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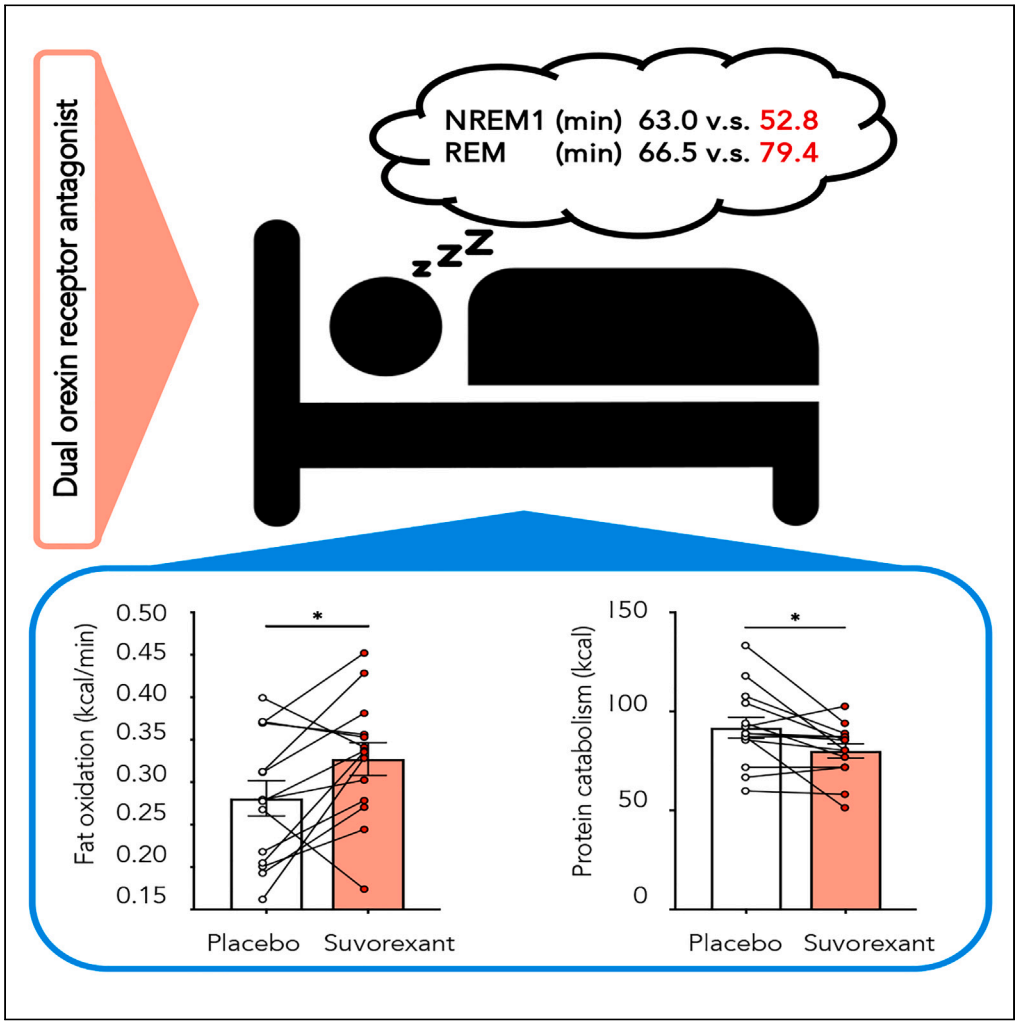

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Highlights

Orexin receptor antagonist increased fat burning during sleep and following morning

Orexin receptor antagonist decreased protein catabolism

Orexin system affects fat oxidation independent of its roles in sleep regulation

Orexin receptor antagonists may aid body weight management in clinical practice

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Orexin receptor antagonist increases fat oxidation and suppresses protein catabolism during sleep in humans

Insung Park,[1] Rikako Yoshitake,[2] Kazuki Kioka,[2] Asuka Ishihara,[1] Katsuhiko Yajima,[3] Fusae Kawana,[1] Toshio Kokubo,[1] Ichiyo Matsuzaki,[1] Takashi Kanbayashi,[1] Masashi Yanagisawa,[1] and Kumpei Tokuyama[1,4,*]

SUMMARY

Suvorexant is an orexin receptor antagonist that targets the wake-promoting system. Orexin is also known to regulate energy metabolism in rodents, but its role in humans remains largely unknown. Here, we assessed the effect of suvorexant (20 mg) on energy metabolism during sleep and shortly after awakening in a randomized, double-blind, placebo-controlled, crossover study in 14 healthy men. Suvorexant increased rapid eye movement (REM) but decreased nonrapid eye movement (NREM) stage 1. Energy expenditure during wake after sleep onset (WASO) was higher than that during NREM and REM sleep in the placebo but not in the suvorexant trial, suggesting that the increase in energy expenditure during WASO was due to an activation of the orexin system. Fat oxidation during sleep increased, and its effect remained after waking the next morning. Suvorexant decreased protein catabolism but did not affect overall energy expenditure. The orexin system may affect fat oxidation independent of its roles in sleep regulation in humans.

INTRODUCTION

Orexins are neuropeptides produced in the lateral hypothalamus, and it plays a key role in enhancing feeding behavior and maintaining arousal.[1] Prepro-orexin, the precursor peptide of orexin, produces two neuropeptides of distinct lengths, orexin-A and orexin-B.[2] In mammals, the orexin exerts its effect by binding and activating two subtypes of G-protein-coupled orexin receptors, the orexin-1 and orexin-2 receptors, which have 64% amino acid identity with each other. Orexin-2 receptor activation is necessary to stabilize wakefulness, whereas activation of both orexin-1 and orexin-2 receptors inhibits rapid eye movement (REM) sleep.[3] Suvorexant, the first approved dual orexin receptor antagonist, targets the orexin-mediated wake-promoting system and offers an alternative mechanistic approach to treating insomnia as it blocks both the orexin-1 and orexin-2 receptors.[4]

The close link between sleep and energy metabolism has become increasingly recognized over the past two decades. Insufficient sleep is a risk factor for weight gain,[5] whereas 2 weeks of sleep extension decreases objectively assessed energy intake, body weight, and body fat.[6] Orexin was originally named after its role to induce feeding,[1] but it is also a potent stimulator of wakefulness. In animal experiments, loss of orexin function by administration of orexin receptor antagonists and orexin gene knockout decreases energy expenditure.[7–9] On the other hand, a gain of orexin function in mice by injection of orexin-A into the third ventricle increases energy expenditure.[10] Because activation of the orexin system induces wakefulness, food-seeking activity, and food intake, the effect of orexin to increase energy expenditure in free-moving rodents may or may not be mediated by its effects to change behaviors. In an experimental protocol of human study, differences in behaviors, such as timing of meals and sleep, can be matched.

Support for a role of the orexin system in regulating substrate oxidation is inconsistent. In mice, the respiratory quotient (RQ), which reflects oxidized substrate in the body, is decreased, unaffected, or increased by central administration of orexin-A depending on the time of the orexin-A injection and nutritional state.[7,10] Similarly, a loss of function of the orexin system decreases RQ during the dark phase, but not during the light phase.[8] We previously reported that 20 mg of suvorexant transiently decreased energy expenditure during sleep.[11] This finding, however, may be related to the specific experimental design, which involved forced awakening at 90 min after sleep.

The present study was a randomized, double-blind, placebo-controlled, cross-over trial investigating the effects of the orexin receptor antagonist suvorexant on sleeping energy metabolism under continuous undisrupted sleep (Figure 1). Twenty milligram dose of suvorexant was chosen as for young subjects in Japan. Energy metabolism was assessed using whole-room indirect calorimetry with an improved time

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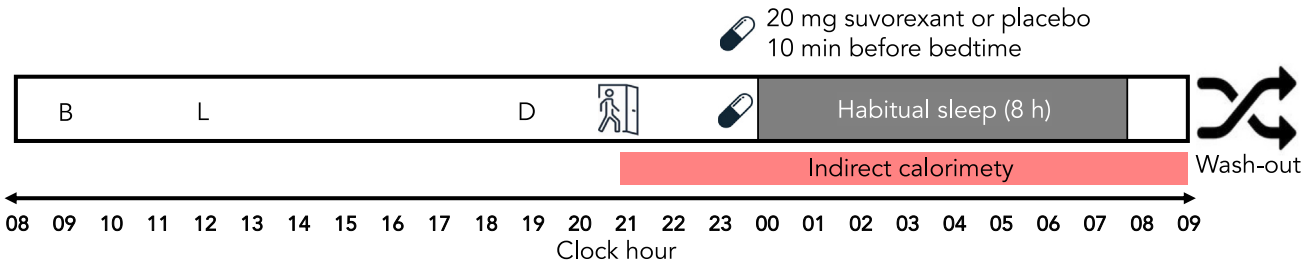

Figure 1. Study protocol

For subjects whose habitual bedtime is 00:00, indirect calorimetry began at 21:00 and ended at 09:00 the next morning. After fitting the subjects with the electrodes for polysomnography, they entered the metabolic chamber and remained sedentary until bedtime. Breakfast, lunch, and dinner are indicated as (B), (L), and (D), respectively. Each trial included a 1-week washout period.

resolution.[12] As effects of suvorexant on sleep architecture were expected, energy metabolism between the two trials was compared by adjusting the effect of the sleep stage and time after sleep onset on energy metabolism using a semi-parametric regression analysis.[13]

RESULTS

Participant characteristics

Participant characteristics are shown in Table 1. All participants completed the two trials, and no significant differences in weight, body fat, or BMI were detected between trials. All participants met the inclusion/exclusion criteria in their entirety.

Sleep

The sleep architecture was similar between the two trials, except for a longer duration of REM sleep and a shorter duration of N1 observed in the trials with suvorexant administration (Table 2). In both trials, the time course of the sleep architecture showed characteristic changes; slow-wave sleep (SWS) gradually decreased and was replaced by nonrapid eye movement (NREM) stage 2 and REM sleep (Figure 2).

Energy metabolism

Suvorexant administration was associated with a significant increase in the average fat oxidation over the entire sleeping period (effect size = 0.613; 2-tailed t test, $p = 0.033$), but no changes in energy expenditure, carbohydrate oxidation, and RQ were observed (Figures 3A–3D). A 2-factor repeated measures analysis of variance (ANOVA) identified a significant main effect of time ($p < 0.0001$) and trial ($p = 0.033$) in the hourly mean values of fat oxidation, but no significant interaction between time and trial. The hourly mean values of energy expenditure, carbohydrate oxidation, and RQ during sleep showed a significant main effect of time ($p < 0.001$), but no significant main effect of trial or interaction between time and trial. During the first hour after waking the next morning in the suvorexant trial, the increased fat oxidation (2-tailed t test, suvorexant trial: 0.39 $\pm$ 0.04 kcal/min vs. placebo trial: 0.29 $\pm$ 0.04 kcal/min, $p = 0.0003$) and decreased RQ (2-tailed t test, suvorexant trial: 0.87 $\pm$ 0.01 vs. placebo trial: 0.88 $\pm$ 0.01, $p = 0.011$) remained significant (Figure 3E). Protein catabolism was significantly suppressed in the suvorexant trial (effect size = 0.696; 2-tailed t test, $p = 0.019$) (Figure 3F).

Table 1. Physical characteristics of subjects

Anthropometric variables	Mean $\pm$ SEM
Age (years)	24.5 $\pm$ 1.0
Height (cm)	171.7 $\pm$ 1.4
Body weight (kg)	66.1 $\pm$ 3.2
BMI (kg/m^2)	22.3 $\pm$ 0.9
Body fat (%)	19.5 $\pm$ 0.1
Questionnaire variables	**Mean $\pm$ SEM**
PSQI	3.5 $\pm$ 0.4
MEQ	52.9 $\pm$ 2.5
ESS	5.1 $\pm$ 0.7

A total of 14 subjects were recruited.
BMI, body mass index; PSQI, Pittsburgh Sleep Questionnaire Index; MEQ, Morningness-Eveningness Questionnaire; ESS, Epworth Sleepiness Scale.

Table 2. Sleep architecture

	Placebo	Suvorexant	
Parameters	Mean ± SEM	Mean ± SEM	*p*
Total bedtime (min)	480.0	480.0	—
Total sleep time (min)	443.10 ± 07.5	455.60 ± 04.7	0.076
Sleep efficiency (%)	92.30 ± 01.6	94.90 ± 01.0	0.076
Wakefulness (min)	23.60 ± 05.6	15.60 ± 03.9	0.251
Stage 1 (min)	63.00 ± 04.2	52.80 ± 03.6	0.032*
Stage 2 (min)	246.10 ± 09.9	249.00 ± 07.6	0.685
SWS (min)	67.50 ± 07.4	74.50 ± 07.6	0.095
REM sleep (min)	66.50 ± 06.1	79.40 ± 06.1	0.009**
Sleep latency (min)	13.40 ± 04.2	8.80 ± 02.2	0.085
Stage 2 latency (min)	4.70 ± 00.7	3.80 ± 00.5	0.214
SWS latency (min)	28.10 ± 04.3	25.00 ± 02.7	0.402
REM sleep latency (min)	105.90 ± 06.8	97.50 ± 11.7	0.530

SWS, slow-wave sleep; REM, rapid eye movement.
*$p < 0.05$ and **$p < 0.01$.

Semi-parametric analysis of energy metabolism

The effect of the sleep stage on energy metabolism, which is independent of the effect of time after sleep onset by definition of semi-parametric analysis, is shown in Figures 4, 5, 6, and 7. A significant main effect of sleep stage ($p < 0.0001$) and a significant interaction between sleep stage and trial ($p = 0.019$) on energy expenditure were observed. In the multiple comparisons, energy expenditure during wake after sleep onset (WASO) in the placebo trial was significantly higher than that during N1, N2, SWS, and REM (Figure 4A). However, the difference in energy expenditure among different sleep stages in the suvorexant trial was not statistically significant ($p > 0.999$). The energy expenditure during the WASO phase in the placebo trial, after applying the false discovery rate (FDR) correction, remained significantly higher than the levels observed during N1, N2, SWS, and REM (Table S1). A significant main effect of sleep stage ($p < 0.001$) on fat oxidation (Figure 5A), carbohydrate oxidation (Figure 6A), and RQ (Figure 7A) was observed. However, there was no significant main effect of trial on fat oxidation ($p = 0.089$), carbohydrate oxidation ($p = 0.575$), and RQ ($p = 0.249$). The interaction between sleep stage and trial on fat oxidation ($p = 0.312$), carbohydrate oxidation ($p = 0.397$), and RQ ($p = 0.528$) was not significant.

The effect of time after sleep onset on energy expenditure, carbohydrate oxidation, fat oxidation, and RQ during each sleep stage was also evaluated. A significant main effect of time on energy expenditure (Figures 4B–4F), carbohydrate oxidation (Figures 6B–6F), and RQ

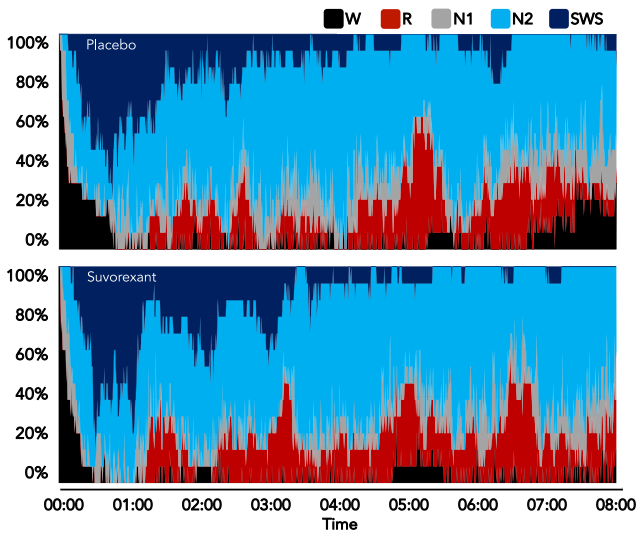

Figure 2. Time course of sleep architecture

Cumulative sleep architecture of the 14 subjects during the placebo trial (upper panel) and the suvorexant trial (bottom panel). The percentage of subjects in stage W (wakefulness; black), stage N1 (gray), stage N2 (light blue), SWS (dark blue), and stage REM (red) changed with the sleep time.

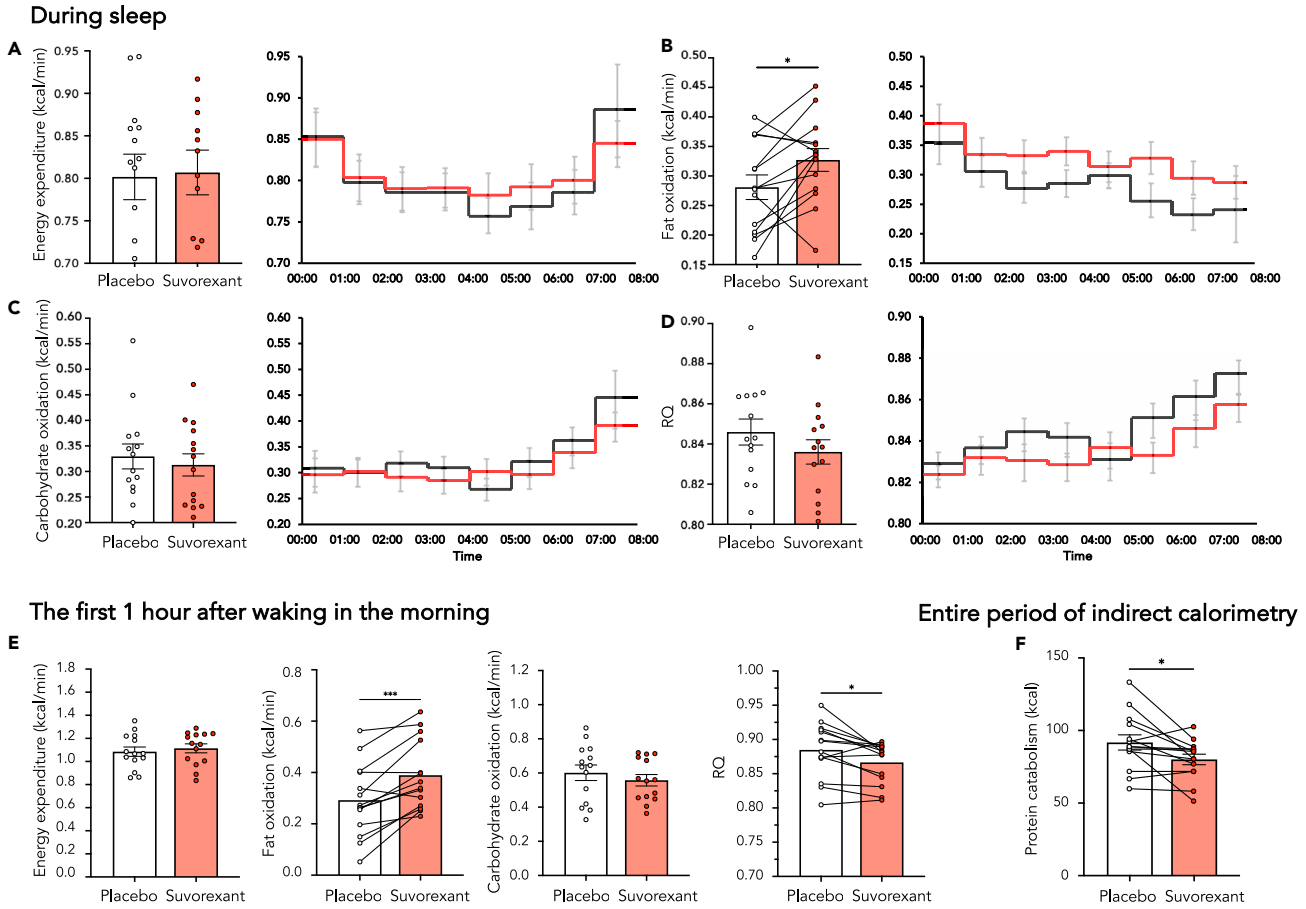

Figure 3. Energy expenditure and substrate oxidation

(A–F) The mean of energy expenditure (A), fat oxidation (B), carbohydrate oxidation (C), and RQ (D) during sleep is shown as the bar graph with individual plots in black and red for the placebo and suvorexant trials, respectively. The time course of an hourly average energy metabolism during sleep is shown as line graph in black and red for the placebo and suvorexant trials, respectively. The mean energy metabolism during the first hour after waking in the morning is shown (E). Protein catabolism during the entire period of indirect calorimetry with individual plots connected by a line (F).

Values are mean ± SEM. The asterisk represents a statistically significant difference between the placebo and suvorexant trials by a paired t test (*$p < 0.05$, ***$p < 0.001$).

(Figures 7B–7F) was observed in all sleep stages ($p < 0.001$). However, there was no significant main effect of trial or an interaction between the time and trial. A significant main effect of time ($p < 0.0001$) and trial ($p = 0.025$) on fat oxidation during N2 was observed, but no significant interaction between time and trial (Figures 5B–5F). During N1, SWS, REM, and WASO, a significant main effect of time on fat oxidation was observed ($p < 0.001$), but there was no significant main effect of trial or interaction between time and trial. The supplemental information contains the list of all statistical results for the semi-parametric analysis of energy metabolism after applying the FDR correction.

Urinary metabolites

We conducted a urine metabolome analysis using urinary samples collected during the entire indirect calorimetry period of the two trials. The analysis encompassed various urinary metabolites, including those related to glycolysis, the TCA cycle, amino acids, acylcarnitines, and vitamins (Table 3). However, the differences in urinary metabolites between the two trials were not statistically significant.

DISCUSSION

The major finding of the present study in healthy human subjects is that suvorexant administration affected energy metabolism, specifically enhancing fat oxidation and suppressing protein catabolism during sleep.

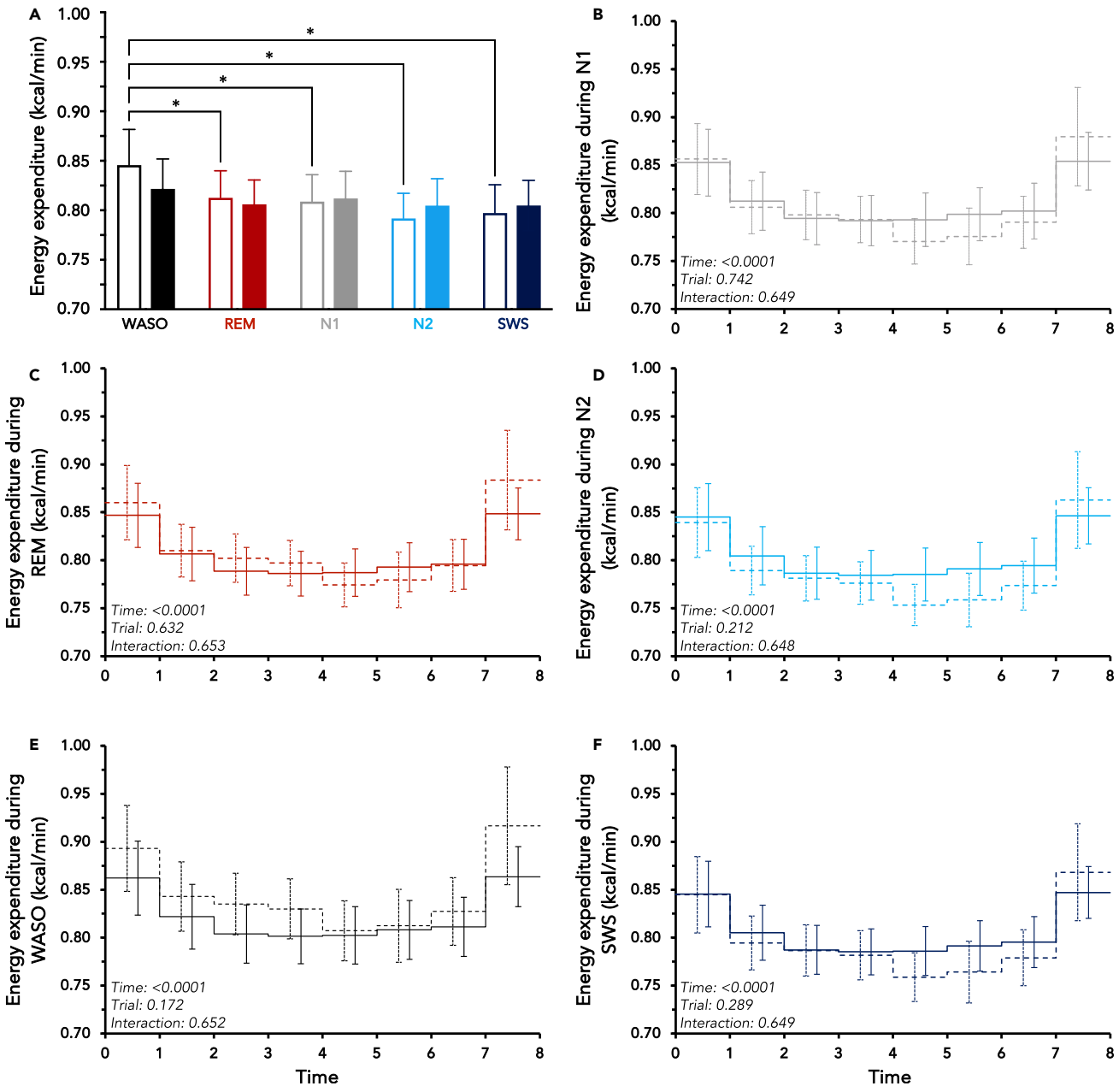

Figure 4. Semi-parametric analysis of energy expenditure

(A) Energy expenditure during sleep was decomposed into the effect of time and the effect of sleep stage by a semi-parametric regression analysis. Average energy expenditure during the entire sleeping period in each sleep stage is shown (A). The lines above the bars represent statistically significant differences among sleep stages (*$p < 0.05$).

(B–F) The effect of time after sleep onset on energy expenditure in each sleep stage is shown as an hourly average in each panel as solid line in the suvorexant trial and in the placebo trial as dotted line.

Values are mean ± SEM. The p value in the panel represents a statistical difference between time course of energy expenditure of two trials by a two-factor repeated measures analysis of variance (ANOVA). The q-value, according to the FDR correction, is represented in Table S1.

Energy expenditure during sleep was not affected by suvorexant in the present study. However, semi-parametric analysis of sleeping energy metabolism in the present study revealed that energy expenditure during WASO was higher than that during NREM (N1, N2, and SWS) and REM sleep in a placebo-controlled trial. Interestingly, no differences in energy expenditure between WASO and other sleep stages were observed in the suvorexant trial. These observations suggest that energy expenditure is increased during WASO due to activation of the

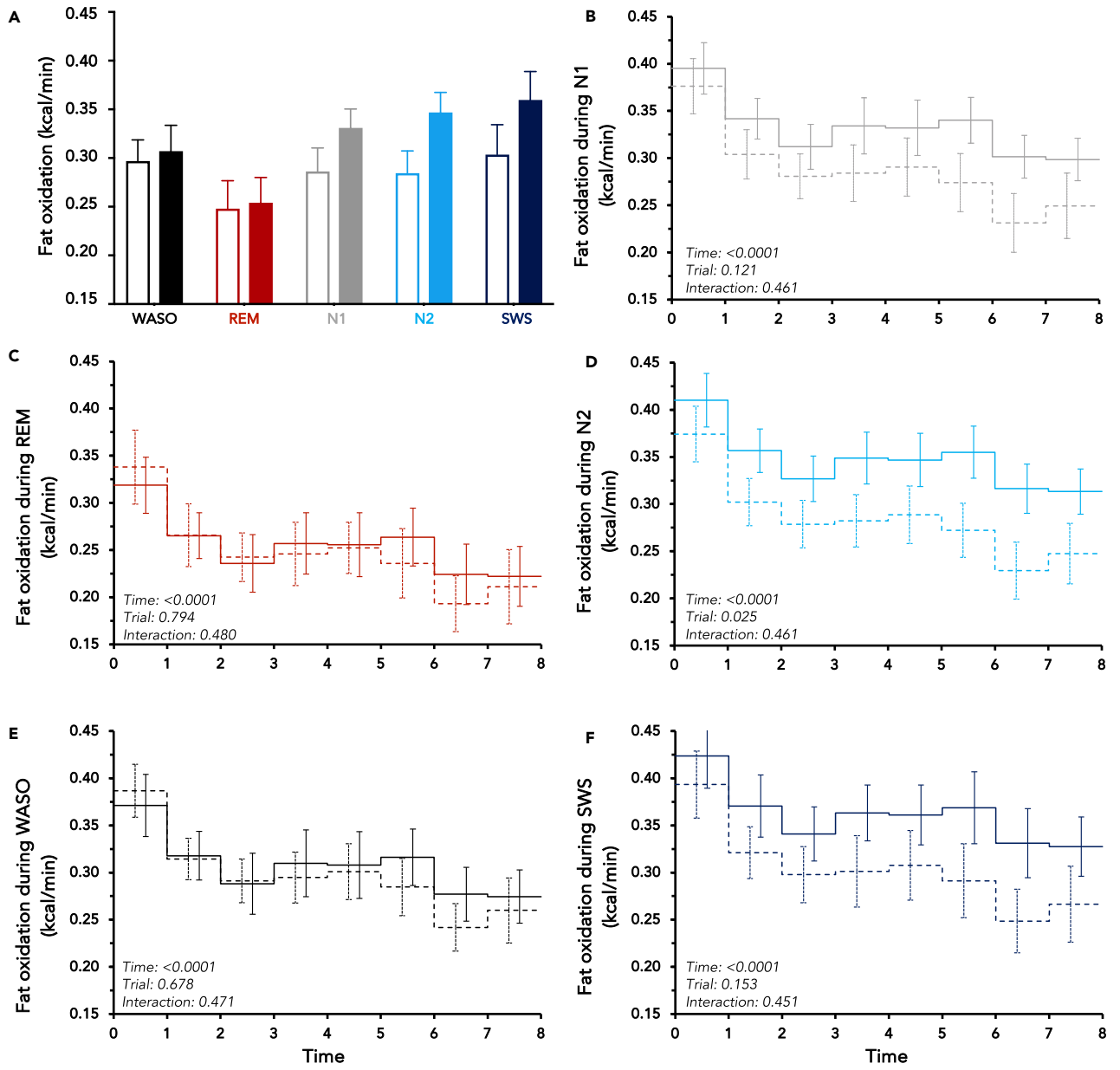

Figure 5. Semi-parametric analysis of fat oxidation

(A) Fat oxidation during sleep was decomposed into the effect of time and the effect of sleep stage by a semi-parametric regression analysis. Average fat oxidation during the entire sleeping period in each sleep stage is shown (A).

(B–F) The effect of time after sleep onset on fat oxidation in each sleep stage is shown as an hourly average in each panel as solid line in the suvorexant trial and in the placebo trial as dotted line.

Values are mean ± SEM. The *p* value in the panel represents a statistically difference between time course of fat oxidation of two trials by a two-factor repeated measures analysis of variance (ANOVA). The *q*-value, according to the FDR correction, is represented in Table S1.

orexin system, but suppressed in the presence of the orexin receptor antagonist suvorexant. Likely due to the short duration of WASO, no effect of suvorexant on energy expenditure was detected over the entire sleeping period.

The present results are apparently inconsistent with previous human study.[11] We previously reported that 20 mg of suvorexant transiently decreased energy expenditure during sleep in healthy male subjects.[11] This finding may be related to the specific experimental design, in which subjects were forced awake at 90 min after sleep onset to perform physical/cognitive tests. When the subjects were allowed to go back to sleep after the forced awakening, the sleep latency was shorter under the influence of suvorexant (~2 min) compared

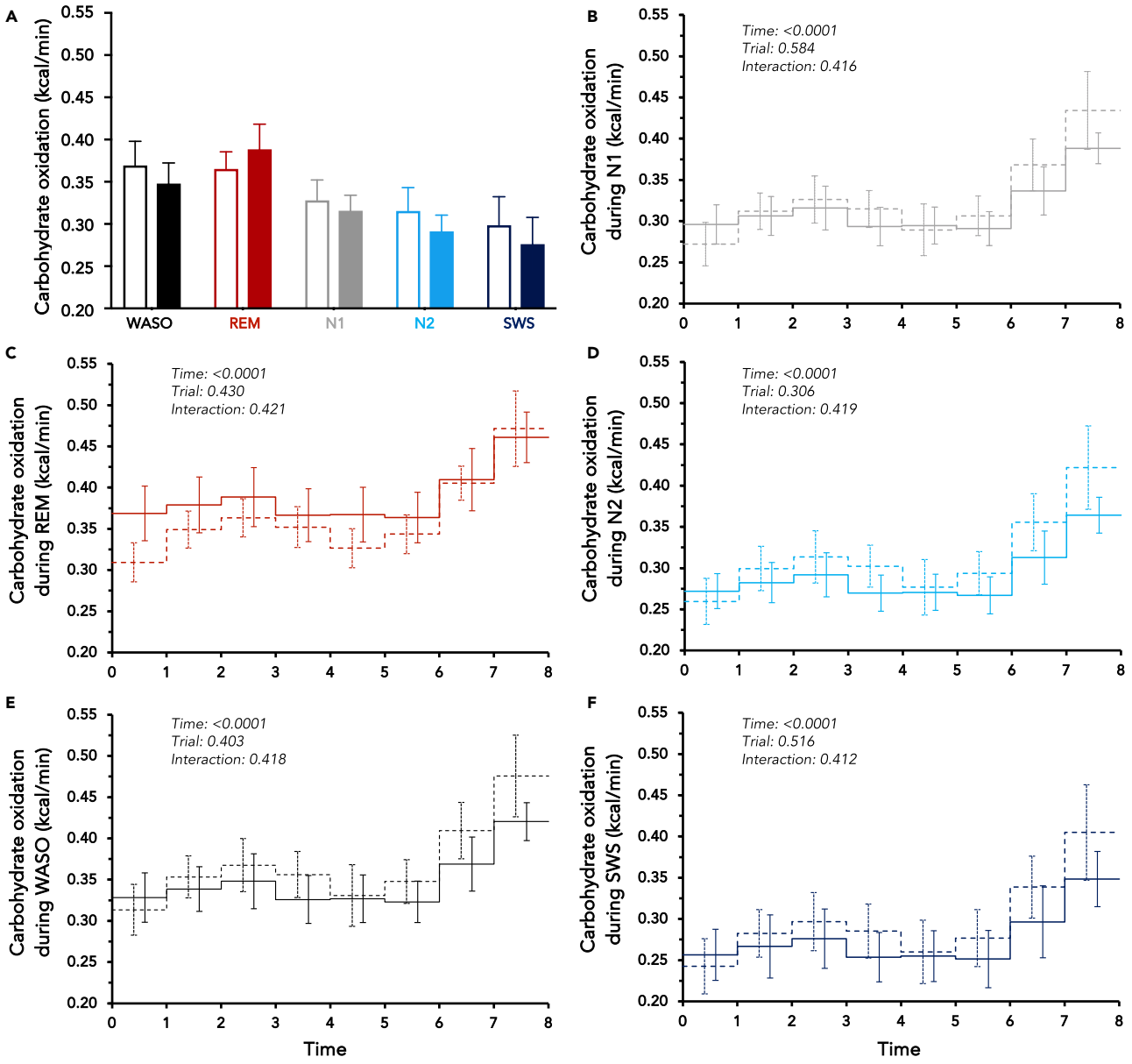

Figure 6. Semi-parametric analysis of carbohydrate oxidation

(A) Carbohydrate oxidation during sleep was decomposed into the effect of time and the effect of sleep stage by a semi-parametric regression analysis. Average carbohydrate oxidation during the entire sleeping period in each sleep stage is shown (A).

(B–F) The effect of time after sleep onset on carbohydrate oxidation in each sleep stage is shown as an hourly average in each panel as solid line in the suvorexant trial and in the placebo trial as dotted line.

Values are mean ± SEM. The *p* value in the panel represents a statistical difference between time course of carbohydrate oxidation of two trials by a two-factor repeated measures analysis of variance (ANOVA). The *q*-value, according to the FDR correction, is represented in Table S1.

with that in the placebo trial (24 min), presumably reflecting an activated orexin system by forced awakening. The effect of a loss of function of the orexin system on energy metabolism has been studied in patients with narcolepsy. Although the measurement period was only 30 min in the morning,[14–16] the basal metabolic rate of patients with narcolepsy was found to be similar to that of control subjects. Furthermore, in two cross-sectional studies, the male/female ratio,[14] age, and BMI[15] were not matched, potentially affecting the interpretation of the findings.

Suvorexant administration increased fat oxidation during sleep without affecting energy expenditure. Adopting a semi-parametric analysis, the effects of sleep stage and time after sleep onset were simultaneously investigated to address whether an effect of suvorexant on fat

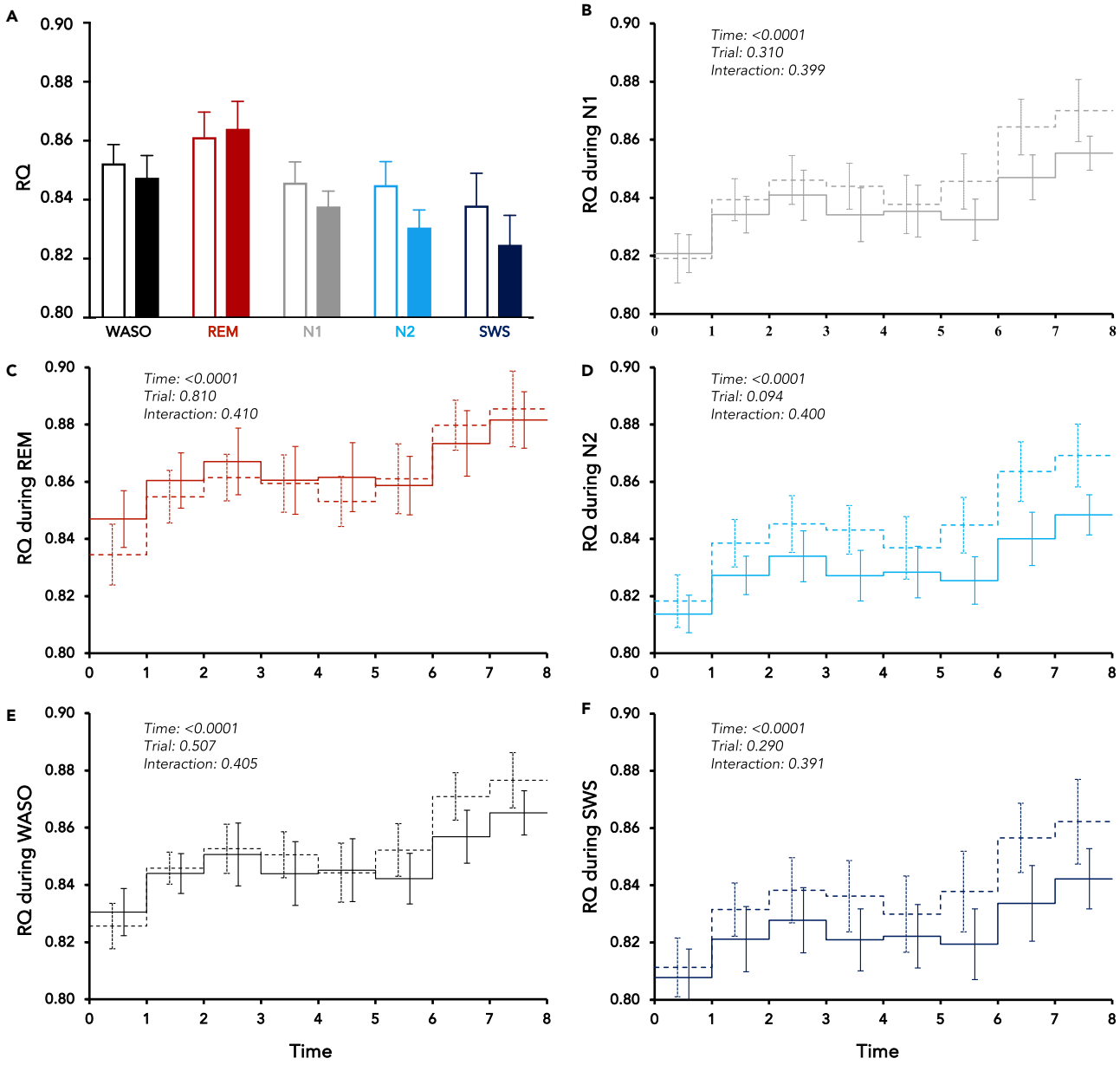

Figure 7. Semi-parametric analysis of RQ

(A) RQ during sleep was decomposed into the effect of time and the effect of sleep stage by a semi-parametric regression analysis. Average RQ during the entire sleeping period in each sleep stage is shown (A).

(B–F) The effect of time after sleep onset on RQ in each sleep stage is shown as an hourly average in each panel as solid line in the suvorexant trial and in the placebo trial as dotted line.

Values are mean ± SEM. The *p* value in the panel represents a statistically difference between time course of RQ of two trials by a two-factor repeated measures analysis of variance (ANOVA). The *q*-value, according to the FDR correction, is represented in Table S1.

oxidation is observed during any specific sleep stage and/or time after sleep onset. A two-way ANOVA on fat oxidation during each sleep stage revealed no statistically significant effect of suvorexant (main effect of trial, $p = 0.089$ in Figure 5A). When the time course of fat oxidation during N2 was isolated and compared, a significant main effect of suvorexant was detected (Figure 5D). No significant effect of suvorexant on fat oxidation was detected during other sleep stages. Sleep stage N2 is the main component of sleep including during the period of presumed peak plasma concentration of suvorexant at approximately 90 min after ingestion[17] (see Figure 2). This may be why the difference in fat oxidation was statistically significant in N2. It remains to be determined whether the effect of suvorexant on fat oxidation is specific

Table 3. Concentration of urine metabolites

		Placebo	Suvorexant	
Pathway	Compounds	Mean ± SEM	Mean ± SEM	*q*
Glycolysis	(nmol/mg creatine)			
	Glucose 6-phosphate	101.187 ± 13.238	88.807 ± 10.981	0.922
	Fructose 6-phosphate	127.694 ± 17.459	119.028 ± 12.047	0.970
	Glycerol 3-phosphate	173.408 ± 42.892	139.261 ± 25.043	0.922
	Glucose 1-phosphate	55.350 ± 6.960	50.528 ± 5.656	0.922
	Glyceraldehyde 3-phosphate	38.217 ± 9.295	42.090 ± 12.520	0.970
	Pyruvic acid	2.000 ± 0.684	1.755 ± 0.580	0.922
	Lactate	19.166 ± 2.384	34.342 ± 14.174	0.922
TCA cycle	(nmol/mg creatine)			
	Citrate	203.411 ± 52.729	153.019 ± 40.160	0.922
	Malate	0.846 ± 0.209	0.925 ± 0.211	0.922
	Succinate	40.327 ± 5.872	48.871 ± 5.538	0.746
	α-Ketoglutarate	12.878 ± 1.898	19.258 ± 4.208	0.922
Amino acids	(μmol/mg creatine)			
	Lysine	1.356 ± 0.356	1.020 ± 0.184	0.922
	Alanine	1.043 ± 0.240	0.788 ± 0.121	0.922
	Tryptophan	0.030 ± 0.008	0.022 ± 0.004	0.922
	Arginine	0.234 ± 0.028	0.241 ± 0.027	0.922
	Histidine	19.361 ± 3.171	18.463 ± 2.545	0.922
	Asparagine	2.674 ± 0.343	2.558 ± 0.373	0.922
	Glutamine	6.449 ± 0.956	6.548 ± 1.196	0.922
	Glycine	2.568 ± 0.478	1.970 ± 0.171	0.922
	Valine	0.671 ± 0.170	0.518 ± 0.073	0.922
	Leucine	1.359 ± 0.330	1.018 ± 0.128	0.922
	Isoleucine	0.507 ± 0.115	0.362 ± 0.045	0.922
	3-Methyl-histidine	6.633 ± 1.585	4.921 ± 0.933	0.746
Acylcarnitines	(nmol/mg creatine)			
	C2-AC	192.407 ± 26.870	185.095 ± 24.300	0.922
	C3-AC	4.947 ± 0.598	5.109 ± 0.723	0.925
	C4-AC	11.051 ± 2.428	13.709 ± 4.539	0.922
	C5-AC	8.104 ± 1.466	8.759 ± 1.836	0.922
	C6-AC	0.148 ± 0.026	0.183 ± 0.043	0.922
	C8-AC	0.311 ± 0.056	0.425 ± 0.120	0.922
	C10-AC	0.141 ± 0.024	0.195 ± 0.058	0.922
	C12-AC	0.004 ± 0.001	0.005 ± 0.002	0.922
	C14-AC	0.003 ± 0.001	0.005 ± 0.002	—
	C16-AC	0.039 ± 0.014	0.060 ± 0.029	0.922
	C18-AC	0.072 ± 0.028	0.107 ± 0.058	0.922
Vitamins	(nmol/mg creatine)			
	Nicotinamide	0.436 ± 0.148	0.301 ± 0.042	0.922
	Pantothenic acid	4.968 ± 1.890	4.327 ± 1.500	0.970
	Riboflavin	0.058 ± 0.021	0.040 ± 0.011	—

Metabolite concentrations were normalized by the excretion of creatine. *q*-values adjusted for false discovery rate (FDR) are shown. AC, acylcarnitine.

to N2. The effect of suvorexant on fat oxidation continued for up to 1 h after waking the next morning, suggesting that the effect of suvorexant to increase fat oxidation during sleep is not a secondary result of a change in the sleep architecture. It is of note that there is no previous study assessing fat oxidation during sleep under the influence of other hypnotic agents such as GABA agonist.

An unexpected finding of the present study was the effect of suvorexant to reduce protein catabolism. To our knowledge, this is the first study to demonstrate the effect of suvorexant on protein metabolism. It remains to be determined whether the decreased protein catabolism is resulting from increased fat oxidation. To gain insight into the mechanism underlying inhibitory effect of suvorexant on protein catabolism, urinary metabolome analysis was performed in the present study. However, the differences in all urinary metabolites between the two trials did not reach statistical significance. Urinary excretion of acylcarnitines negatively correlates with fat oxidation,[18] but it was not affected by suvorexant in the present study. The effect of suvorexant on urinary metabolites required further studies with larger number of subjects. The present study revealed that the orexin system plays a role in the control of energy metabolism; energy expenditure, and substrate oxidation, and the underlying mechanisms remain to be studied.

Our findings in the present study were based on the effect of the dual orexin receptor antagonist on energy metabolism. The distinct effects of selective orexin receptor antagonists on energy metabolism remain to be studied. Animal studies of selective orexin receptor antagonists showed that the orexin-2 receptor pathway is involved in energy expenditure regulation,[7] and the orexin-1 receptor is implicated in motivational responses and reward-based feeding.[19] To confirm the present results, experiments with orexin receptor agonists such as danavorexton[20] remain to be performed. We applied semi-parametric analysis to consider the effect of differential sleep stages and time after sleep onset. A study to clarify the potential effect of suvorexant on energy metabolism, aside from its effect on sleep, requires indirect calorimetry during the daytime after the administration of an orexin receptor antagonist or agonist.

The proportion of patients prescribed suvorexant has been steadily increasing since its approval in 2014.[21,22] The present study evaluated the acute effects of a single dose of suvorexant on sleeping energy metabolism. The actual prescription practice of hypnotic agents for patients with insomnia warrants a study assessing the chronic effects of suvorexant on sleeping energy metabolism. One recent study reported a decreased abdominal circumference after taking 20 mg of suvorexant for 14 weeks.[23] These findings suggest the potential clinical implementation of medications containing dual orexin receptor antagonists on body weight management.

Limitations of the study

One potential limitation to consider in the interpretation of the findings is the need for generalizing the effects of orexin system on energy metabolism. To gain further insights into these effects, future studies should investigate aged males and young and aged females to assess the generalizability of our findings to the entire population.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110212.

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AUTHOR CONTRIBUTIONS

I.P., M.Y., T.Ka., T.Ko., I.M., and K.T. were responsible for the overall experimental design; I.P., R.Y., K.K., and A.I. conducted the polysomnographic recordings and indirect calorimetry of energy metabolism; I.P. performed the statistical analysis; K.Y. performed the urinary metabolome analysis; F.K. conducted the electroencephalogram sleep stage analysis; I.P., M.Y., and K.T. interpreted the results and wrote the paper, which was reviewed by all the authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
GraphPad Prism	GraphPad	https://www.graphpad.com/
R	R Core Team (2023)	http://cran.us.r-project.org
RStudio	Rstudio Team (2023)	http://cran.us.r-project.org

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled the lead contact, Kumpei Tokuyama (tokuyama.kumpei.gf@u.tsukuba.ac.jp).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Data: All data reported in this paper will be shared by the lead contact upon request.
Code: This paper does not report any original code.
Additional information: Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Participants

In this study, 14 healthy young Asian men were selected to participate based on strict inclusion criteria: age between 20 and 40 years, BMI within 18.5 to 25 (kg/m^2), regular sleep/wake patterns, and regular exercise no more than twice a week. Exclusion criteria were self-reported sleep quality (Pittsburgh Sleep Quality Index score >5.5), extreme chronotypes (Morningness-Eveningness Questionnaire score <30 or >70), excessive daytime sleepiness (The Epworth Sleepiness Scale >10), recent shift work or transmeridian travel, smoking, excessive alcohol intake, ongoing medication for certain diseases, and the use of medications affecting normal sleep or energy metabolism. The study's sample size of participants was determined by sample size calculations based on our previous study for the effect of suvorexant on energy expenditure.[11] Fourteen participants allow us to observe significant differences in a paired t-test at 90% power and a 5% alpha level as determined by G-Power 3.1.9 software. This study was conducted in accordance with the Declaration of Helsinki guidelines. The study protocol was approved by the Ethics Committee of University of Tsukuba Hospital (approval number: CRB3180028) and registered with the Japan Registry of Clinical Trials (jRCT ID numbers: jRCTs031210023, 07/04/2021). All participants provided written informed consent before study commencement.

METHOD DETAILS

Procedures

This study was designed as a randomized, double-blind, placebo-controlled, crossover intervention study to investigate the effect of suvorexant on energy metabolism during sleep in healthy young males. The study was conducted in 2 trials separated by a 1 week washout period. Prior to the experiment, all participants spent an adaptation night in a whole-room metabolic chamber, where sensors and electrodes were attached for polysomnographic recordings. Participants maintained an 8-h sleep/16-h wake schedule for 5 days prior to the experiment, following their habitual bed and wake times. During the study period, participants were instructed to abstain from consuming caffeine or alcohol, and from engaging in high-intensity physical activity. Compliance with instructions was monitored with sleep diaries, daily diaries, and wrist actigraphy (GT3X-BT, AMI, VA, NY). On the day of the experiment for placebo and suvorexant trial, participants arrived at the laboratory and consumed the specified experimental meals at designated times (breakfast 1 h after waking, lunch 4 h after waking, and dinner 5 h before bedtime). The identical meals were served between trials and monitored for completion. Following dinner, the polysomnographic recording system electrodes were applied before participants entered the whole-room metabolic chamber. The participants swallowed a capsule containing either suvorexant (20 mg) or placebo 10 minutes before bedtime and then slept for 8 g while their metabolic rates were measured in the whole-room metabolic chamber (Figure 7). The specified meals were based on the estimated energy requirements of each participant, which were calculated using their basal metabolic rate with a physical activity level of 1.5 on the day prior to the

experiment. The macronutrient composition of the experimental meals was 15% protein, 30% fat, and 55% carbohydrates. The contributions of breakfast, lunch, and dinner to total 24-h energy intake were 30%, 30%, and 40%, respectively.

Measures

Indirect calorimetry

An airtight metabolic chamber (2.00 × 3.45 × 2.10 m; FHC-15S, Fuji Medical Science Co., Ltd., Chiba, Japan) was used and air in the chamber was pumped out at a rate of 80 L/min. The temperature and relative humidity of the incoming fresh air were controlled at 25°C and 50%, respectively. The chamber was furnished with an adjustable mattress, desk, chair, and toilet. The concentrations of oxygen (O_2) and carbon dioxide (CO_2) in the outgoing air were measured with high precision by online process mass spectrometry (Prima PRO; Thermo Scientific Co., Winsford, UK). The precision of the mass spectrometry, defined as the standard deviation for continuous measurement of the calibrated gas mixture (O_2, 15%; CO_2, 5%), was 0.0016% for O_2 and 0.0011% for CO_2. Every minute, O_2 consumption (VO_2) and CO_2 production (VCO_2) rates were calculated using an algorithm for an improved transient response.[24] Energy expenditure and macronutrient oxidation were calculated from VO_2, VCO_2, and urinary nitrogen excretion.[13,25] Urinary nitrogen excretion (N) was measured using the Kjeldahl method. The rate of urinary nitrogen excretion, an index of protein catabolism, was assumed to be constant during the calorimetry. The RQ was defined as a ratio of VCO_2 to VO_2. Equations for glucose, fat and protein oxidation rates were as follows:

$$\text{Glucose oxidation (g/min)} = 4.55\ VCO_2\ \text{(L/min)} - 3.21\ VO_2\ \text{(L/min)} - 2.87\ N\ \text{(g/min)}$$

$$\text{Fat oxidation (g/min)} = 1.67\ VO_2\ \text{(L/min)} - 1.67\ VCO_2\ \text{(L/min)} - 1.92\ N\ \text{(g/min)}$$

$$\text{Protein oxidation (g/min)} = 6.25\ N\ \text{(g/min)}$$

Polysomnography

The EEG recording system (PSG-1100, Nihon Kohden, Japan) comprised 6 EEG locations (C3-M2, C4-M1, O1-M2, O2-M1, F3-M2, and F4-M1), submental electromyography, and a bilateral electro-oculograms. Sleep parameters were categorized at 30-s intervals as wakefulness and NREM sleep stage 1 (N1), N2, SWS, and REM sleep according to the standard criteria of the American Academy of Sleep Medicine.[26] In addition, total sleep time, sleep onset latency, REM sleep latency, and sleep efficiency were evaluated.

Urinary metabolites

The protocol for assaying urinary metabolites was previously described.[27] Briefly, urinary metabolites were assayed using a liquid chromatograph coupled with a Shimadzu LCMS-8050 tandem mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization source. The liquid chromatograph system was equipped with a CTO-40C column oven (maintained at 40°C, Shimazu, Kyoto, Japan), LC-40D XS pumps (Shimazu, Kyoto, Japan), and an SCL-40 system controller (Shimazu, Kyoto, Japan). Conditions of the liquid chromatographic analysis are described in the Table S2. All urinary metabolites were assayed using the internal standard and the results were normalized to urinary creatinine excretion.[28] Metabolites were quantified using Shimadzu LC Solution Software (Version 1.22 SP1 software, Shimadzu, Kyoto, Japan).

QUANTIFICATION AND STATISTICAL ANALYSIS

Energy metabolism during sleep was analyzed using a semi-parametric regression model, i.e., a parametric analysis for the effect of sleep stage (N1, N2, SWS, REM, and WASO) and a nonparametric analysis for the effect of time after sleep onset were simultaneously applied using the SemiPar package of the statistical software package R (ver 4.2.3).[29]

The results are expressed as mean ± standard error of the mean (SEM). Paired t tests were used to compare the mean values of energy expenditure, substrate oxidation, and sleep parameters between trials. Because of the large individual variation in urinary metabolites, we applied FDR correction to the *q*-value to assess the significance of the features. The effects of suvorexant on the time course of energy metabolism and differences between sleep stages were assessed by 2-way repeated-measures ANOVA with Bonferroni's correction applied for multiple comparisons. We also confirmed FDR correction for the results of semi-parametric analysis of energy metabolism (Table S1). Data analysis was conducted using Prism 10 (GraphPad Software, San Diego, CA), or R (https://www.r-project.org/), and differences were considered significant when the error probability was less than 0.05.

ADDITIONAL RESOURCES

This study was registered at the Japan Registry of Clinical Trials (jRCT ID numbers: jRCTs031210023, 07/04/2021).