Short-term sleep deprivation with nocturnal light exposure alters time-dependent glucagon-like peptide-1 and insulin secretion in male volunteers

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regulation of L cell activity in rats is highly sensitive to circadian disruption, as exposure to constant light conditions abrogates the normal variations in GLP-1 and insulin nutrient-induced responses and significantly impairs glucose tolerance (18). These findings are consistent with previous studies in rodents showing that light exposure during the dark period has deleterious consequences for both insulin secretion and insulin sensitivity (9, 34). In contrast, similar studies investigating the effects of nocturnal light exposure on GLP-1 and insulin secretion in humans are lacking.

Circadian disruption has been identified as a major risk factor for metabolic disorders in humans (16, 33, 42). Specifically, maintenance of a sleep deprivation regimen for 1–2 wk causes impaired glucose tolerance in healthy humans (6, 32, 41), whereas insulin resistance is detectable after just one night of total or even partial sleep deprivation (12, 19). However, whether these alterations are accompanied by parallel defects in insulin secretion remains controversial. Hence, some studies have reported no significant effects of sleep restriction on insulin secretion (6, 32), whereas others have found either potentiation (38, 44) or attenuation (41) of insulin excursions in sleep-deprived participants. These inconsistencies might be explained by differences in the sleep restriction regimens that the volunteers are forced to follow or in the nature of the stimulatory tests conducted to examine insulin secretion. Moreover, sleep deprivation is not conducted under dark conditions in most studies, but it is usually associated with irregular light-dark cycles. Therefore, it is difficult to determine whether any observed effects on glucoregulatory mechanisms are generated by the sleep restriction per se or by the associated changes in the light-dark cycle.

The goal of the present study was twofold. First, we investigated whether a rhythmic regulation of GLP-1 secretory responses is found in the human L cell, as demonstrated previously for the rodent L cell (18). To this end, the existence of a cell-autonomous clock and a variation in the GLP-1 responses to different stimuli was interrogated in a widely used in vitro model of the human L cell, the NCI-H716 cells (3, 36). The relevance of the in vitro findings was then tested by determination of the basal levels and GLP-1, insulin, and glucose excursions in response to identical meals (850 kcal) served 12 h apart (2300 and 1100) in healthy male volunteers maintained under standard sleep-wake and light-dark cycles in a controlled environment. Second, basal and postprandial GLP-1, insulin, and glucose levels were examined in the same volunteers under the influence of two different circadian disruptors, sleep deprivation alone and sleep deprivation with nocturnal light exposure. Therefore, the three different groups

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METHODS

In vitro studies. NCI-H716 cells (from a male subject; American Type Culture Collection, Manassas, VA) are a well-validated model of the enteroendocrine L cell (3, 36). The cells were propagated in RPMI-1640 with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. For gene expression, cells were synchronized 2 days after plating on Matrigel by overnight starvation in RPMI with 0.5% FBS, followed by a 1-h shock with 10 μM forskolin in RPMI with 10% FBS. After the shock, the cells were washed and incubated in RPMI with 10% FBS; this was considered time 0. Total RNA was then extracted every 6 h for 42 h using the RNeasy Plus Mini kit with QiaShredder (Qiagen, Germantown, MD). Clock gene, glucose-dependent insulinotropic peptide (GIP) receptor, and melanocyte receptor mRNA levels were determined by qRT-PCR, using Hist1H3a as the internal control, as described (18). The following primers (Invtigene, Burlington, ON, Canada) were used: Artml, Hs00154147_m1; Per2, Hs00256143_m1; Hist1H3a, Hs00543854_s1; GIPR, Hs00609210_m1; Mttn1a, Hs00195567_m1; and Mttn1b, Hs00173794_m1. For secretion studies, cells were plated, synchronized as above, and at 0, 24, 36, and 48 h after synchronization were incubated for 2 h in RPMI with 0.5% FBS alone or containing 10−3 M bethanechol (Sigma-Aldrich) or 10−7 M GIP (Bachem Americas, Torrance, CA). Total media and cell GLP-1 content were determined by RIA (Millipore, Billerica, MA), as described (18).

Subjects and experimental design of the human study. The protocol was approved by the Research Ethics Board of Mount Sinai Hospital, Toronto, ON, Canada. Eight healthy male volunteers (18–26 yr old) were recruited by public advertisement at the University of Toronto; all gave written, informed consent and self-reported that they were nonsmokers, were on no medications, and had no known medical conditions or sleep disorders. Volunteers were admitted to the Toronto Centre for Advanced Reproductive Technology (TCART) on four consecutive Fridays at 1700 to 21 continuous hours. Participants were asked to not drink alcohol within 24 h or eat within 3 h of admission and to follow a regular sleep schedule (1100–0800 ± 1 h) during the preceding 5 days. The subjects’ anthropometric characteristics are shown in Table 1. Four different conditions were tested with subjects being randomized between two test (T) conditions each week: T1 or T2 in weeks 2 and 4 and T3 and T4 in weeks 1 and 3 (Fig. 2).

In T1 (normal light-dark cycle with regular sleep), volunteers were maintained under standard bright light (600 lux) until 2200, when the lights were switched off, and subjects remained under dim-red light (dark, <2 lux) until 0800, when the regular bright lights were turned on until the end of the study. During the periods of wakefulness, all the volunteers remained in the same room, seated around a circular table, while playing board games and/or maintaining lively conversations with each other. At least two researchers were in the room at all times to collect blood samples and ensure compliance with the wakefulness requirements. At midnight, volunteers were moved to another room and allowed to sleep in a bed, wherein they fell asleep within 20 min and slept continuously until 0600, as visually monitored by two investigators who remained in the room. All the beds were located in the same room but were separated individually by a black curtain to grant some privacy to the volunteers. Participants were never wakened during blood sampling.

In T2 (sleep deprivation in the dark), participants were kept under the same light conditions as above but remained under wakefulness conditions throughout, as described above.

In T3 (sleep deprivation with nocturnal light exposure) and T4 (sleep deprivation with nocturnal filtered-light exposure), participants were kept under standard bright light and remained awake throughout, as described above. At 2200, the volunteers donned optical filters, which were not removed until 0800, to mimic the timing of lights-off and -on in T1 and T2. In T3, the optical filters were placebo and allowed the transmission of wavelengths from the entire light spectrum; in T4, the glasses completely filtered wavelengths <480 nm (=0% transmission).

Of the eight volunteers that began the study, two withdrew after the first day and were replaced; thus these replacement subjects did not complete one study each (T3 and T4, respectively). One of the original participants also missed one of the studies (T4), and one of the substitutes was later excluded from the study due to noncompliance with meal requirements. As a result, six subjects were included in the statistical analysis of T1–T3 whereas, to reduce the risk for type 2 errors in the analysis, the data from T4 were not included due to the failure of one remaining subject to complete this study.

Upon admission to TCART, an intravenous catheter was inserted in the forearm by a certified phlebotomist. Basal blood samples (4 ml) were taken every 2–3 h into heparin-coated tubes for melatonin and cortisol measurements. Two identical standard meals (each consisting of a combination of two convenience food dishes; Table 2) containing a total of 850 kcal (53.7% carbohydrate, 26.5% fat, and 19.8% protein) were offered at 2300 and 1100 to be consumed within 10 min [meal tolerance test (MTT)]. Blood samples (2 ml) were collected into 200 μl of 0.1 mM diprotin A (Sigma-Aldrich, Oakville, ON, Canada), 5,000 KIU/ml aprotinin (Sigma-Aldrich), and 0.03 M EDTA immediately before and every 10–30 min for 180 min after the meal. Plasma was used for insulin, GLP-1, and glucose measurements. In addition, orange-flavored glucose drinks (300 kcal) were provided at 1800 and 0600 to fulfill the daily energy requirements of the participants (2,300 kcal/24 h) and to preclude any confounder effects of different fasting periods prior to the meal tolerance tests (4). Blood

Table 1. Anthropometric characteristics of the study volunteers

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Weight, kg</th>
<th>Height, m</th>
<th>BMI, kg/m²</th>
<th>Waist Circumference, cm</th>
<th>Hip Circumference, cm</th>
<th>WHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.1 ± 0.9</td>
<td>79.1 ± 3.3</td>
<td>1.8 ± 0.1</td>
<td>23.9 ± 1.0</td>
<td>79.6 ± 3.8</td>
<td>89.1 ± 4</td>
<td>0.89 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. WHR, waist-to-hip ratio.

Table 2. Nutrient composition of the combined meal served at 2300 and 1100

<table>
<thead>
<tr>
<th>Nutrition Facts</th>
<th>PC Cashew Chicken</th>
<th>PC Butter Chicken</th>
<th>Combined Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total calories</td>
<td>400</td>
<td>450</td>
<td>850</td>
</tr>
<tr>
<td>Total fat/lipid</td>
<td>11</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>2</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Trans fat</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>40</td>
<td>65</td>
<td>105</td>
</tr>
<tr>
<td>Sodium</td>
<td>960</td>
<td>690</td>
<td>1,650</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>54</td>
<td>60</td>
<td>114</td>
</tr>
<tr>
<td>Fiber</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Sugars</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Protein</td>
<td>22</td>
<td>20</td>
<td>42</td>
</tr>
</tbody>
</table>

PC (President’s Choice) is a registered trademark of Loblaw Canada.
samples were also taken before and after consumption of the glucose drinks.

Assays and measurements. Plasma glucose was determined by enzymatic assay (Analox Instruments, Lunenburg, MA). Plasma insulin and total GLP-1, melatonin, and cortisol were measured using kits from MesoScale Discovery (Gaithersburg, MD), Rocky Mountain Diagnostics (Colorado Springs, CO), and DRG International (Springfield, NJ), respectively.

Calculations and statistical analysis. Data are expressed as means ± SE. Areas under the curves (AUC) were calculated by the trapezoid rule. Insulin resistance was estimated by two different surrogate measurements: the homeostatic model assessment (HOMA-IR) index, calculated from fasting plasma insulin (FPI) and fasting plasma glucose (FPG) concentrations [FPI (mU/l) × FPG (mmol/l)/22.5], and Matsuda’s composite index, calculated using both fasting and postprandial concentrations of insulin and glucose [10,000/(FPI × FPG × mean insulin levels during MTT × mean glucose levels during MTT)1/2]. Because the relationship between insulin and glucose concentrations during the fasting state reflects mainly the balance between hepatic glucose output and insulin secretion, it is generally accepted that the HOMA-IR is an indicator of hepatic insulin resistance (28, 29, 39, 46). However, during the postabsorptive state, insulin sensitivity equally reflects both suppression of hepatic glucose production and glucose disposal by all tissues in the body, and therefore, Matsuda’s composite index is generally considered an indicator of whole body insulin sensitivity (28, 39). Accordingly, whole body insulin resistance is estimated by calculating the inverse number of Matsuda’s composite index. To compare the different groups in the volunteers, either one-way or two-way ANOVA with repeated measurements were conducted, as appropriate; the in vitro data were analyzed by one-way ANOVA. Homoscedasticity of the data was checked by the Bartlett’s test, and a Holm-Sidak multiple comparison test was run for post hoc analysis. Significance was set at P < 0.05. GraphPad Prism 6 software was used to perform the statistical analyses.

RESULTS

Rhythmic activity of the human enteroendocrine L cell in vitro. The existence of a cell-autonomous independent clock in the human L cell was first established in the NCI-H716 cell line, which demonstrated rhythmic oscillations in the mRNA levels of two canonical clock genes, Bmal-1 (Fig. 1A) and Per-2 (Fig. 1B), over a 42-h period. These patterns were more robust during the first 24 h and waned over time, which was likely due to loss of synchrony, as reported for other endocrine cells (27). A variation in the GLP-1 secretory response to two well-established L cell secretagogues, bethanechol and GIP (3, 40), was also observed in these cells such that the GLP-1 response to identical bethanechol doses was significantly higher at times 24 and 48 h after synchronization than at times 0 and 36 h (P < 0.01; Fig. 1C). Similarly, GLP-1 secretion induced by the same concentrations of GIP was also significantly higher at times 24 and 48 h than at 36 h after synchronization (P < 0.05-0.01; Fig. 1D). Finally, to determine whether GLP-1 secretion might be modulated by melatonin, a key circulating hormone that follows a circadian rhythm, the presence of mRNA transcripts for the receptors Mel1A and Mel1B was examined in the NCI-H716 cells. Compared with the GIP receptor, which was rapidly detectable, no amplification was detected for either of the melatonin receptors (Fig. 1E). Together, these findings suggest that the existence of a cell-autonomous clock in the human L cell that may govern rhythmic secretory activity independently of melatonin signaling.

Differential effects of acute sleep deprivation with or without nocturnal light exposure on the daily profile of melatonin and cortisol levels. As expected, control subjects maintained under a normal light-dark cycle (T1; Fig. 2) demonstrated a bell-shaped curve in circulating melatonin levels, with levels rising to a plateau between 0200 and 0800 and quickly returning to baseline once the lights were turned on. No difference in the melatonin profile was observed between these individuals and those who were sleep deprived in the dark (T2). In contrast, nocturnal light exposure (T3) significantly reduced (P < 0.01–0.05) the nighttime rise (from 2300 to 0600) in melatonin levels (Fig. 3A).

Control subjects exhibited the expected cortisol pattern, with the lowest levels detected at the beginning of the night and a peak in the early morning, followed by a rapid decline (Fig. 3B). The morning peak was significantly reduced (P < 0.01 at 0600) following sleep deprivation, irrespective of the light conditions, and cortisol levels remained steadily elevated afterward, resulting in significantly augmented circulating levels at 1400 (P < 0.05). Subjects under continuous light exposure also showed significantly (P < 0.05) elevated cortisol levels during the early night (0200) compared with the normal sleep group, as reported previously (35).

Differential effects of acute sleep deprivation with or without nocturnal light exposure on basal insulin, glucose, and GLP-1 levels. Basal levels of all parameters were determined at 2300, 0600, and 1100; these time points were selected to allow ≥5 h after the last caloric ingestion in order for insulin and GLP-1 levels to return to baseline (14, 24, 45). When the participants were maintained under the regular light-dark cycle and allowed to sleep, a diurnal variation in insulin basal levels was found (Fig. 4A), with concentrations peaking at 0600 (P < 0.001). No significant differences in basal glucose levels were observed (Fig. 4B), although significantly elevated HOMA index values were detected at 0600 (P < 0.001; Fig. 4C), consistent with the elevated insulin levels. Sleep deprivation in the dark significantly reduced insulin concentrations (P < 0.01) and HOMA values (P < 0.05) at 0600, and the diurnal variation in these parameters was suppressed. The profiles of basal insulin levels and HOMA index values exhibited by the sleep-deprived group under nocturnal light exposure were comparable with those observed in the sleep-deprived subjects in the dark, with only small increments that did not reach significance. Interestingly, and consistent with the profile in insulin basal levels, a peak in insulin concentrations was also found at 0600 (P < 0.001; Fig. 4D). Although this peak was consistently observed under all different conditions, the magnitude was reduced significantly (P < 0.05) in the subjects exposed to continuous light compared with the control group.

Differential effects of acute sleep deprivation with or without nocturnal light exposure on the insulin, glucose, and GLP-1 responses to meal ingestion. The time course profiles of insulin (Fig. 5, A and B), glucose (Fig. 6, A and B), and GLP-1 (Fig. 7, A and B) were examined in response to identical meals at 2300 (under dark conditions in both the control and sleep-deprived subjects in the dark groups but under light conditions in the nocturnal light group) and 1100 (under bright light conditions in all groups). When the volunteers slept normally in between the meals, the expected rhythm in insulin and glucose responses was observed (17, 21, 47). Hence, the first
phase (30 min) of the insulin response was significantly enhanced (by 54%) in the morning ($P < 0.05$; Fig. 5C), in association with significantly better glucose tolerance (by 14%, $P < 0.05$; Fig. 6C), compared with the night. In contrast, the diurnal variation in the first phase of insulin response was not detected in subjects who were sleep deprived in the dark (Fig. 5C), although the variation in glucose tolerance was maintained in these participants (13% difference, $P < 0.05$; Fig. 6C). Furthermore, although sleep deprivation per se did not affect the integrated (120 min) insulin response at any time, it was significantly affected by light (Fig. 5D) such that, at 2300, when the subjects had been exposed to only 1 h of nocturnal light, the AUC of the insulin response was significantly increased by 68% ($P < 0.05$) compared with the same meal served under dark conditions. This potentiation of the integrated insulin response was further magnified when the meal was served at 1100, after an entire night under continuous light exposure, such that the insulin response was enhanced by 92% ($P < 0.01$). However, this enhancement in the insulin response did not result in any improvement in glucose tolerance at 2300, and indeed, glucose levels were not appropriately decreased at 1100 (Fig. 6C), which was likely due to significantly elevated insulin resistance at both time points ($P < 0.01–0.001$; Fig. 6D).

A variation in the first phase of postprandial GLP-1 responses (30 min) was also observed in the control subjects, although surprisingly, the changes were opposite with respect to the pattern observed in the insulin response. Thus, the AUC of the GLP-1 response was significantly higher (by 23%, $P < 0.05$) at 2300 compared with 1100 (Fig. 7C). This variation was maintained in the subjects with sleep deprivation under dark conditions (28% difference, $P < 0.01$) but was lost when the participants were exposed to nocturnal light. Consistent with the potentiation of insulin responses induced by nighttime light, the integrated (120 min) GLP-1 postprandial responses were also augmented in response to nocturnal light, reaching significance at 1100 (by 25%, $P < 0.05$; Fig. 7D).
Finally, the insulin, glucose, and GLP-1 responses to an oral glucose tolerance test (OGTT) administered at 0600 were also studied in control and sleep-deprived subjects under both dark and nocturnal light conditions; however, no significant differences in any of these parameters were found between the different conditions tested (Table 3).

DISCUSSION

Recently, we have demonstrated the existence of a functional cell-autonomous clock in the rodent L cell that is responsible for rhythmic variations in the cell sensitivity to stimuli (18). Hence, we found both a circadian variation in GLP-1 secretory responses to well-established secretagogues in the murine L cell in vitro and a diurnal variation in the GLP-1 responses to oral nutrients in rats in vivo (18). Herein, we show that canonical clock genes are also expressed and rhythmically regulated in a well-established model of the human L cell. Furthermore, both a fluctuation in the GLP-1 responses of these cells to known secretagogues and a diurnal variation in basal as well as GLP-1 responses to identical meals in male volunteers were found, suggesting that a rhythmic regulation in L cell secretion also exists in humans.

Since both GLP-1 and insulin secretory responses are highly sensitive to circadian disruption in rodents, we separately tested the effects of two well-established circadian disruptors, sleep deprivation and nocturnal light exposure, on the basal and postprandial levels of these hormones in the same volunteers. We found that acute sleep deprivation per se is sufficient to alter some of the regulatory mechanisms of glucose homeostasis, including the daily profiles of basal cortisol and insulin levels, as well as the diurnal variation in postprandial insulin responses. However, the most marked effects were detected when acute sleep deprivation was combined with nocturnal light exposure such that both insulin and GLP-1 postprandial levels were significantly lower than in regular sleep conditions (Fig. 3).

![Fig. 2. Schematic representation of the experimental design for the in vivo studies. Short arrows, oral glucose load (OGL); long arrows, meal tolerance test (MTT).](image)

![Fig. 3. Daily profiles of melatonin (A) and cortisol (B) plasma levels in subjects with regular sleep (RS; ○) and in sleep-deprived volunteers maintained in the dark (SD; gray circles) or under full-spectrum light (NL; □) from 2200 to 0800. Light and dark periods are indicated by the open bars and solid bars at the bottom of the graphs (n = 6). *P < 0.05 and **P < 0.01 vs. regular sleep conditions; $P < 0.05 and $$$P < 0.01 vs. sleep deprivation in the dark.](image)
elevated, and the diurnal variation in the responses of both hormones was disrupted in association with a significant increase in insulin resistance and a dampening of the variation in glucose tolerance. Remarkably, the same alterations were not observed in sleep-deprived participants maintained under dark conditions, suggesting a direct effect of light on factors that regulate glucose homeostasis. Consistent with such a possibility, previous studies have found that pro-

Fig. 4. Basal insulin levels (A), basal glucose levels (B), HOMA index (C), and basal GLP-1 levels (D) at 2300, 0600, and 1100 in subjects with RS (black bars) and SD (gray bars) or NL subjects (open bars) from 2200 to 0800 ($n = 6$). *$P < 0.05$ and **$P < 0.01$ vs. regular sleep conditions; #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ vs. the other 2 time points within the same group.

Fig. 5. Time course profile of the postprandial insulin response to identical meals served at 2300 (A) and 1100 (B) to subjects allowed to sleep between the meals (RS; ○) and in SD (gray circles) or NL subjects (○) from 2200 to 0800. Area under the curves (AUC) of the first 30 min (C) and the entire insulin response (120 min; D) of the time course profiles shown above ($n = 6$). *$P < 0.05$ and **$P < 0.01$ vs. RS conditions; $P < 0.05$ and $$P < 0.01$ vs. sleep-deprivation in the dark; #P < 0.05 vs. the other time point within the same group.
longed exposure of rats to abnormal light patterns causes disrupted circadian rhythms in the islets, leading to impaired insulin secretion (34).

Both a diurnal variation in postprandial insulin levels, with greater responses in the morning (17), and a pattern in basal insulin levels, with higher levels at the end of the sleep period, the so-called “dawn phenomenon” (5), have been reported previously. In the present study, these characteristic patterns in insulin levels were found when the volunteers were allowed to sleep at night but were notably altered when the participants were sleep deprived in the dark. The mechanisms by which sleep loss might affect insulin levels are not clear. However, the daily profile of cortisol levels was also altered by a sleepless night in the present study, with the early morning peak in cortisol being attenuated, followed by an elevation in circulating levels the next afternoon, as also reported by others (23). Reductions in the amplitude of the cortisol rhythm have been associated with a decreased diurnal variation in the insulin secretion rate in response to constant glucose infusion (43). Thus, alterations in the normal cortisol rhythm may be responsible for the reduced basal insulin levels at 0600 and the suppression of the normal variation in insulin postprandial responses found in sleep-deprived subjects.

GLP-1 was investigated as a potential mediator of the diurnal variation in insulin responses in humans, as demonstrated previously in rats (18). However, although a correlation in basal GLP-1 and insulin levels was found, the diurnal variations in GLP-1 responses to identical meals reported herein were not consistently paralleled by those found for insulin. This finding contradicts a previous study in humans reporting that the first phase of both the insulin and the GLP-1 response to identical meals is higher at 0800 than at 1700 (24). These apparent discrepancies might be explained by the different fasting periods between meals (9–10 vs. 5 h in the present study), different caloric loads (524 vs. 850 kcal), and the fact that both meals were offered under light conditions in the previous study. Although our findings suggest that GLP-1 may not be the major regulator of the diurnal variation in insulin responses to a mixed meal in humans, it remains to be determined whether human β-cell sensitivity to GLP-1 fluctuates, as we have demonstrated previously in rats, which show a more potent stimulatory effect of GLP-1 immediately prior to the normal feeding period (18). In this context, a role for melatonin in the insulin secretory response of β-cells to GLP-1 stimulation has been suggested. Thus, it has been shown that short-term exposure to melatonin attenuates the stimulatory effects elicited by GLP-1 in vitro, whereas more prolonged exposure times (12 h), resembling the natural overnight exposure to high melatonin levels, have been associated with enhanced sensitivity to GLP-1 stimulation (11, 22). These findings support the hypothesis that β-cells may be more sensitive to GLP-1 effects in the morning than at the beginning of the dark period. In contrast, a direct role of melatonin regulating GLP-1 secretion seems unlikely, since no expression of either of the two melatonin receptors present in humans was detected.

Short-term nocturnal light exposure was found to greatly increase insulin resistance in our volunteers, as demonstrated by significant potentiation of postprandial insulin responses that were still inadequate to reduce glycemia. Previous studies in rodents have demonstrated similarly that continuous light exposure induces defects in insulin sensitivity and glucose tolerance despite enhanced insulin responses to nutrients (9, 18). These effects appear to be a result of light-induced
dysregulation of the suprachiasmatic nuclei (SCN) (9), which coordinate the function of peripheral organs to maintain energy homeostasis. Consistent with this notion, selective ablation of the SCN causes severe insulin resistance in rats (10). Melatonin is a likely mediator of the effects of SCN on insulin resistance, as reduced melatonin levels are associated with increased insulin resistance in experimental animals (8), whereas nocturnal melatonin secretion is inversely correlated with insulin resistance in healthy humans (30). Furthermore, lower melatonin secretion is associated with a higher risk for type 2 diabetes (31). Because we found insulin resistance only in the sleep-deprived volunteers kept under nocturnal light exposure, who were also the only subjects showing suppression of melatonin levels, these findings provide further support for a role of melatonin in regulating peripheral insulin sensitivity. However, conversely, it has also been reported that oral administration of melatonin prior to an OGTT is associated with impaired glucose tolerance (7, 37). Although this observation is clinically relevant and may have potential therapeutic implications, this effect has been demonstrated only in the context of pharmacological melatonin levels. Whether changes in melatonin levels within the physiological range, as reported in the present study, can affect insulin sensitivity thus still remains to be clarified. Interestingly, upregulation of insulin secretory responses is a well-known adaptive response in the settings of acute insulin resistance (1). Thus, the potentiation of postprandial insulin responses found in sleep-deprived participants under nocturnal light exposure may represent a compensatory mechanism for the increased insulin resistance to avoid hyperglycemia, a response that may be mediated in part through an increased incretin effect.

Previous studies showed that GLP-1 secretory responses in rats are highly sensitive to circadian disruption, such as that caused by continuous light exposure (18). Whether the cell-autonomous clock of the L cell is susceptible to alterations in the light-dark cycle, as reported for the molecular clock (34), is not known. However, we found that GLP-1 postprandial responses rose by 24% after a night of light exposure, and consequently, the augmented GLP-1 output may be partially responsible for the observed potentiation of the insulin responses. In contrast, no differences in the insulin, glucose, or GLP-1 responses to an oral glucose load administered at 0600 were observed between the different conditions tested. However, compared with the 850-kcal mixed meal consumed by the volunteers at 2300 and 1100, the low caloric content (300 kcal) and/or the single-nutrient composition of the glucose drink may have been insufficient to induce differential incretin responses.

The major limitation of the present study is the small sample size that complicates the interpretation of nonsignificant findings. Thus, dysregulation of the suprachiasmatic nuclei (SCN) (9), which coordinate the function of peripheral organs to maintain energy homeostasis. Consistent with this notion, selective ablation of the SCN causes severe insulin resistance in rats (10). Melatonin is a likely mediator of the effects of SCN on insulin resistance, as reduced melatonin levels are associated with increased insulin resistance in experimental animals (8), whereas nocturnal melatonin secretion is inversely correlated with insulin resistance in healthy humans (30). Furthermore, lower melatonin secretion is associated with a higher risk for type 2 diabetes (31). Because we found insulin resistance only in the sleep-deprived volunteers kept under nocturnal light exposure, who were also the only subjects showing suppression of melatonin levels, these findings provide further support for a role of melatonin in regulating peripheral insulin sensitivity. However, conversely, it has also been reported that oral administration of melatonin prior to an OGTT is associated with impaired glucose tolerance (7, 37). Although this observation is clinically relevant and may have potential therapeutic implications, this effect has been demonstrated only in the context of pharmacological melatonin levels. Whether changes in melatonin levels within the physiological range, as reported in the present study, can affect insulin sensitivity thus still remains to be clarified. Interestingly, upregulation of insulin secretory responses is a well-known adaptive response in the settings of acute insulin resistance (1). Thus, the potentiation of postprandial insulin responses found in sleep-deprived participants under nocturnal light exposure may represent a compensatory mechanism for the increased insulin resistance to avoid hyperglycemia, a response that may be mediated in part through an increased incretin effect.

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the absence of statistical significance in the diurnal variation of some of the parameters studied needs to be interpreted carefully, since this may represent an undesired consequence of low statistical power. Therefore, larger studies are required to confirm our results. However, the magnitude of the significant effects reported herein, including the upregulation of insulin and GLP-1 postsprandial responses and the increment in insulin resistance provoked by nocturnal light exposure, is large enough to be considered physiologically relevant. Another important limitation is the dependence on surrogate measures (HOMA-IR and Matsuda’s composite index) to estimate insulin resistance in our subjects, which were utilized because the gold standard methodology to calculate insulin sensitivity, the euglycemic hyperinsulinemic clamp, was beyond the scope of the present study. Nevertheless, the use of these measures as effective indicators of insulin resistance has been validated repeatedly in several studies involving both large and small sample sizes by comparing them with the results obtained by the euglycemic insulin clamp technique (28, 46). A third limitation is that we did not determine the rate of gastric emptying during the MTT, which can affect GLP-1 secretion (26). However, because more rapid gastric emptying is generally associated with enhanced GLP-1 responses, and gastric emptying rate is significantly higher in the morning than in the evening (20), it seems unlikely that changes in gastric activity might be driving the diurnal variation in GLP-1 responses reported herein. Whether sleep deprivation or nocturnal light exposure may also affect gastric emptying has not been well defined; therefore, a potential contribution of this parameter to the potentiation of GLP-1 postprandial levels promoted by nocturnal light remains to be determined. Finally, because sex differences in glucose tolerance have been reported (15), only Caucasian young males were recruited for the study to examine a group as homogeneous as possible. However, this age group was of particular interest, as students are among the individuals with most frequent episodes of voluntary sleep restriction (25). Nevertheless, further studies are required to determine whether our findings may be extended to other populations known to undergo circadian disruption, such as shift workers.

In an attempt to prevent the metabolic alterations caused by short-term disruptions of the light-dark cycle, we also conducted an additional study (T4; data not shown) in which short wavelengths (<480 nm) were filtered during nocturnal light exposure. The reduced number of subjects who completed this condition precluded inclusion of this group in the final analysis. However, because this intervention has previously been shown to restore light-induced effects on melanotonin and cortisol levels (35), further studies will be required to determine whether short-wavelength filtration or alternative interventions may ameliorate the deleterious effects of disrupted cycles of the light-dark and sleep-wake cycles, as is most commonly seen with shift work.

In summary, we have determined temporal variations in GLP-1 secretory responses to identical stimuli in both an in vitro model of the enteroendocrine L cell and in human volunteers under regular sleep-wake and dark-light cycles. These findings suggest that the activity of human L cell is subject to circadian regulation, as demonstrated previously for the rodent L cell (18). The diurnal variation in GLP-1 responses to identical meals was disrupted by short-term nocturnal light exposure, which also increased insulin resistance and markedly upregulated insulin and GLP-1 postprandial levels. Because the same alterations were not observed when the participants were sleep deprived under dark conditions, our data demonstrate that irregular light-dark cycles can affect multiple mechanisms involved in the regulation of glucose homeostasis. Moreover, given that voluntary sleep restriction and 24- to 30-h periods of continuous wakefulness are not an uncommon behavior in modern-day society, particularly in shift workers (2), approaches directed to control the exposure to nocturnal light might have beneficial effects for the health of these workers.

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DISCLOSURES

M. Gil-Lozano, P. M. Hunter, B. Gladanac, L. A. Behan, and P. L. Brubaker have no conflicts of interest, financial or otherwise, to declare. R. F. Casper is a founder of and has stock in Circadian/ZircLight.

AUTHOR CONTRIBUTIONS


REFERENCES


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