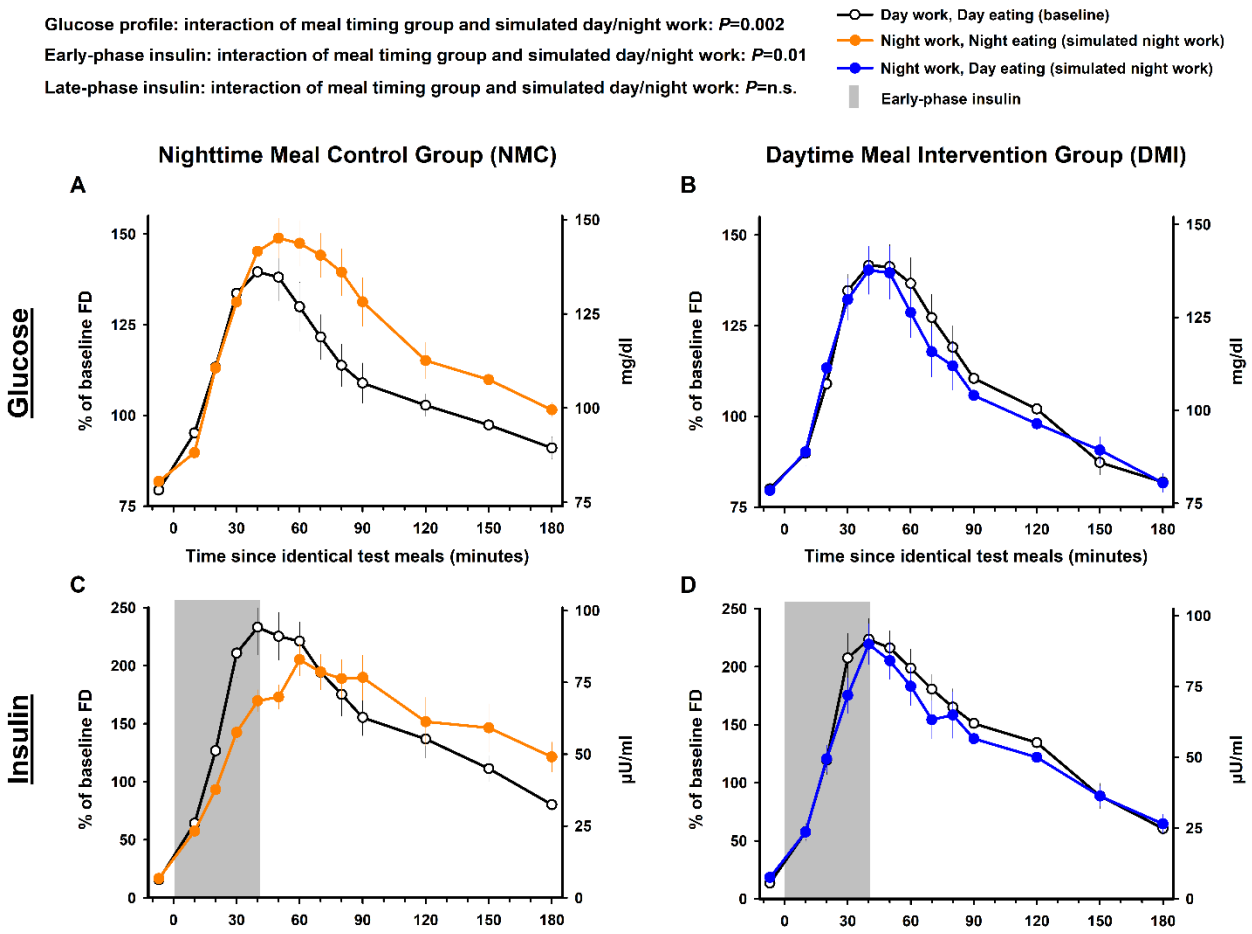

Figure S3. Effects of meal timing intervention on glucose tolerance averaged across test meals. The meal timing intervention significantly modified the impact of simulated night work on the 3-h postprandial glucose and early-phase insulin profiles (average of breakfast and dinner test meals). Simulated night work in the Nighttime Meal Control Group (NMC) adversely influenced the 3-h postprandial glucose profile (**A**) and early-phase postprandial insulin (panel **C**, grey bar). In contrast, no such effects occurred in Daytime Meal Intervention Group (DMI) (**B**, **D**, grey bar). Moreover, the meal timing intervention did not significantly modify the impact of simulated night work on the late-phase insulin profile (**C, D**). Data are the average (mean ± SEM) across participants per simulated day/night work condition and per meal timing group (n=10 in the NMC Group and n=9 in the DMI Group).

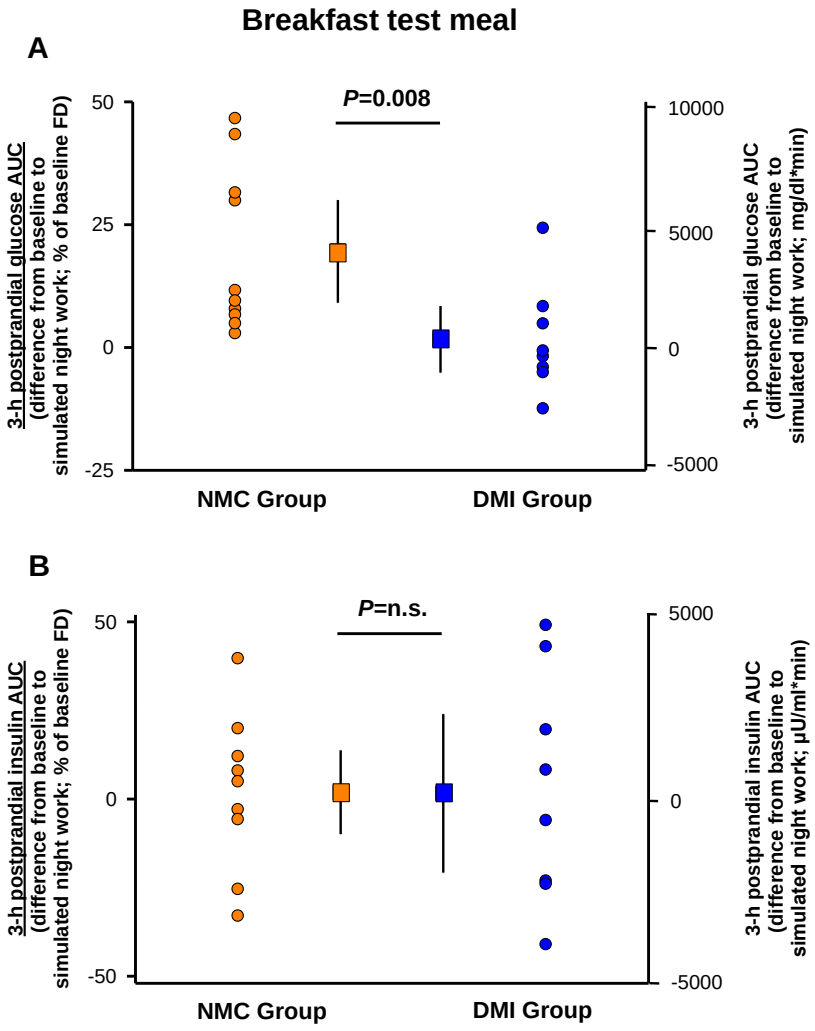

Figure S4. Modulation by meal timing intervention on postprandial glucose and insulin area under the curve (AUC) during the breakfast test meals. The change from baseline (simulated day work) to simulated night work in the 3-h postprandial glucose AUC differed between meal timing groups during the breakfast test meals. Accordingly, all participants in the Nighttime Meal Control Group (NMC) had an increase in postprandial glucose AUC during the breakfast test meal, whereas this did not occur in the Daytime Meal Intervention Group (DMI) (**A**). Conversely, the change from baseline to simulated night work in the 3-h postprandial insulin AUC during the breakfast test meals did not differ between meal timing groups (**B**). Individual AUC values are presented as the difference from baseline to simulated night work (% of baseline FD in left y axes and change in absolute values in the right y axes [average across all individuals in both groups]). Circles correspond to individual data and squares are the mean ± 95% confidence intervals across participants per group. *P*-values correspond to the two-sided, unpaired *t*-test comparisons of the meal timing group effect.

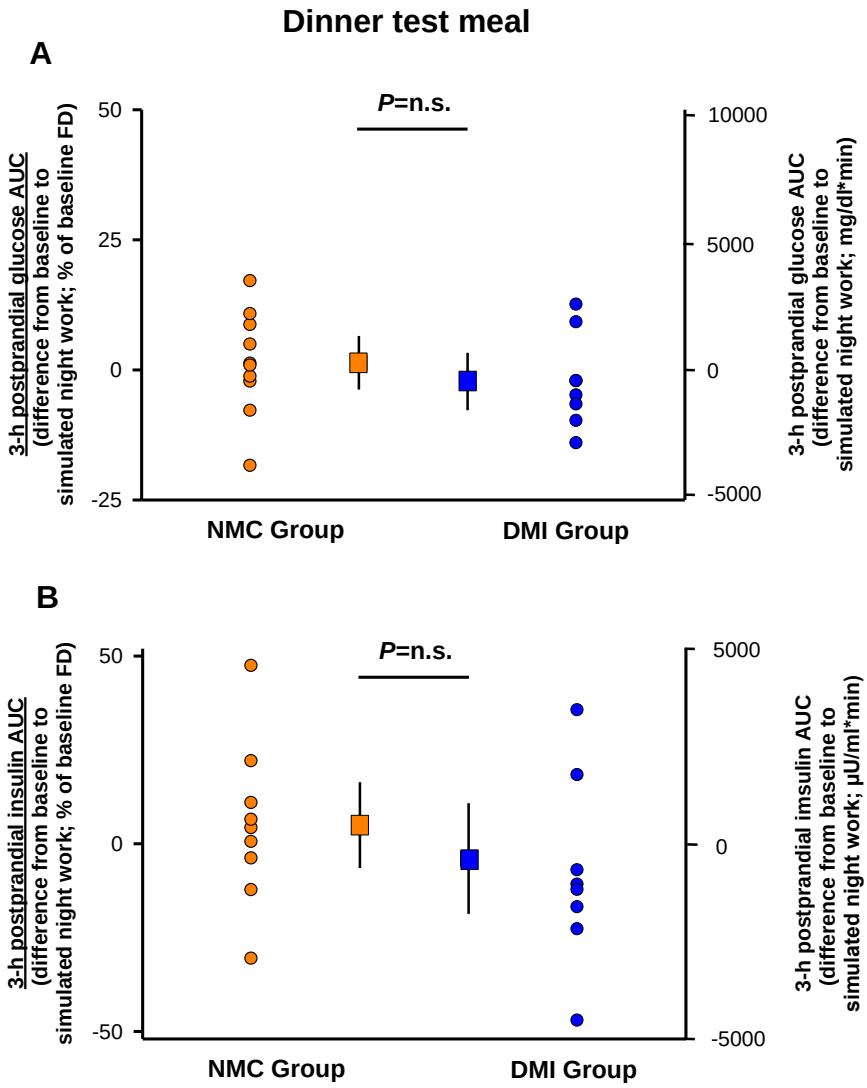

Figure S5. Modulation by meal timing intervention on postprandial glucose and insulin area under the curve (AUC) during the dinner test meals. The change from baseline (simulated day work) to simulated night work in the 3-h postprandial glucose (**A**) and insulin AUC (**B**) did not differ between meal timing groups during the dinner test meals. Individual AUC values are presented as the difference from baseline to simulated night work (% of baseline FD in left y axes and change in absolute values in the right y axes [average across all individuals in both groups]). Circles correspond to individual data and squares are the mean ± 95% confidence intervals across participants per group. *P*-values correspond to the two-sided, unpaired *t*-test comparisons of the meal timing group effect.

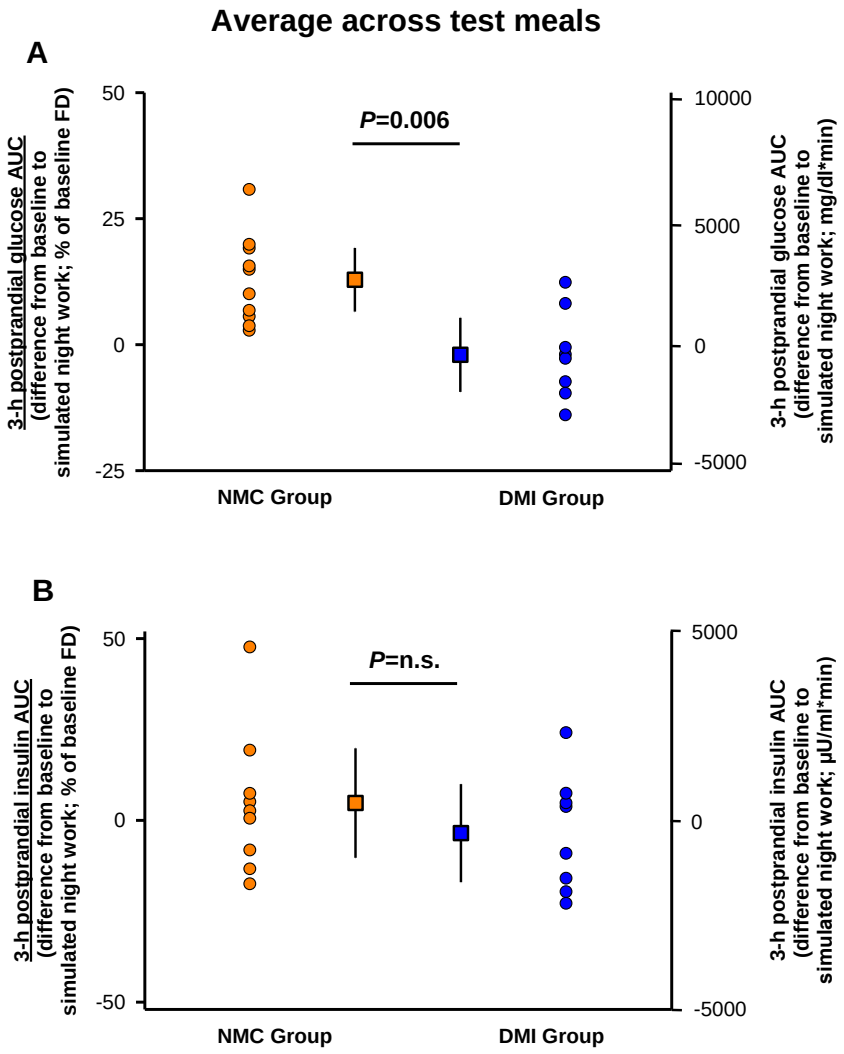

Figure S6. Modulation by meal timing intervention on postprandial glucose and insulin area under the curve (AUC) averaged across test meals. The change from baseline (simulated day work) to simulated night work in the 3-h postprandial glucose AUC differed between groups, such that all participants in the Nighttime Meal Control Group (NMC) had an increase in glucose AUC averaged across test meals, whereas this did not occur in the Daytime Meal Intervention Group (DMI) (**A**). Conversely, the change from baseline to simulated night work in the 3-h postprandial insulin AUC averaged across test meals did not differ between groups (**B**). Individual AUC values are presented as the difference from baseline to simulated night work (% of baseline FD in left y axes and change in absolute values in the right y axes [average across all individuals in both groups]) averaged across breakfast and dinner test meals. Circles correspond to individual data and squares are the mean ± 95% confidence intervals across participants per group. *P*-values correspond to the two-sided, unpaired *t*-test comparisons of the meal timing group effect.

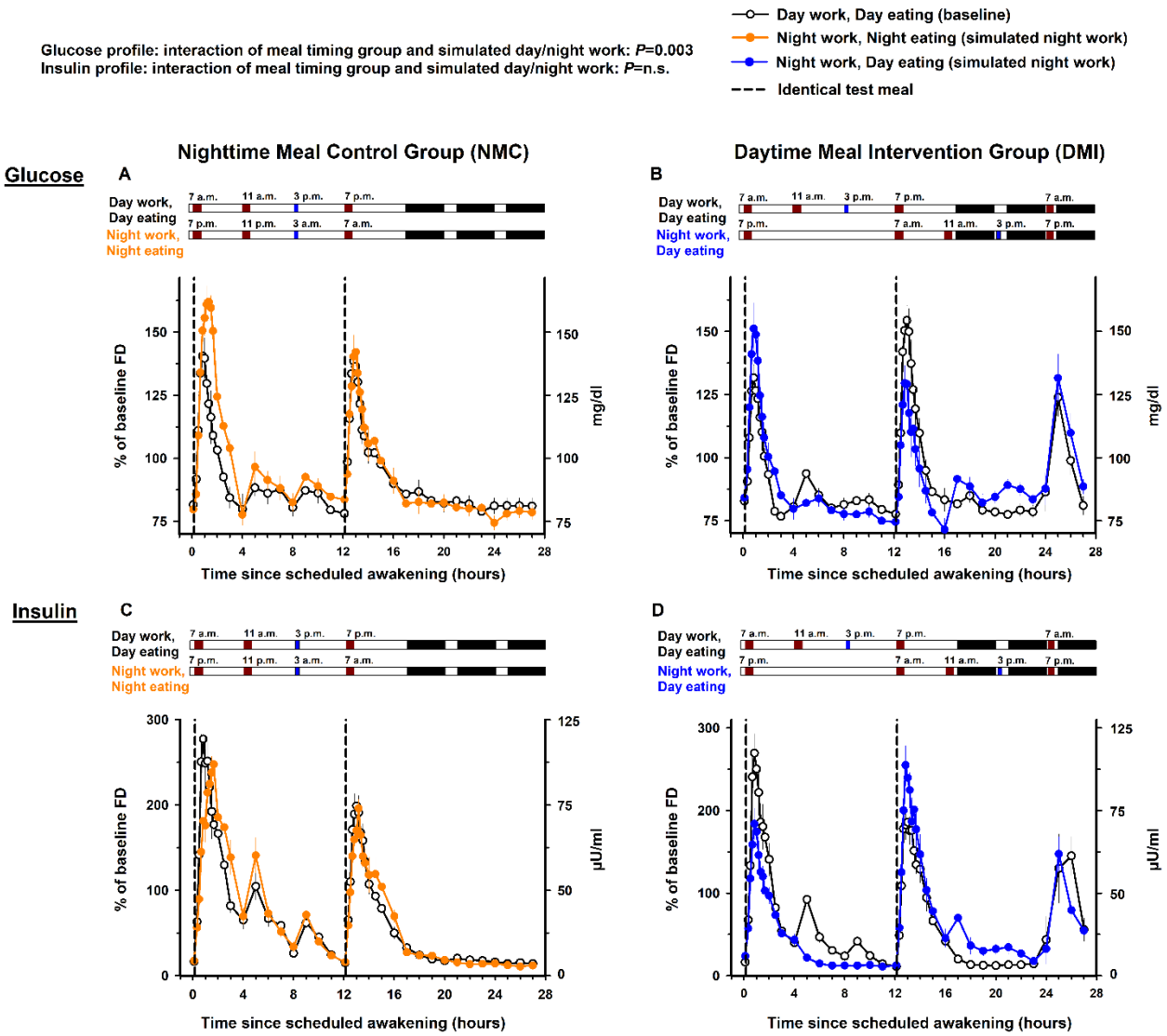

Figure S7. Effects of meal timing intervention on 28-h profiles of glucose and insulin. The meal timing intervention significantly modified the impact of simulated night work on the glucose profile but not of insulin. Accordingly, simulated night work in the Nighttime Meal Control Group (NMC) adversely influenced the average glucose profile (**A**), but not that of insulin (**C**). In contrast, simulated night work in the Daytime Meal Intervention Group (DMI) did not adversely affect glucose (**B**) or insulin profiles (**D**). Data plotted across 28-h to show the direct comparison of baseline and simulated night work, matched up by time into the sleep/wake cycle. Top X axes indicate relative scheduled meal, sleep and wake times (simulated day work on top and night work below); bottom X axes indicate time since schedule awakening for all participants. Data are the average (mean ± SEM) across participants per simulated day/night work condition and per meal timing group (n=10 in the NMC Group, n=9 in the DMI Group).

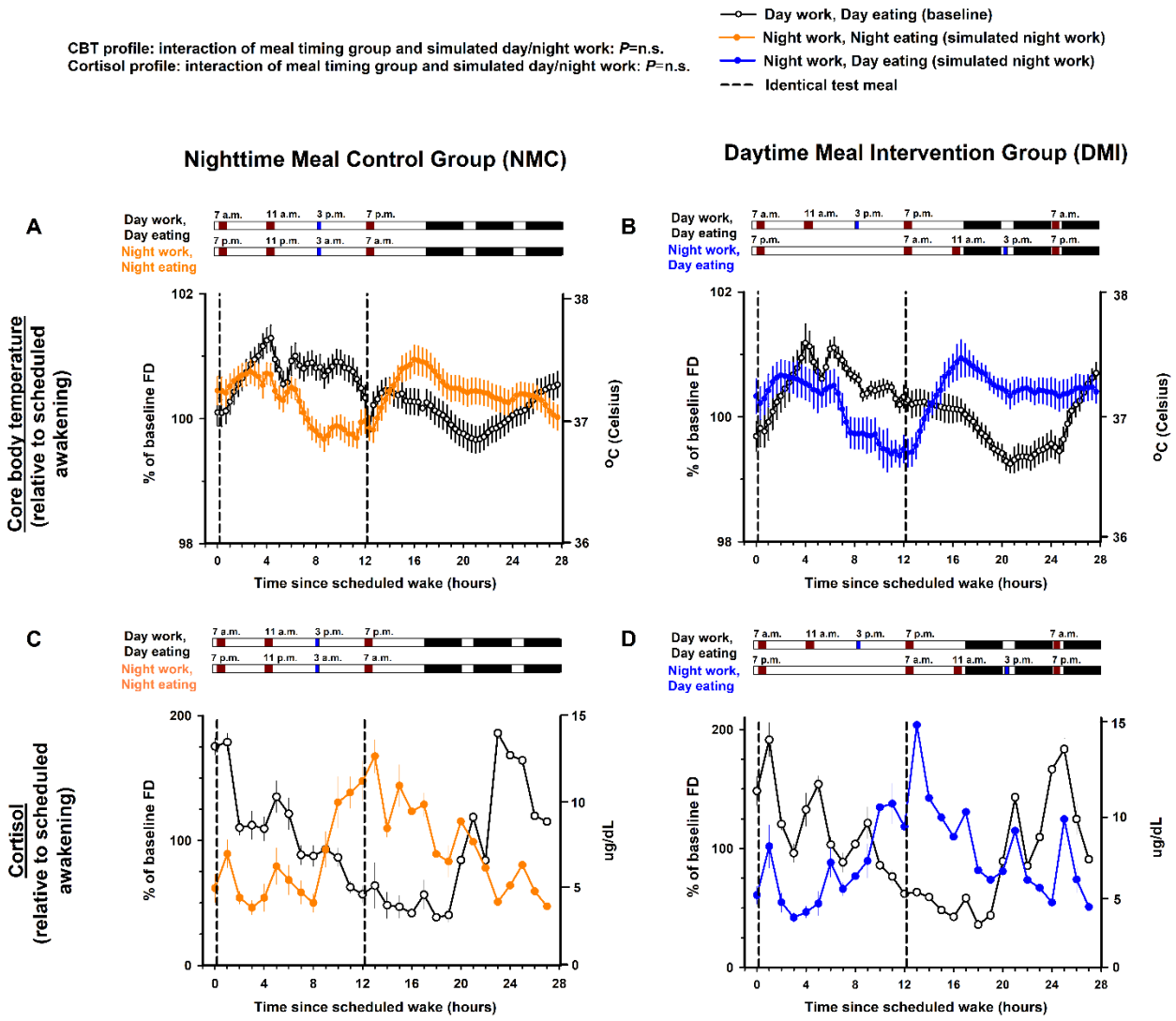

Figure S8. Effects of meal timing intervention on 28-h profiles of core body temperature (CBT) and cortisol. The meal timing intervention did not significantly modify the impact of simulated night work on the CBT and cortisol profiles. Accordingly, simulated night work did not significantly affect the average CBT (**A, B**) and cortisol (**C, D**) profiles, as compared to baseline, in either group. Data plotted across 28-h to show the direct comparison of baseline and simulated night work conditions, matched up by time into the sleep/wake cycle. Moreover, as expected for outputs under strong central circadian control, CBT and cortisol profiles closely follow the circadian clock. Data correspond to the average (mean ± SEM) across participants per simulated day/night work condition and per meal timing group (n=10 in the NMC Group, n=9 in the DMI Group).

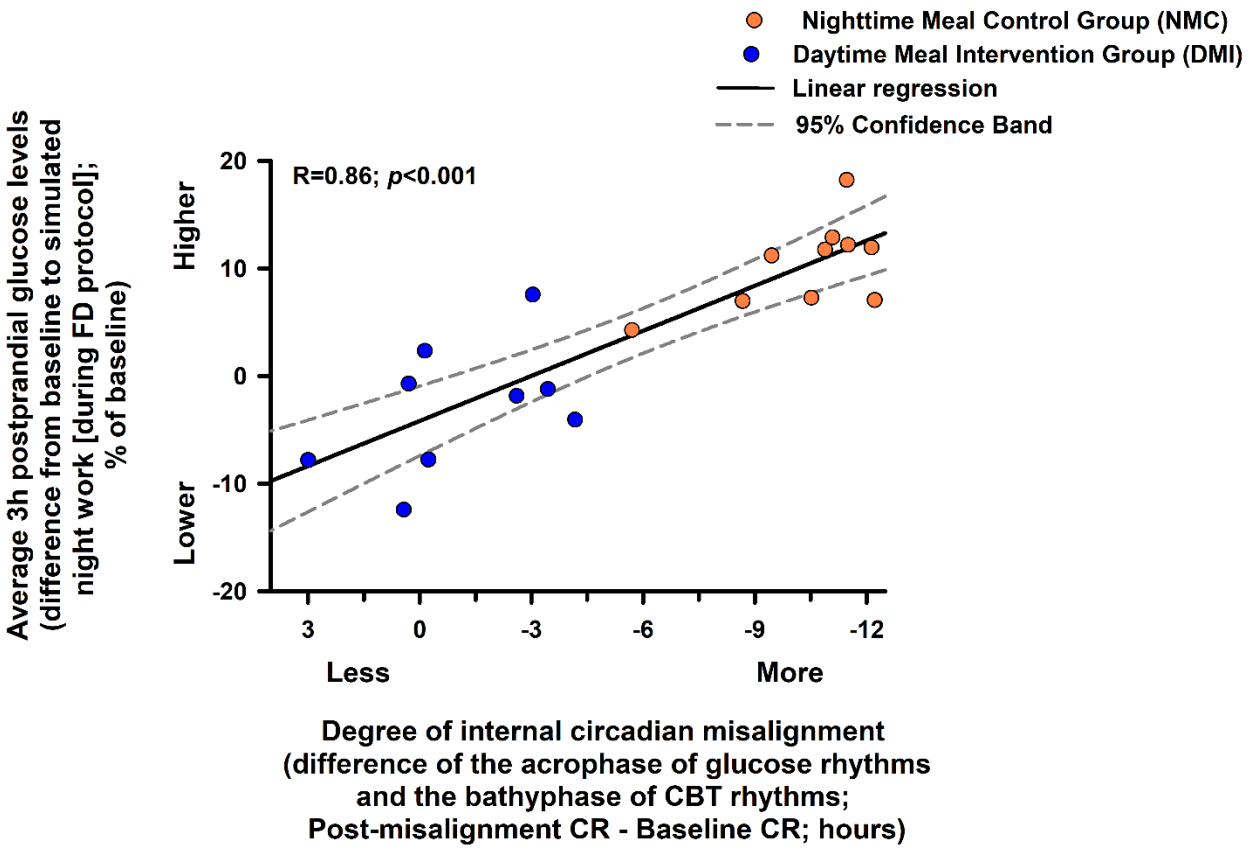

Figure S9. Association of magnitude of internal circadian misalignment with that of impaired glucose tolerance during circadian misalignment. The degree of internal circadian misalignment was positively associated with impaired glucose tolerance during circadian misalignment induced by the 28-h Forced Desynchrony protocol (r=0.86; P<0.001) across participants in both meal timing groups. Circles correspond to individual data and solid and dashed lines correspond to, respectively, linear regression models and the 95% confidence interval. Bottom X axes: Degree of internal circadian misalignment as indexed by the change in the phase difference between the acrophase of circadian glucose rhythms and the bathyphase of circadian body temperature rhythms (expressed as the difference from Baseline CR to Post-misalignment CR, in hours). Y axes: average of the 3-h postprandial glucose levels, expressed as the change from baseline to simulated night work (during the FD protocol; % of Baseline FD).

Table S1. Baseline demographics and study-related characteristics assessed during participant screening (before the laboratory circadian protocol) between meal timing groups.

	Nighttime Meal Control Group (NMC)	**Daytime Meal Intervention Group (DMI)**	*P*-value*
n	10	9	
Age (y)	27.0 (23.8 to 30.2)	26.2 (23.0 to 29.4)	0.70
Sex	4 women; 6 men	3 women; 6 men	0.99
Body-mass index (kg/m^2)	22.5 (21.2 to 23.8)	23.1 (21.1 to 25.0)	0.61
Diet	2 vegetarians; 8 non-vegetarians	1 vegetarian; 8 non-vegetarians	0.99
Race	1 African-American; 1 Asian-American; 8 Caucasians	1 African-American; 8 Caucasians	0.99
Ethnicity	10 non-Hispanics	7 non-Hispanics; 2 Hispanics	0.41
Pittsburg Sleep Quality Index	2.2 (1.5 to 2.9)	2.1 (1.4 to 2.8)	0.83
Epworth Sleepiness Scale	4.2 (1.2 to 7.1)	5.2 (2.6 to 7.8)	0.54
Chronotype (Horne-Osberg)	58.5 (52.8 to 64.2)	57.1 (50.2 to 64.0)	0.72
Beck Depression Inventory	1.4 (0.2 to 2.9)	1.1 (0.1 to 6.1)	0.59
TSH (uIU/mL)	1.4 (0.9 to 1.8)	1.7 (1.1 to 2.3)	0.41
Glucose (mg/dL)	84.9 (78.5 to 91.2)	82.4 (76.5 to 88.4)	0.55
Heart rate (bpm)	80.6 (69.8 to 91.4)	72.9 (60.2 to 85.2)	0.30
Systolic blood pressure (mmHg)	125.3 (107.7 to 125.8)	117.1 (107.4 to 126.8)	0.38
Diastolic blood pressure (mmHg)	70.9 (64.5 to 77.3)	68 (58.4 to 77.5)	0.56
Hemoglobin A1C (%)	5.2 (4.9 to 5.4)	5.2 (5.0 to 5.4)	0.86

Data are Mean and 95% Confidence intervals.

Yates's chi-squared tests for sex, diet, race and ethnicity. *T*-tests for independent groups for the other outcomes.

Table S2. Sleep structure characteristics assessed during the sleep before baseline and simulated night work conditions in the Nighttime Meal Control Group (NMC) and the Daytime Meal Intervention Group (DMI).

	Nighttime Meal Control Group (NMC)		**Daytime Meal Intervention Group (DMI)**		**Group**	**Condition**	**Interaction**
Sleep structure	Day work, Day eating (baseline; Day 6)	Night work, Night eating (simulated night work; Day 10)	Day work, Day eating (baseline; Day 6)	Night work, Day eating (simulated night work; Day 10)	*P*-value[*]	*P*-value[**]	*P*-value[***]
N1 (min)	20.5 (14.3 to 26.8)	19.1 (16.9 to 21.2)	23.7 (17.3 to 30.1)	21.1 (17.2 to 24.9)	0.31	0.41	0.81
N2 (min)	164.4 (132.2 to 196.6)	187.3 (150.8 to 223.8)	143.7 (121.1 to 166.2)	158.0 (124.6 to 191.4)	0.13	0.26	0.79
N3 (min)	88.9 (66.2 to 111.6)	113.9 (88.7 to 139.1)	90.5 (71.6 to 109.5)	99.0 (82.4 to 115.7)	0.55	0.13	0.45
REM sleep (min)	84.3 (68.9 to 99.5)	86.6 (74.5 to 98.8)	96.9 (84.3 to 109.5)	78.1 (62.7 to 93.4)	0.54	0.14	0.46
Wake (min)	120.3 (87.8 to 152.8)	152.5 (100.1 to 204.9)	119.7 (73.1 to 150.3)	201.9 (153.1 to 250.8)	0.36	0.001	0.20
Sleep onset N1 (min)	31.8 (20.2 to 43.4)	32.3 (18.8 to 45.7)	32.9 (20.2 to 45.8)	37.0 (18.4 to 55.6)	0.69	0.75	0.81
Sleep onset N2 (min)	36.5 (25.1 to 47.8)	37.1 (22.5 to 51.5)	40.9 (26.3 to 55.4)	40.3 (20.8 to 59.7)	0.81	0.79	0.73
Sleep efficiency (%)	74.8 (68.1 to 81.6)	72.7 (63.3 to 82.1)	75.8 (67.6 to 82.9)	67.8 (60.1 to 77.5)	0.34	0.10	0.24
TST (min)	358.1 (325.7 to 390.5)	406.9 (354.5 to 459.4)	354.8 (325.1 to 384.7)	356.2 (308.5 to 403.9)	0.50	0.45	0.70

Data correspond to the actual descriptive data, and are presented as mean and 95% confidence intervals; N1-N3: non-REM sleep stages 1-3; wake: wakefulness after lights off; TST: total sleep time; time in bed for sleep before baseline was 480 min and 560 min before simulated night work conditions in both meal timing groups.

[*] *P*-values correspond to main effect "Meal timing group" in mixed-model analyses.

[**] *P*-values correspond to main effect "Circadian alignment/misalignment condition" in mixed-model analyses.

[***] *P*-values correspond to interaction of "Meal timing group" vs. "Circadian alignment/misalignment condition" in mixed-model analyses.