Book Chapter

Acute Impact of Light at Night and Exogenous Melatonin on Subjective Appetite and Plasma Leptin

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Abstract

This study investigates the possible effect of exogenous melatonin on appetite control by investigating plasma leptin and subjective appetite parameters. Nine healthy male participants [26 ± 1.3 years, body mass index (BMI) 24.8 ± 0.8 kg/m2] (mean ± SD) were recruited. The study was designed as a randomized three-way cross-over design; light (>500 lux) (LS), dark (<5 lux) + exogenous melatonin (DSC), and light (>500 lux) + exogenous melatonin (LSC), with an interval of at least 7 days between each session. Each session started at 18:00 h and ended at 06:00 h the following day. Participants were awake and in a semi-recumbent position during each clinical session. The meal times were individualized according to melatonin onset from 48 h sequential urine collection, whereas melatonin intake was given 90 min before the evening meal. Subjective appetite parameters were collected at 30 min intervals during each session. Plasma leptin was collected at specific time points to analyze pre-prandial and postprandial leptin. Subjective hunger and desire to eat were reported higher in LS than DSC and LSC (P = 0.03, and P = 0.001). Plasma leptin showed a significant increase in LSC and DSC (p = 0.007). This study suggested a positive impact of exogenous melatonin on subjective appetite and plasma leptin.

Keywords

Metabolism; Melatonin; Light At Night; Leptin; Appetite

Abbreviations

ALAN-Artificial Light at Night; α MT6s-6-Sulfatoxymelatonin; CIU-Clinical Investigation Unit; PSQI-Pittsburgh Sleep Quality Index; HÖ-Horne-Östberg; RIA-Radioimmunoassay; TAUC-Total Area Under the Curve; DLMO-Dim Light Melatonin Onset

Introduction

Artificial light at night (ALAN) has grown exponentially over modern societies' natural nocturnal lighting levels. Although ALAN has provided substantial benefits to humankind, the adverse biological impacts of ALAN have been widely investigated [1–3]. Extensive studies have been conducted to understand ALAN's mechanism and health hazards against human physiology. The link between ALAN and disruption of circadian rhythm has been well established [4,5], which can contribute to alterations in metabolism [6,7], and increased risk of chronic disorders such as obesity and type 2 diabetes [8]. Furthermore, light can directly affect endocrine signaling from circadian dysregulation or impaired melatonin production [9].

Melatonin has been associated to various biological processes due to its widely distributed melatonin receptors. Various studies have linked melatonin to lipid and glucose metabolism [10,11], vascular function [12], appetite [13], and behaviors [14]. Therefore, melatonin suppression as a result of ALAN may potentially have an impact on metabolism. Melatonin acts as a hormonal mediator of photoperiodic information, regulating energy homeostasis by balancing energy intake and energy expenditure. Add to this, the diversity of melatonin binding sites in gastrointestinal tracts suggest various possible function of melatonin in appetite regulation.

Leptin is a hormone that is mainly released by adipose tissue to maintain energy balance and appetite regulation. Melatonin's role in the release of leptin has been highlighted in several animal studies. The administration of melatonin in middle-aged goldfish and rats results in a decrease in plasma leptin levels, and this impact was inverted in goldfish by the administration of a

melatonin antagonist [15,16]. Other contradictory results revealed that consistent light exposure in rats could increase food intake consumption [17] or abolish day/light variation of leptin levels [18]. Despite the controversial results in animal studies, the majority of studies suggested that melatonin administration may increase the circulating leptin levels [19]. The role of melatonin in controlling appetite and weight has been discussed by Buonfiglio et al. [20] showing that deletion of melatonin receptor type 1 in the hypothalamus was associated with the malfunction of leptin signaling and leptin resistance [20].

There are few and controversial findings from human studies that discuss the impact of melatonin on appetite hormones such as leptin and ghrelin. Figueiro et al. [21] reported a boost in leptin levels and a drop in ghrelin in sleep-restricted individuals after morning light exposure [21]. Similarly, an increased sense of hunger [22,23], and reduced leptin [24,25] were associated with sleep restriction or sleep debt. A recent study has shown a reduced body weight and body mass index (BMI) among overweight night shift workers after melatonin supplementation [26], whereas eating habits among female night shift workers with excessive weight have not changed after melatonin administration [27]. In addition, a recent meta-analysis included seven clinical trials and 244 cases did not support the melatonin impact on body weight and appetite [28]. We have previously shown that exogenous melatonin/ALAN was associated with reduced glucose tolerance, insulin insensitivity, and changes in lipid profile among healthy young males [10]. We hypothesize that exogenous melatonin can alter subjective appetite and plasma leptin. Therefore, this study was conducted to assess the effects of exogenous melatonin administration on subjective appetite and plasma leptin among healthy young males.

Methods
Ethic Statement and Recruitment

The ethics committee at the University of Surrey approved all parts of the study (UEC/2015/021/FHMS). The methodology and experimental design were discussed during an induction session for participants who met the inclusion and exclusion criteria

(Supplementary material A). The chosen participants signed a written consent form before attending the clinical session, confirming they were aware of the potential hazards and discomforts. All included participants were a young male student from University of Surrey (Guildford, UK). All participant information was tagged and rigorously kept in accordance with the Data Protection Act (1998).

Screening Procedures

Participants were required to complete multiple questionnaire such as general health questionnaire, Horne-Ostberg (HÖ) evaluation, Pittsburgh sleep quality index (PSQI), Munich chronotype questionnaire (MCTQ), and daily sleep diary. HÖ was completed to assess the chronotype of participants [29]. PSQI evaluates the sleep quality assessment for all participants using PSQI was evaluated [30], whereas Munich chronotype was completed to evaluate sleep schedule during working/free days [31]. Before the clinical session, participants wore Actiwatches to track their sleep-wake cycle. Furthermore, participants were not on night duties, or crossed more than 2 time zones in the month prior to the study session.

Caffeinated beverages, alcohol, strenuous activity, and analgesic medicine usage were prohibited for 24 h prior to the laboratory sessions. In addition, cosinor analysis was used to determine acrophase of 6-sulfatoxymelatonin (aMT6s) (Stockgrand Ltd., University of Surrey, Guildford, UK), by analyzing a 48-h sequential urine collection from all participants. This will assist in determining the rising phase of participants' endogenous melatonin, thus allowing meal intakes (supper) to be individually assigned for each participants (Supplementary materials B,C). Participants who met the inclusion and exclusion criteria, plus passing the screening procedures were allowed to join the clinical sessions.

Meal and Circadin Timings

Table 1 shows the composition and macronutrients of meals served during the clinical session. Breakfast was served at 08:00

h, whereas lunch and the evening meal "supper" were tailored to the acrophase time of urinary aMT6s. All meals were served at the Clinical Investigation Unit, University of Surrey. The average fasting period between lunch and the super was 9–10 h. Circadin prolonged-release melatonin (Neurim Pharmaceuticals Ltd.) containing 2 mg melatonin was utilized to maintain increased melatonin levels for 8–10 h [32]. Circadin was administered orally 90 min before the supper to ensure sufficient melatonin levels throughout the session (Supplementary material B).

Table 1: Carbohydrate, protein, fat, fiber, and energy for each of the meals and overall composition of all three meals.

Meal/g	Energy Kcal	Protein (g)	CHO (g)	Fat (g)	Fiber (g)
Breakfast	627	15	98	16	14
Lunch	927	25	115	38	19
Test meal "Supper"	1,066	38	104	54	7
Total	2,620	78	317	105	40
% composition*		15%	59%	19%	7%

*Percentages were calculated proportionally from the total daily consumption.

Study Session

Participants were randomized using a three-way cross-over design protocol: light session (LS) (>500 lux), light + Circadin session (LSC) (>500 lux) and dark + Circadin session (DSC) (<5 lux), with at least 7-day washout period. Each session began at 18:00 h and ended at 06:00 h the next day. Participants were assigned to one of two groups: A or B. Group A attended LS first, followed by DSC and then the LSC session, whereas Group B started with LSC first, followed by DSC and then the LS session (Supplementary material D). Sequencing effects were statistically evaluated in the data. Figure 1 depicts the study protocol procedures. During the investigation, both body movement and posture were rigorously controlled. The participants were instructed to remain semi-recumbent (being at

around 45°). They were also allowed to use the toilet following sample collection. Nonetheless, they had to rest in a semi-recumbent position for around 15 min before the next sample collection to ensure that all participants' bodily motions and energy consumption were consistent during the study sessions. The light intensity and irradiance were recorded and maintained during the three sessions. The Supplementary Table E. 1 shows total photon flux (photons/cm2/cm), light intensity (lux), and irradiance (w/m2).

Figure 1: Study protocol. The schematic figure represents the study protocol for a participant with plasma melatonin onset at 20:30 h. All interventions (see key) were relative to each participants' melatonin onset.

At 08:00 h, each laboratory session began with breakfast. Participants were allowed to leave the CIU after breakfast, but to return at lunch time. After lunch, all participants were asked to stay at the CIU until the clinical session starts. The lunch and late evening test meals were tailored to the estimated melatonin onset from urinary aMT6 acrophase timing. Circadin was given 90 min before the late evening test meal in the DSC and LSC sessions to ensure that melatonin levels remained elevated throughout the session. Plasma leptin was collected hourly, beginning at 18:00 h and continuing until the late evening test meal. The samples were taken every 15 min for the first hour following the meal then every 30 min until the session was over. The meal VAS questionnaire was recorded using a visual

analogue scale (VAS PRO-Diary) at 30 min intervals throughout the session (Figure 1). VAS-Pro-Diary measured three types of subjective appetite: hunger, desire to eat, and fullness. Human leptin radioimmunoassay kit from Millipore Company (Billerica, MA, USA) was used to measure plasma leptin. The inter-assay and coefficient variation for plasma leptin was 4.1% for low-level quality control and 6.7% for high-level quality control. In contrast, Intra-assay for low and high-level quality controls were 5.5 and 4.2%, respectively.

Statistical Analysis

Based on data from a prior investigation [7], a power calculation was performed using PS software (Vanderbilt University, Nashville, TN, US) with a power of 80% and a significance level of 0.05. This power estimate indicated that 12 or more participants were required; however, data sets were only retrieved from $n = 9$. The cosinor analysis to determine the peak of aMT6s was performed using a built-in calculation developed by Dr. D S Minors at University of Manchester (Supplementary material F).

Normality test using the D'Agostino-Pearson omnibus was assessed (GraphPad, San Diego, CA, USA). Mean, standard deviation, and standard error were calculated for all data. All hormonal and metabolic data were subjected to three-factor repeated measures (light, melatonin, and time) ANOVA, followed by Tukey's honest significance test using statistical analysis software (SAS) software SAS Institute Inc., Cary, NC, USA. The total area under the curve (TAUC) and Incremental area under the curve (IAUC) were calculated using the trapezoidal and incremental rule (TAUC and IAUC). TAUC and IAUC were used to examine the hormonal and metabolic data, which was then followed by one-way ANOVA and Tukey's multiple comparison testing. The level of significance was fixed at $p \leq 0.05$.

Results

Twelve participants were recruited for this study, and only nine completed the study. All nine participants were males with an average age of 26 ± 1.3 and BMI of 24.8 ± 0.8 (Table 2). Participants' demographics, such as smoking and caffeine consumption, are shown in Table 2. The average score of PSQI was 3.5 ± 0.4, indicating good sleep quality, whereas HÖ scored 54.5 ± 2.6. In addition, three participants were classified as moderate morning type, whereas the remaining six were neither morning nor evening type. The mid-sleep time during the free days ranged between 03:30 to 06:30 h, and the average of all participants was 04:46 ± 00:22. Sleep parameters analyzed by Actiwatches showed no significant difference in sleep latency, efficiency and fragmentation index prior to each session (Supplementary material G). Participants have a sleep duration of approximately 6 h, with sleep efficiency above 70% and a fragmentation index over 40.

Table 2: Participant demographics.

	All participants ($n = 9$) (mean ± SEM)
Age (year)	26 ± 1.3
Body weight (Kg)	75.3 ± 3.1
Height (m)	1.7 ± 0.02
BMI (kg/m^2)	24.8 ± 0.8
Caffeine/wk	6.2 ± 2.5
Alcohol/wk	0.2 ± 0.2
PSQI[a]	3.5 ± 0.4
HÖ[a]	54.5 ± 2.6
MCTQ[a] (h)	04:46 ± 00:22
RBC[a] (10^3/mm^3)	5.1 ± 0.1
WBC[a] (10^3/mm^3)	5.6 ± 0.4
PLT[a] (10^3/mm^3)	235 ± 11.7
HGB[a] (g/dl)	13.8 ± 0.3

Values are mean ± SEM. RBC, red blood cell; WBC, white blood cell; PLT, platelet; HGB, hemoglobin.
[a]Values given are those obtained during the screening session.

Subjective Appetite Scores

Figure 2 shows the repeated measure ANOVA of subjective hunger, desire to eat and fullness score in all three sessions (Figure 2). Participants felt more hunger and desire to eat in LS than in DSC and LSC (P = 0.03, and P = 0.001), respectively, whereas no significant difference was shown in the fullness score (P = 0.9). The significant differences were only reported at postprandial times (+270, +300, +330, and +360 min). The significant effects of times were reported in all three subjective parameters during all three sessions (P < 0.001).
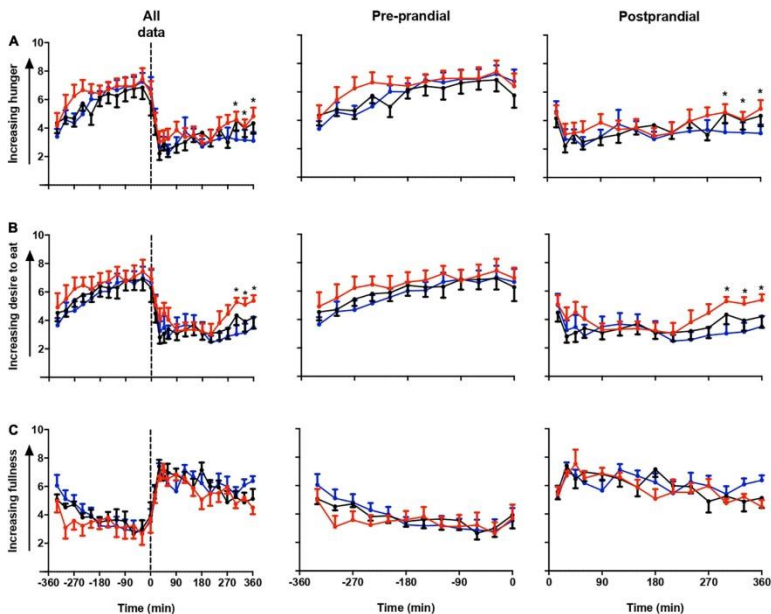
Figure 2: Subjective appetite parameters. Subjective hunger (A), desire to eat (B), and fullness (C) ratings (mean ± SEM) prior to and after a standard evening meal (time = 0 black dotted line) during LS (●), DSC (●), and LSC (●) in all participants (n = 9) (T = −330 to T = +360 min).

Pre-prandial and Postprandial of Plasma Leptin

Figure 3 shows the significant difference in plasma leptin during pre-prandial and postprandial times. There was a significant effect of melatonin (p = 0.007) and time (p < 0.001) between the

three session. Plasma leptin showed significant increase in LSC and DSC compared to LS (p = 0.007). Significant differences were spotted at the postprandial time at +210 and +330 min (Figures 3A–C). Better visualization of leptin response can be seen when the data were plotted as a % leptin of T = 0 or T = −360 (Figures 4A–B). Further details about the plasma leptin for all participants are shown in Supplementary material I.
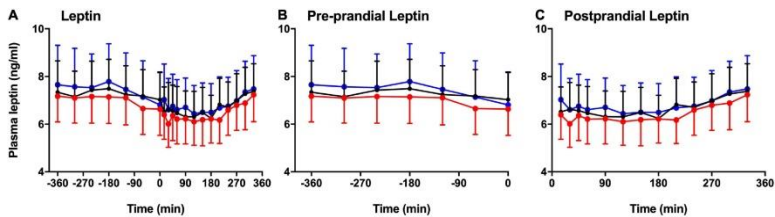
Figure 3: Pre-prandial and postprandial plasma leptin. Pre-prandial and postprandial plasma leptin (mean ± SEM) levels prior to and after a standard evening meal (time = 0 black dotted line) during LS (—●—), DSC (—●—), and LSC (—●—) in all participants (n = 9). (A) Plasma leptin between −360 to +330 min, (B) pre-prandial leptin between −360 to 0 min, and (C) postprandial leptin between +15 to +330 min [F(1,496) = 11.54, p = 0.007].
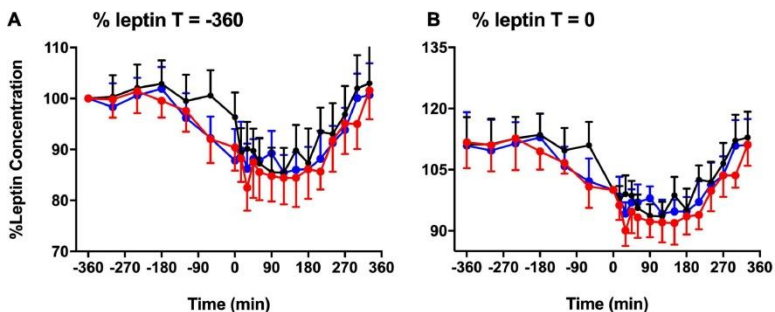
Figure 4: Basal leptin concentration. % basal leptin (mean ± SEM) levels directly prior to the test meal (T = 0) (A) and at time −360 (B) during LS (—●—), DSC (—●—), and LSC (—●—) (n = 9) (T = −360 to T = +330 min) [F(1,496) = 11.54, p = 0.007].

Total Area under the Curve and Incremental Area under the Curve of Plasma Leptin

Total area under the curves and IAUCs of plasma leptin are presented in Figures 5A–B. Plasma leptin was considerably

higher in DSC and LSC than LS (p = 0.01). The Tukey's multiple comparison testing showed the significant differences between LS and DSC (p = 0.04) and between LS and LSC (p = 0.01). TAUCs of plasma leptin in LS were 137.9 ± 21.1, 143.6 ± 22.2 in DSC, and 146.7 ± 27.5 ng/ml.min in LSC. No significant differences were reported in IAUC between all three session (p = 0.13). IAUC of plasma leptin in LS was 15.5 ± 2.5, 25.1 ± 7.1 in DSC, and 28.2 ± 8.1 ng/ml.min in LSC. Further details about the TAUC and IAUC of plasma leptin for all participants are shown in Supplementary materials J–K.
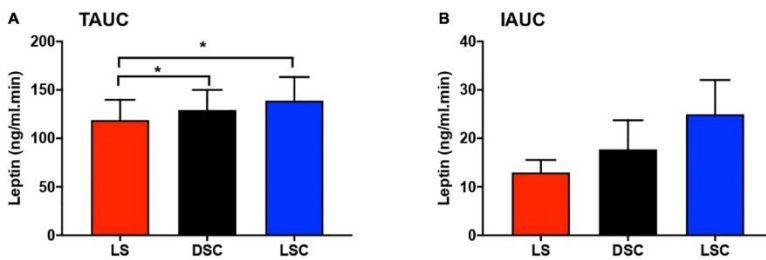
Figure 5: Total area under the curve (TAUC) and incremental area under the curve (IAUC) of plasma leptin. TAUC (A) and IAUC (B) for plasma leptin (mean ± SEM) during LS (■), DSC (■), and LSC (■) in all participants (n = 9) [TAUC: $F_{(1.5,12.1)}$ = 7.06, p = 0.01; IAUC: $F_{(1.9,15.3)}$ = 2.28, p = 0.13]. * Means p < 0.05.

Discussion

This study investigated the effect of exogenous melatonin on plasma leptin as the primary appetite hormone was investigated. This is the first study to assess the acute effect of exogenous melatonin on subjective appetite score and plasma leptin among healthy young males. This is part of a large clinical study that investigated the impact of exogenous melatonin/ALAN on hormones and metabolites [10]. Data from recent studies revealed the presence of contradictory results on melatonin-leptin interactions. In some mammals, melatonin administration has increased leptin levels [33,34], whereas leptin was decreased in middle-aged rats and goldfish [15,16,19]. However, these factors influenced the above controversies due to: (1) Different species; (2) Melatonin had adverse action on the adrenal glands, which triggered changes in glucocorticoids levels [35], or

elevated the hypothalmo-pituitary-adrenal axis as result of pinealectomy [36]; and (3) leptin suppression due to weight reduction [15,37]. Add to this, the possible differences in the physiological meaning of melatonin between nocturnal and diurnal animals could explain the discrepancy in the melatonin-leptin results reported in animal and human studies.

Consequently, our results revealed a notable increase in leptin level with melatonin administration because leptin was significantly higher in DSC and LSC than in LS. Eventually confirmed by TAUC and IAUC, this rise was quite evident when the data were split into "before and after meal periods" or "pre and post-prandial periods." Contrarily, our study conflicts with that of Figueiro et al. [21], who reported that leptin levels increased after morning light exposure [21]. It likewise contradicts Cheung et al. [38], who reported no changes in leptin or ghrelin levels after exposure to blue-enriched light [38]. Investigating the melatonin levels would have shed more light on their findings but both studies did not do that. Besides, their study designs did not cover the melatonin profile window. This opens a question whether changes reported above were due endogenous melatonin exerting metabolic effects.

The expression and release of the leptin gene were stimulated by glucocorticoid administration [39]. We previously investigated cortisol in this study, yet cortisol's possible stimulatory effects were absent, as there were no apparent differences between the three sessions [10]. Moreover, insulin-leptin interaction have been more controversial [40–42]. Yet, Alonso-Vale et al. [43] showed that while insulin acted in synergy with melatonin to enhance the expression of the leptin gene, insulin or melatonin alone did not affect leptin gene expression. They further reported that through the Pertussis toxin (PTX)-sensitive Gi protein-coupled membrane receptor, melatonin could obstruct forskolin's inhibitory effect on both leptin synthesis and secretion in adipocytes [43]. Insulin signaling is involved in leptin expression via melatonin receptor type I (MTI), and melatonin could increase its signaling effect [43]. Although with melatonin administration, insulin levels were much lower [10],

the melatonin-insulin action upon adipocytes cannot be unnoticed.

It is likewise essential to note that endocrine and metabolic status can be aggravated during major depression [44,45]. A recent meta-analysis have indicated metabolic and inflammatory dysregulation were strongly associated with atypical depression [46]. One example is the report that those in a depressed mood tend to take more food [47]. However, in this study, participants reported no difference in depressive mood between the three sessions. Meanwhile, this hypothetical mechanism is at odds with the notable increase in the subjective miserable score during LSC compared to LS (Supplementary material H). Nonetheless, analyzing short-term appetite control associated with other appetite biomarkers like cholecystokinin (CCK) and ghrelin could be more helpful in this condition. According to Spiegel et al. [23], the effect of insomnia or sleep deprivation on leptin levels is well documented [23]. Nevertheless, during all three sessions in this study, participants were sleep deprived. This implies that leptin changes are not really influenced by sleep deprivation.

In this study, with the opposite in fullness score, compared to DSC and LSC, we saw significant increases in subjective hunger and desire to eat scores in LS. Our data revealed a notable effect of melatonin administration on subjective appetite scores. The significant differences shown in plasma leptin are explained by subjective hunger and desire to eat. These results contradicts to that of Cheung et al. [38], who reported that after exposure to blue-enriched light administration, both in the morning and evening, there were no changes in subjective hunger [38]. This opens a question of whether melatonin intake can reduce body weight as reported in early type chronotype night shift workers [26]. Our plasma leptin and subjective parameters support these findings, yet it is noteworthy to indicate that the evening test meal was served in the same room during the three sessions. Therefore, the smell of food by subjects or subjects with earlier dim light melatonin onset (DLMO) who had their meals could potentially influence our food VAS results.

There was an evident melatonin suppression increase in LS, shown in subjective appetite scores for hunger and desire to eat. Melatonin administration in this study, strengthened the possibility of a melatonin-leptin interaction. So, most likely because of the lower plasma leptin, participants reported higher hunger and desire to eat. So, according to Alonso-Vale et al. [43], forskolin's inhibitory effect on leptin synthesis and secretion in adipocytes is prevented by melatonin. Melatonin also increases the signaling power involved in insulin-induced leptin expression via MTI [43]. Defined as the hormonal regulatory loop involving leptin-insulin interaction, never ignore the importance of the adipo-insular axis.

A key strength of the present study was the highly controlled in-laboratory and strict session monitoring of participants prior to attending the clinical session. For instance, melatonin intake and supper timing were individualized to ensure sufficient circulating melatonin when meal is given. A total of 9–10.5 h of fasting period was crucial to allow for sufficient collection for pre-prandial samples, and to ensure returning to basal fasting level at the beginning of the session. Light intensity and irradiance were well-controlled and monitored at the horizontal and vertical levels to ensure the same amount and correct intensity were delivered. Additionally, other non-photic factors capable of affecting melatonin, such as posture and calorie intake, were restricted. In contrast, there are multiple limitations that worth to be noted. This study was conducted only in males, with a low sample size. It is imperative to investigate the effect of melatonin in both genders and in a larger sample size to assess the significance level and minimize the limitations of statistical power. Various eating patterns as a result of adopting eating patterns, such as in athletes or amongst mixed cultural backgrounds, were not considered, which may influence the appetite analysis [48–50]. The meals were served in the same room, thus the smell of food affecting the food VAS results cannot be ignored. Short-term appetite hormones such as ghrelin and CCK would add significant value to this study.

According to the major findings of this study, our data suggested a positive impact of exogenous melatonin on subjective appetite

and plasma leptin. Our recent findings along with previously reported evidences demonstrated could possibly influence leptin release by modulating insulin levels [10]. These findings also indicate the short-term effect of ALAN, which may become aggravated in long-term exposure, such as in shift work. Furthermore, helping to minimize obesity, the ability of melatonin to alter subjective hunger and desire to eat, plus influencing plasma leptin, may regulate food intake. Finally, although melatonin is available "over the counter" in some countries, it is noteworthy mention that the greater efficacy of MT1/MT2 receptor agonist may be effective for improving appetite control.

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Supplementary Material

Supplementary A

All participants were students at the University of Surrey. Strict inclusion and exclusion criteria were applied during recruitment. Participants who were eligible to take part in the study were provided with the study participant information sheet.

The inclusion criteria were as follows:
Aged between 18–60 years
BMI between 18.5–28.0 kg/m2
Maintenance of selected regular sleep--wake cycle
Sleep duration between 6.5 to 8.0 hours
Must refrain from alcohol, caffeine, heavy exercise and carbonated drinks 24 hours before each session
The exclusion criteria were as follows:
Confirmed or possible pregnancy
Familial or personal history of psychiatric disorders
History of infectious conditions
History of drug or alcohol abuse.
History of taking any medication other than minor analgesics within 2 weeks prior to the study
Participation in any experimental drug trial within the last 3 months before the start of the study
Donation of blood within the last 3 months before the start of the study.

Supplementary B

Table B.1 showed the average excretion rate and the amount of aMT6s excreted over 48 hours. Participants showed a substantial amount of aMT6s produced before joining the study as a part of screening procedures. Melatonin onset obtained from urinary aMT6s was compared with salivary melatonin onset which was calculated as described in Figure B.1. There were at least 1-hour differences between urinary melatonin onset and salivary melatonin onset.

The following calculations were used to determine meal timing for each partici-
pant:

| Plasma acrophase = Urinary acrophase - 1(h) | DLMO = Plasma Acrophase - 4(h) |
| Supper time = DLMO + 30(mins) | Lunch Time = Supper time - 10(h) |

Figure B.1: Calculation of meal timings.

Table B.1: aMT6s urinary screening.

subject	Average aMT6s excretion rate ng/h		aMT6s mole		S-melatonin onset	U-melatonin onset
S001	1st day	390	1st day	2128	22:02	23:15
	2nd day	464	2nd day	4211		
S002	1st day	1063	1st day	37933	20:32	22:45
	2nd day	737	2nd day	23922		
S004	1st day	2021	1st day	39250	21:50	22:35
	2nd day	1002	2nd day	26394		
S006	1st day	995	1st day	25736	20:35	22:00
	2nd day	1073	2nd day	32043		
S007	1st day	767	1st day	39568	21:05	22:30
	2nd day	1021	2nd day	40154		
S008	1st day	530	1st day	3490	22:30	23:00
	2nd day	387	2nd day	11860		
S009	1st day	160	1st day	5895	23:00	23:45
	2nd day	207	2nd day	5639		
S011	1st day	258	1st day	11105	23:00	00:45
	2nd day	336	2nd day	19121		
S012	1st day	1323	1st day	24927	23:05	00:00
	2nd day	667	2nd day	14822		

Supplementary C

Table C.1: Plasma melatonin onset and meals timing of the participants in the study 2.

Subject	Acrophase aMT6s	Acrophase plasma	DLMO	Lunch	Supper
S001	04:15	03:15	23:15	14:45	23:45
S002	03:45	02:45	22:45	14:15	23:15
S004	03:35	02:35	22:35	14:00	23:00
S006	03:00	02:00	22:00	13:30	22:30
S007	03:30	02:30	22:30	14:00	23:00
S008	04:00	03:00	23:00	14:30	23:30
S009	04:45	03:45	23:45	15:00	00:00
S011	05:45	04:45	00:45	15:15	01:15
S012	05:00	04:00	00:00	15:00	00:30

Supplementary D

Carryover Effect
Data obtained from plasma melatonin have been used to examine the carryover "sequence" effect. This analysis was performed using Two-way RM ANOVA by testing the source of variations such as treatment, subject, sequence, and sequence x treatment.
Table D.1:

ANOVA Table	SS	DF	MS	P value
Sequence x Treatment	20.64	2	10.32	0.45
Sequence	1.215	1	1.21	0.85
Treatment	19.90	2	9.95	0.43
Subject	247.7	7	35.39	0.04
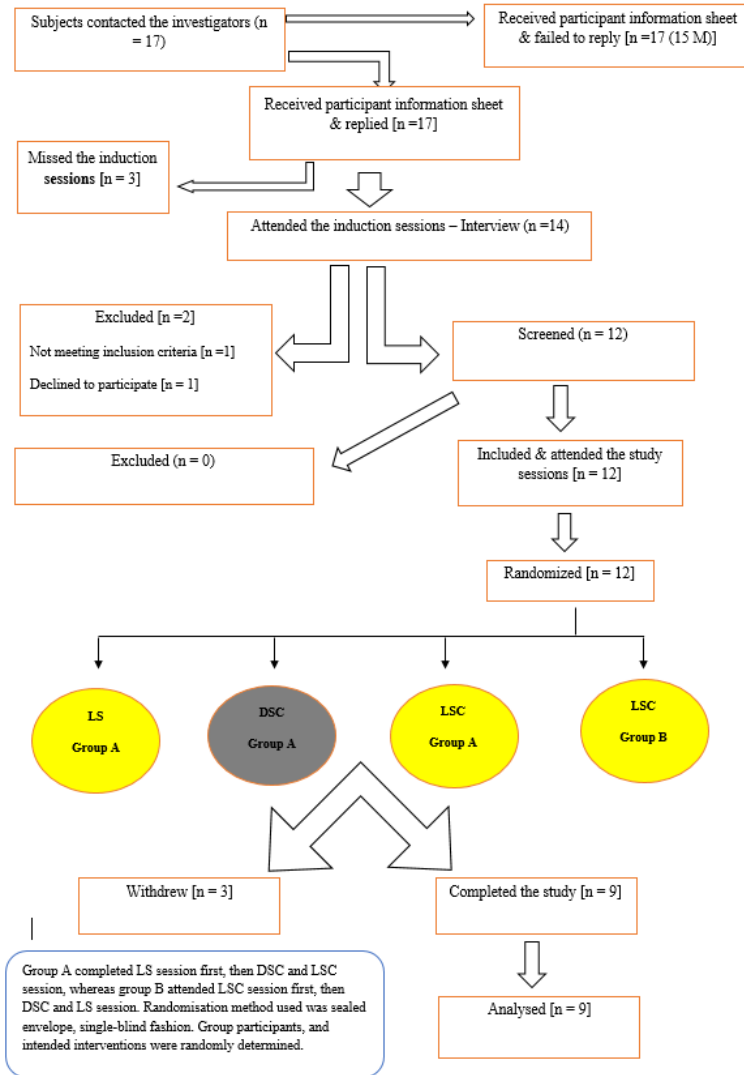
Figure D.1: Study flow chart.

Supplementary E

Table E.1: Light measurements in LS, DSC and LSC.

Condition	Level	LS	DSC	LSC (n = 243)
Light intensity lux	Horizontal (n = 243)	563 ± 12	1.32 ± 0.2	548 ± 10
	Vertical (n = 243)	342 ± 12.2	1.02 ± 0.03	350 ± 10.5
Light irradiance w/m2	Horizontal	0.981	0.0012	0.981
	Vertical	0.73	0.0008	0.73
Photon flux photons/cm2/sec	NA	3.12 x 1019	2.63 x 1019	3.12 x 1019

Supplementary F

Table F.1: Consinor analysis.

	Section #	Start Sample	End Sample	Period (h)	Mesor	Amplitude	Acrophase (Degrees)	Acrophase (Time)	P-Value	% Rhythm
	1	1	6	24.0	8.45	9.78	-66.14	4.41	0.006382	96.6
S001	2	6	11	24.0	8.03	9.73	-62.83	4.19	0.006224	96.6
	3	1	11	24.0	8.13	9.68	-63.85	4.26	0.000002	96.1
	1	1	6	24.0	15.22	27.71	-52.77	3.52	0.092331	79.6
S002	2	6	10	24.0	16.05	24.91	-57.85	3.86	0.166110	83.4
	3	1	10	24.0	16.05	26.69	-56.57	3.77	0.002228	82.5
	1	1	5	24.0	18.02	22.50	-115.93	7.73	0.340036	66.0
S012	2	2	6	24.0	10.43	13.62	-76.41	5.09	0.008723	91.3
	3	1	6	24.0	14.90	21.51	-95.53	6.37	0.312565	53.9
	1	1	6	24.0	20.47	26.66	-48.15	3.21	0.256216	59.7
S004	2	5	11	24.0	17.14	20.10	-55.69	3.71	0.002585	94.9
	3	1	11	24.0	19.26	25.03	-49.71	3.31	0.008838	69.3
	1	1	7	24.0	17.11	21.17	-44.80	2.99	0.002907	94.6
S006	2	7	10	24.0	6.65	6.30	-44.11	2.94	0.186134	96.5
	3	1	10	24.0	9.72	11.24	-50.39	3.36	0.160005	40.8
	1	1	6	24.0	16.56	26.36	-59.41	3.96	0.014134	94.2
S007	2	6	11	24.0	17.45	24.30	-43.98	2.93	0.007599	96.1
	3	1	11	24.0	16.67	24.19	-53.52	3.57	0.000012	94.1
	1	1	6	24.0	10.76	12.83	-50.66	3.38	0.056630	85.3
S008	2	6	11	24.0	8.83	9.89	-76.01	5.07	0.020027	92.6
	3	1	11	24.0	10.34	12.39	-60.31	4.02	0.000542	84.7
	1	1	7	24.0	4.33	4.29	-52.83	3.52	0.002978	94.5
S009	2	7	12	24.0	4.10	5.23	-77.41	5.16	0.024712	91.5
	3	1	12	24.0	4.12	4.75	-72.28	4.82	0.000059	88.5
	1	1	9	24.0	8.41	10.19	-81.58	5.44	0.000029	96.9
S011	2	8	14	24.0	5.80	7.76	-89.22	5.95	0.000546	97.7
	3	1	14	24.0	6.85	9.15	-87.02	5.80	0.000005	89.0

Supplementary G

Table I.1: screening sleep data.

	LS	DSC	LSC	P
Sleep starta, h:min	24:40 ± 00:23	24:48 ± 00:16	24:51 ± 00:24	0.9
Sleep enda, h:min	07:47 ± 00:23	07:58 ± 00:23	07:57 ± 00:26	0.9
Sleep durationa, h:min	05:46 ± 00:14	05:53 ± 00:15	05:50 ± 00:19	0.9
% Sleep efficiencya	77.4 ± 2.6	74.9 ± 2.5	75.9 ± 3.2	0.2
Sleep latencya, h:min	00:17 ± 00:03	00:42 ± 00:10	00:30 ± 00:14	0.1
Fragmentation indexa	41.48 ± 5.4	40.1 ± 5.1	41.2 ± 6.7	0.6

aValues are mean ± SEM, calculated by one-way ANOVA (n = 9). aValues were obtained from 7 days prior to LS, DSC and LSC sessions.

Supplementary H
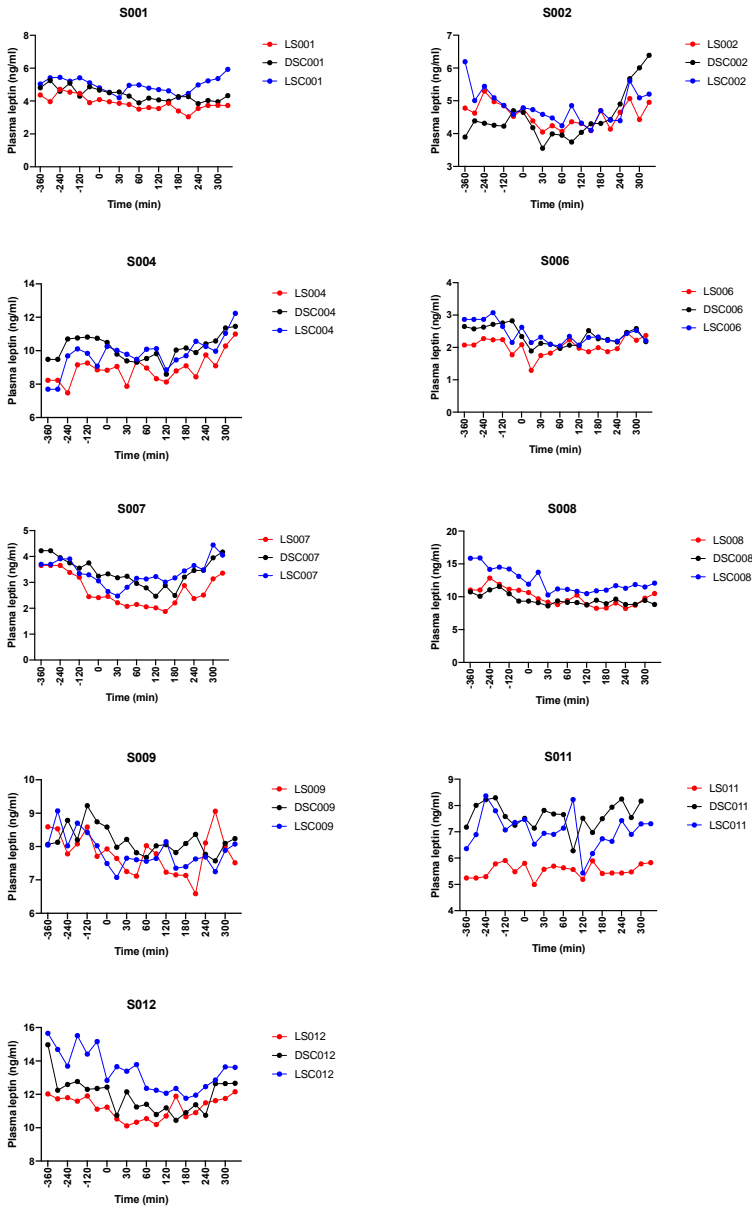
Pre-prandial and postprandial KSS, mood and alertness
Schematic figure H.1 illustrates subjective KSS, alertness and moos scores at pre-prandial and postprandial times during LS, DSC and LSC. No significant differences were reported in KSS, alertness, mood2 and mood 3 (P = 0.2, P = 0.3, P = 0.3 and P 0.3) respectively. Participants were significantly feeling miserable in LSC compared to DSC and LS (P = 0.01). Post-hoc test revealed significant differences at test meal (T = 0) and at postprandial times (+30, +60, +180 and +210). All 5 subjective parameters showed significant effects of time (P < 0.001).
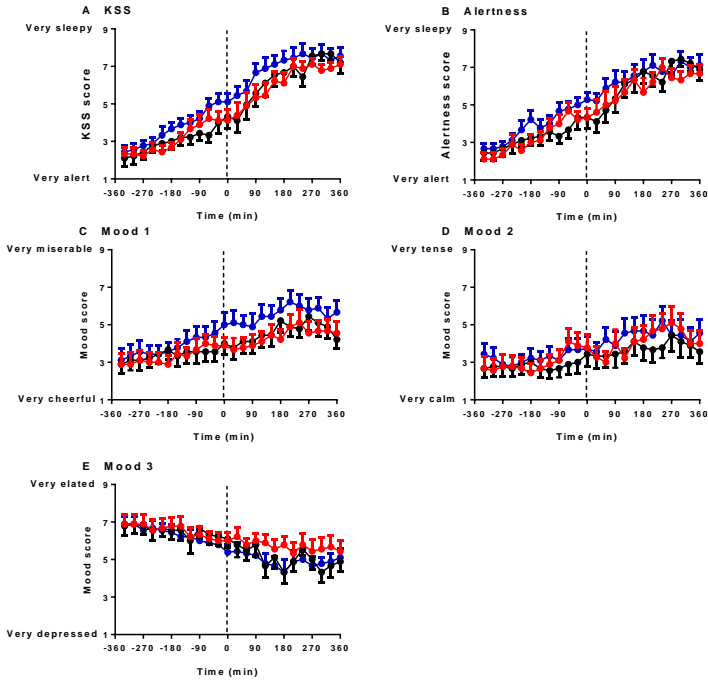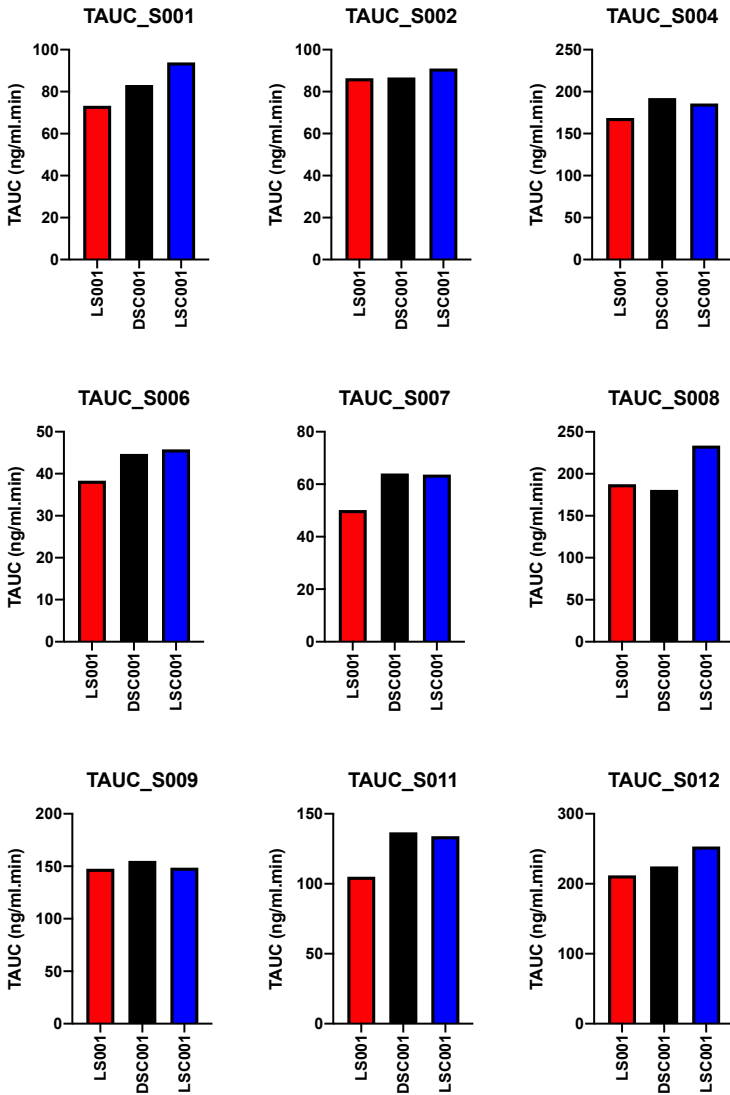
Figure H.1: Subjective mood and alertness.

Subjective KSS (A), alertness (B), mood 1 (C), 2 (D) and 3 (E) (mean ± SEM) ratings prior to and after a standard evening meal (time = 0 black dotted line) during LS (━●━), DS (━●━) and LSC (━●━) in all participants (n=9).

Supplementary I

Supplementary J

Supplementary K

IAUC_S001

IAUC_S002

IAUC_S004

IAUC_S006

IAUC_S007

IAUC_S008

IAUC_S009

IAUC_S011

IAUC_S012