

Dark Nights Reverse Metabolic Disruption Caused by Dim Light at Night

L.K. Fonken, Z.M. Weil and R.J. Nelson

Objective: The increasing prevalence of obesity and related metabolic disorders coincides with increasing exposure to light at night. Previous studies report that mice exposed to dim light at night (dLAN) develop symptoms of metabolic syndrome. This study investigated whether mice returned to dark nights after dLAN exposure recover metabolic function.

Design and Methods: Male Swiss-Webster mice were assigned to either: (1) standard light-dark (LD) conditions for 8 weeks (LD/LD), (2) dLAN for 8 weeks (dLAN/dLAN), (3) LD for 4 weeks followed by 4 weeks of dLAN (LD/dLAN), and (4) dLAN for 4 weeks followed by 4 weeks of LD (dLAN/LD).

Results: After 4 weeks in their respective lighting conditions both groups initially placed in dLAN increased body mass gain compared to LD mice. Half of the dLAN mice (dLAN/LD) were then transferred to LD and vice versa (LD/dLAN). Following the transfer dLAN/dLAN and LD/dLAN mice gained more weight than LD/LD and dLAN/LD mice. At the conclusion of the study dLAN/LD mice did not differ from LD/LD mice with respect to weight gain and had lower fat pad mass compared to dLAN/dLAN mice. Compared to all other groups dLAN/dLAN mice decreased glucose tolerance as indicated by an intraperitoneal glucose tolerance test at week 7, indicating that dLAN/LD mice recovered glucose metabolism. dLAN/dLAN mice also increased MAC1 mRNA expression in peripheral fat as compared to both LD/LD and dLAN/LD mice, suggesting peripheral inflammation is induced by dLAN, but not sustained after return to LD.

Conclusion: These results suggest that re-exposure to dark nights ameliorates metabolic disruption caused by dLAN exposure.

Obesity (2013) **21**, 1159-1164. doi:10.1002/oby.20108

Introduction

Metabolic disorders are increasing in prevalence worldwide and represent a major global health threat. The metabolic syndrome is categorized by the development of several metabolic abnormalities that increase the risk of coronary artery disease, stroke, and diabetes. Hypercaloric food intake and physical lethargy are known to underlie the development of metabolic syndrome and obesity. However, additional nontraditional factors are likely involved. The increasing prevalence of metabolic disorders coincides with increasing exposure to light at night (1-3). Recent epidemiological and experimental studies implicate the introduction of artificial light in the development of metabolic syndrome (4). Indeed, shift-workers who experience high levels of light at night are at increased risk for cardiovascular disease (5,6) and elevated body mass index (7). Even brief behavioral and circadian misalignment alters metabolic homeostasis in humans, resulting in hyperglycemia, hyperinsulinemia, and postprandial glucose levels comparable to a prediabetic state (8). Moreover, in rodent models exposure to light at night produces changes in metabolism (9-11).

Metabolic processes fluctuate throughout the day. The suprachiasmatic nuclei (SCN) of the hypothalamus comprise the master circadian clock in mammals and control physiological and behavioral circadian rhythms. Photoc input to the SCN is the dominant cue for entraining the circadian clock. Light travels directly from intrinsically photosensitive Retinal Ganglion Cells (ipRGCs) to the SCN via the retino-hypothalamic tract (RHT). Prior to the wide-spread adoption of electrical lighting the circadian system was principally synchronized to the solar cycle. In contrast, modern light exposure occurs in a variety of patterns. Because of the importance of light in synchronizing the circadian system, exposure to aberrant light schedules disrupt circadian activity. Disruption in the clock gene network is linked to changes in sleep, body mass, locomotor activity, and food intake. Homozygous Clock mutant mice have significant increases in energy intake and body weight, and total arrhythmicity when housed in constant darkness (12). These mutants also showed dyslipidemia, hyperglycemia, and hypoinsulinemia- all markers of metabolic dysregulation (12). Manipulation of other genes in the clock gene family similarly cause metabolic abnormalities (13).

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Disclosure: The authors declare no conflicts of interest.

Funding agencies: This research was supported by a grant from the National Science Foundation to RJN and an American Heart Association Predoctoral Fellowship to LKF.

Received: 16 July 2012 **Accepted:** 5 September 2012 **Published online** 10 May 2013. doi:10.1002/oby.20108

Interactions between metabolism and the circadian system appear to be reciprocal as diet induced obesity alters the period of the central clock and dampens diurnal rhythm in locomotor activity (14,15). Both short and long duration exposure to light at night or constant light also produce symptoms of metabolic syndrome (9,10,16).

Although the SCN is the dominant brain region involved in driving circadian activity, peripheral clock mechanisms are present throughout the body. Metabolic tissues such as liver, adipose, pancreas, and muscle all display independent rhythmic clock gene expression. The SCN principally regulates peripheral clock activity through neural and endocrine signaling pathways (17,18). Extra-SCN clock activity occurs in a tissue-specific manner which enables organs to cope with local physiological demands and respond to local factors. Multiple signals related to feeding and fasting entrain clock activity in metabolic tissue. Environmental cues that occur at aberrant times may lead to asynchronous activity within or between tissues which can lead to organ dysfunction (19). For example, changes in peripheral clock function contribute to symptoms of metabolic syndrome such as body weight gain and reduced glucose tolerance (20-22).

We previously reported that constant light (LL) and a bright/dim light cycles (dLAN) alter metabolic parameters in mice (9). Mice housed in LL and dLAN increase body mass and white adipose tissue, impair glucose processing, and alter food intake patterns compared to mice housed in LD. dLAN mice consume more food during the light period than at night which is atypical in nocturnal rodents (9). Altered timing of food intake could be the mechanism by which light at night induces weight gain as it has previously been shown to induce metabolic disorder (23) and uncouple central and peripheral clock gene expression (24). The goal of the present experiment was to determine whether or not changes in metabolism dissipate after removal of the aberrant light schedule. Studies in shift-workers offer contrasting views about whether removing circadian disruption can produce a return to baseline state. For example, current shift-workers increase systemic markers of inflammation, but former shift workers do not differ from day shift controls (25). However, former shift workers have increased risk for obesity (25). Thus, we tested whether metabolic disruption that occurs with light at night is an enduring effect following placement back in a standard light dark cycle. Mice were housed under dim light at night for 4 weeks and then transferred back to a standard light dark cycle. Placement back into dark nights ameliorated the effects of exposure to dim light at night. This suggests that changes in metabolism that occur with nighttime light exposure are not necessarily permanent.

Methods and Procedures

Animals

Sixty male Swiss-Webster mice (~8 weeks of age) from Charles River Kingston were used in this study. Nocturnal rodents were used in this study to investigate the effects of nighttime light exposure independent of sleep disruption. The mice were individually housed in polypropylene cages (dimensions: 27.8 × 7.5 × 13 cm³) at an ambient temperature of 22°C ± 2°C and provided with Harlan Teklad 8640 food (Madison, WI) and filtered tap water ad libitum. All experimental procedures were approved by The Ohio State University Institutional Animal Care and Use Committee, and animals were maintained in accordance with the recommendations of the *National Institutes of Health* and the *Guide for the Care and Use of*

Laboratory Animals. All mice appeared healthy throughout the study and showed no signs of sickness behavior.

Mice were assigned to one of four groups: (1) a control group that remained in standard light-dark conditions [14-h light (150 lux): 10-h dark (0 lux); LD/LD] for the 8-weeks study, (2) a group housed in dim light at night for 8 weeks [14-h light (150 lux): 10-h dim (5 lux); dLAN/dLAN], (3) a group housed in LD for 4 weeks, then 4 weeks of dLAN (LD/dLAN), and (4) a group housed in dLAN for 4 weeks then 4 weeks of LD (dLAN/LD). All mice were housed in LD for 1 week to entrain to the local light-dark cycle and recover from the effects of shipping prior to entering the study. On day 1 of the experiment mice were weighed and transferred from an LD room to a cabinet with either an LD or dLAN light cycle. Body mass was measured weekly and glucose tolerance was evaluated after 7 weeks in experimental conditions. After 6 weeks in lighting conditions timing of food intake was measured for 4 consecutive days. At the conclusions of the study mice were anesthetized with isoflurane vapors and rapidly decapitated. Epididymal fat pads of mice that underwent the glucose tolerance test were collected and weighed and then flash frozen for qPCR analyses.

Real-time quantitative PCR (qRT-PCR)

The mRNA levels of MAC1, IL6, and TNF α were assayed in epididymal fat pads as an index of peripheral inflammation (26). A small portion of the distal epididymal fat pad was collected and flash frozen at the conclusion of the study. Total RNA was extracted using a homogenizer (Ultra-Turrax T8, IKAWorks, Wilmington, NC) and an RNeasy Mini Kit (Qiagen, Austin, TX). RNA was reverse transcribed into cDNA with M-MLV Reverse Transcriptase enzyme (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Gene expression for MAC1, IL6, and TNF α was determined using inventoried primer and probe assays (Applied Biosystems, Foster City, CA) on an ABI 7500 Fast Real Time PCR System using Taqman[®] Universal PCR Master Mix. The universal two-step RT-PCR cycling conditions used were: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Relative gene expression of individual samples run in duplicate was calculated by comparison to a relative standard curve and standardized by comparison to 18S rRNA signal.

Intraperitoneal glucose tolerance test (GTT)

After 7 weeks in experimental light conditions a subset of each group of mice was administered an intraperitoneal glucose bolus (1.5 g kg⁻¹ body mass) after an 18-h fast. Blood samples of 5 μ L were collected via submandibular bleed before injection and at 15, 30, 60, 120, and 180 min following injection. Blood glucose was measured immediately with a Contour blood glucose monitoring system and corresponding test strips (Bayer HealthCare, Mishawaka, IN).

Statistical analyses

Body mass and glucose tolerance test were compared between groups using repeated measures analysis of variance (ANOVA). Body mass gain, fat pad mass, gene expression, and food intake comparisons were analyzed using one-way ANOVA. Following a significant F score, multiple comparisons were conducted with Tukey's HSD tests. The above statistical analyses were conducted with StatView software (v. 5.0.1, Cary, NC). In all cases, differences between group means were considered statistically significant if $P \leq 0.05$.

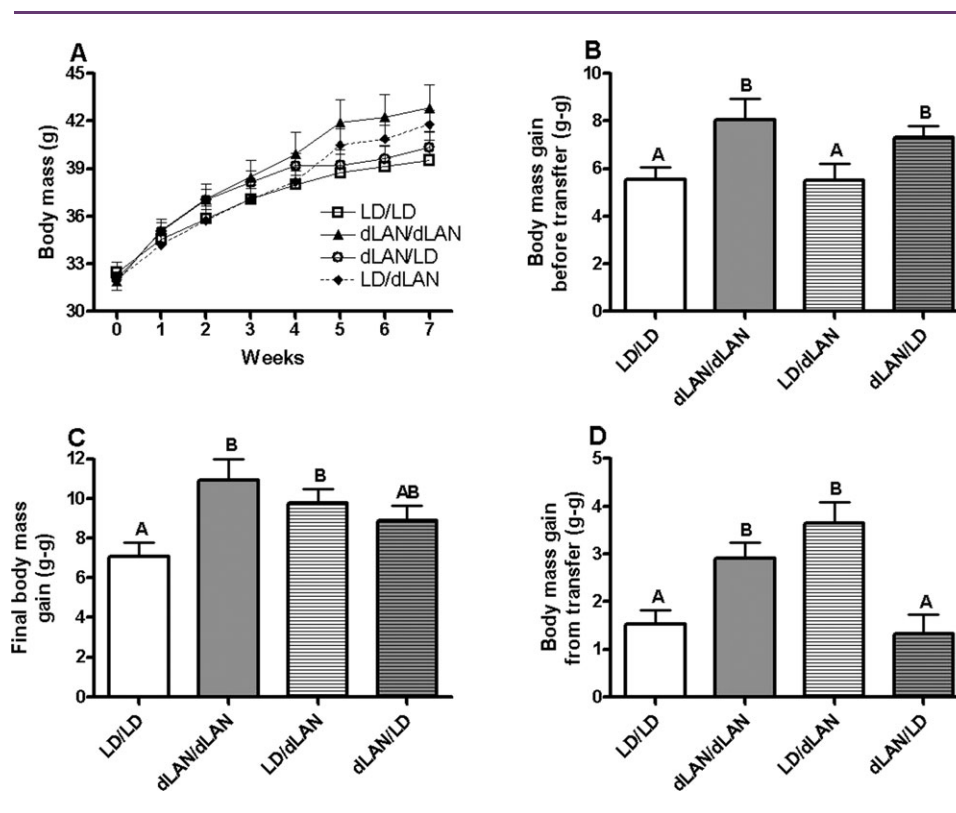


FIGURE 1 (A) Weekly body mass for mice over the course of the study. (B) Body mass gain after 4 weeks of lighting conditions and prior to the transfer. (C) Body mass gain at the conclusion of the study. (D) Body mass gain from the point of the transfer at experimental week 4 (different letters denote differences between groups, $P \leq 0.05$).

Results

Body mass

Body mass was significantly affected by light conditions over the 8 experimental weeks ($F_{21,336} = 3.537$; $P < 0.0001$; Figure 1A). After 4 weeks in their respective lighting conditions, and prior to the light schedule transfer, significant differences in weight gain were observed ($F_{3,50} = 3.710$; $P < 0.05$). Both groups that were initially housed under dLAN showed elevated body mass gain compared to those housed in standard lighting conditions (post hoc analyses; $P < 0.05$; Figure 1B). Directly following collection of week 4 body weights mice in the dLAN/LD and LD/dLAN groups were transferred to the new light schedules. After 3 weeks of the new lighting schedules body mass gain was also significantly affected by lighting conditions ($F_{3,50} = 3.906$ from beginning of study; Figure 1C; $F_{3,50} = 8.862$ from transfer; Figure 1D; $P < 0.05$ respectively). LD/dLAN and dLAN/dLAN mice gained significantly more weight throughout the study as compared to LD/LD mice (post hoc; $P < 0.05$; Figure 1C). dLAN/LD mice did not differ from any group with respect to weight gain when evaluating the entire 7-week period. When evaluating body mass gain following the transfer however, dLAN/LD mice significantly reduced weight gain compared to dLAN/dLAN and LD/dLAN mice (post hoc; $P < 0.05$; Figure 1D).

Relative epididymal fat pad mass (corrected for total body mass), a representative measure of white adipose tissue, significantly differed by the conclusion of the study ($F_{3,31} = 4.089$; $P < 0.05$; Figure

2A). Mice that were housed with dLAN for the entirety of the study showed significantly elevated relative epididymal fat pad mass as compared to LD/LD and dLAN/LD mice. LD/dLAN mice also had significantly elevated fat pad mass as compared to LD/LD mice (post hoc; $P < 0.05$). These results are particularly important because they suggest that increases in body mass among dLAN mice reflect increases in white adipose tissue (27). Furthermore, these results demonstrate that a switch back to a standard light dark cycle after 4 weeks of housing in dLAN allows restoration of fat levels to those of LD/LD mice. Lighting conditions did not affect paired testes, epididymides, spleen, or adrenal mass ($P > 0.05$ in each case).

Gene expression

MAC1 mRNA expression in epididymal fat pads was significantly affected by lighting conditions ($F_{3,31} = 2.948$; $P < 0.05$; Figure 2B). dLAN/dLAN mice elevated MAC1 expression as compared to both LD/LD and dLAN/LD mice (post hoc; $P < 0.05$). Expression of IL6 and TNF α in the fat pads did not significantly differ between groups (data not shown, $P > 0.05$).

Glucose tolerance test

Three weeks after the transfer to the new lighting schedules (7 experimental weeks) mice underwent a GTT. Injection of glucose led to a rapid increase in blood glucose levels in all groups ($F_{5,155} =$

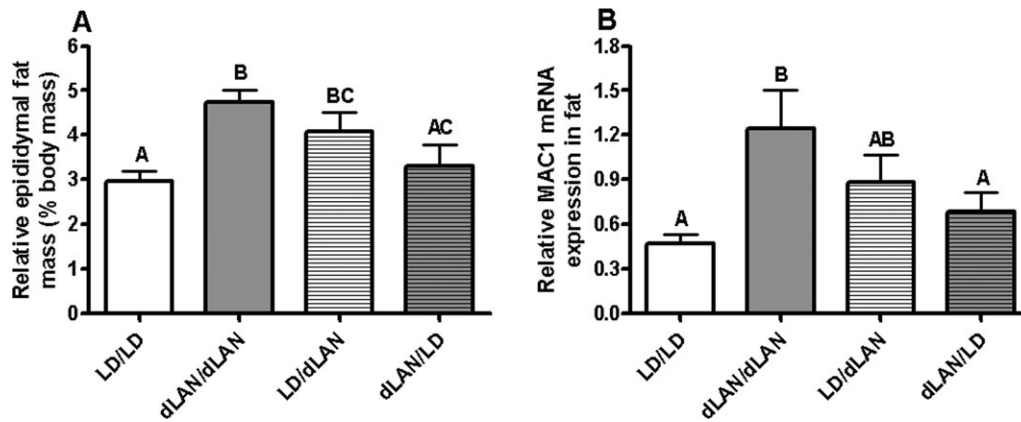


FIGURE 2 dLAN increased relative fat pad mass and macrophage gene expression in fat pads. **(A)** Relative epididymal fat pad mass at the conclusion of the study. **(B)** Relative MAC1 mRNA expression from epididymal fat pads (different letters denote differences between groups, $P \leq 0.05$).

267.514; $P < 0.0001$; Figure 3A). Furthermore, there was a significant interaction between lighting conditions and blood glucose levels over time ($F_{15,155} = 2.427$; $P < 0.005$), such that dLAN/dLAN mice failed to recover glucose levels as effectively as all other groups. Single time point comparisons revealed that glucose levels were significantly elevated in dLAN/dLAN mice as compared to LD/LD mice at T60 and as compared to all other groups at T120 and T180 ($F_{3,31} = 3.285, 4.879, 4.622$, respectively; $P < 0.05$). These results suggest that dysregulation in glucose processing is secondary to increased weight gain as the LD/dLAN groups does not yet show impairments in glucose tolerance. Furthermore, the improvement in glucose processing in the dLAN/LD group suggests that impairments in glucose regulation are reversible among dLAN mice by a transfer back to standard lighting conditions.

Energy intake

Total 24-h food intake did not differ among groups ($P > 0.05$). There was a main effect of light cycle on timing of food intake

($F_{3,31} = 3.912$; $P < 0.05$; Figure 3B); dLAN/dLAN and LD/dLAN mice consumed significantly more of their food during the light phase as compared LD/LD mice (post hoc; $P < 0.05$). LD/dLAN also consumed significantly more food during the light phase than dLAN/LD mice (post hoc; $P < 0.05$).

Discussion

The goal of the current study was to determine whether mice returned to dark nights after dLAN exposure recover metabolic function. This study replicates previous results demonstrating that mice housed with dim light at night develop symptoms of metabolic syndrome (9). As expected, both groups of mice housed in dLAN for the initial segment of the experiment (dLAN/dLAN and dLAN/LD) significantly increased body mass gain compared to LD mice. Half of the dLAN mice (dLAN/LD) were then transferred to LD and vice versa (LD/dLAN). Following the transfer dLAN/dLAN and LD/dLAN mice gained significantly more weight than LD/LD and dLAN/LD mice. At the conclusion of the study dLAN/LD mice did

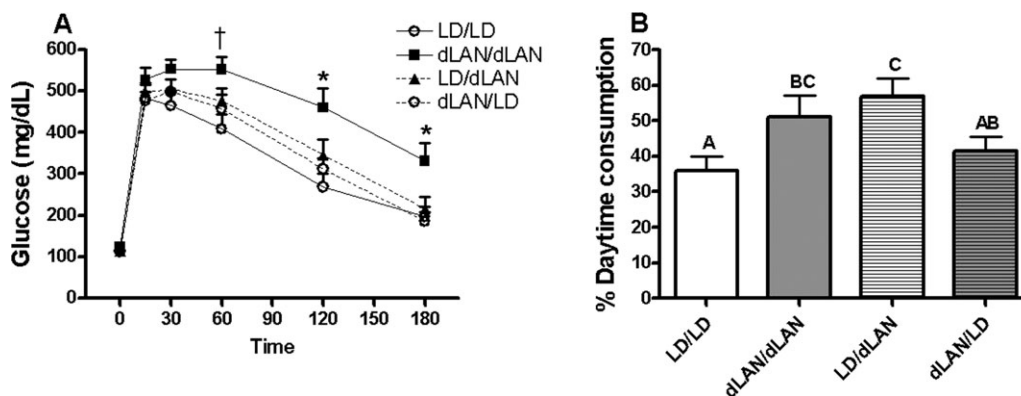


FIGURE 3 **(A)** Glucose tolerance was evaluated after 7 weeks in lighting conditions (+indicates LD/LD and dLAN/dLAN differ, *indicates dLAN/dLAN differs from LD/LD, LD/dLAN, and dLAN/LD). **(B)** Percentage of food consumed during the light phase (different letters denote differences between groups, $P \leq 0.05$).

not differ from either LD/LD or dLAN/dLAN mice with respect to body mass gain. The intermediary results of the dLAN/LD mice may reflect an inability to completely recover body mass after the 4-week exposure to LAN. This would indicate persistent changes in metabolism occur after nighttime light exposure. Former shift workers show symptoms of metabolic dysfunction after return to a day shift schedule which indicates that this may be the case in humans (25). However, in the study on shift workers there was no specified duration of time workers were on the non-shifting schedule. An alternative explanation to our findings is that 3 weeks in LD may be insufficient to completely recover body mass to LD levels. For example, recovery of other metabolic markers can occur prior to reduction in body mass (28). Food intake patterns, epididymal fat pad mass, as well as the GTT results support the latter hypothesis.

In agreement with previous findings, dLAN/dLAN mice shifted the timing of food intake compared to LD/LD controls without changing the amount consumed. dLAN/dLAN mice ate a significantly higher percentage of food during the light phase which is atypical for nocturnal rodents and may contribute to weight gain. Consuming higher amounts of food during the light phase is associated with increased weight gain in rodents (23). Moreover, restricting food intake to the dark phase can prevent weight gain in mice fed a high fat diet (29,30). Three weeks after dLAN/LD mice were moved back to LD, food intake no longer differed from LD/LD controls. In contrast, LD/dLAN mice shifted food intake to the light phase, with similar levels of daytime consumption as dLAN/dLAN mice. These results suggest that 3 weeks of dLAN is sufficient to induce altered timing of food intake.

Reduced glucose tolerance is a key symptom of metabolic syndrome (31). Here we show that impaired glucose clearance abilities associated with dLAN are recovered by 3 weeks of exposure to LD. After 7 weeks in lighting conditions mice underwent a glucose tolerance test. dLAN/dLAN mice showed significantly reduced glucose tolerance compared to all other groups. Importantly, the dLAN/LD group was comparable to LD/LD controls in the GTT. This suggests that dLAN/LD mice recover glucose processing abilities. Although dLAN/LD mice did not show a complete reduction in body mass, the GTT results suggest that metabolic function is restored.

Increases in body mass in dLAN are associated with increased white adipose tissue (9). dLAN/dLAN mice displayed significantly elevated epididymal fat pad masses compared to LD/LD mice indicating increases in body mass in dLAN are due to increased fat depots. At the conclusion of the study, dLAN/LD mice had significantly reduced white adipose tissue compared to dLAN/dLAN mice. Furthermore, LD/dLAN mice had intermediary fat pad mass compared to the LD/LD and dLAN/dLAN groups. Elevated fat mass is associated with widespread chronic low-grade inflammation in peripheral metabolic tissue which led us to evaluate levels of macrophage expression in the epididymal fat pads (32,33). dLAN/dLAN mice significantly elevated expression of MAC1, a marker for macrophages, in the epididymal fat pads. This indicates that there is increased macrophage infiltration into peripheral fat tissue. Peripheral inflammation can lead to disrupted insulin and leptin signaling further propagating fat accumulation (34). LD/dLAN mice had intermediary level of MAC1 expression compared to LD/LD and dLAN/dLAN mice whereas dLAN/LD mice had comparable MAC1 expression to LD/LD controls. This suggests that increases in peripheral inflammation are associated metabolic dysfunction following exposure to dLAN.

This study also provides important insight into the time-course of development of metabolic syndrome in dLAN. For example, altered timing of feeding likely precede changes in glucose clearance as LD/dLAN mice did not differ in the GTT compared to LD/LD controls although they already showed a pattern of food intake similar to dLAN/dLAN mice. As discussed above, timing of food intake is a critical factor in the development of metabolic disease (23,29) and light at night likely elevates body mass gain through altering time of food intake (9). Additionally, changes in weight gain appear to occur prior to the development of glucose intolerance because LD/dLAN mice show elevated weight gain but do not have impairments in the GTT. This would be consistent with other models of obesity in which adipose tissue releases factors (such as nonesterified fatty acids, glycerol, proinflammatory cytokines, etc.) that can contribute to the development of insulin resistance and β -cell dysfunction (31).

Overall, these results demonstrate that re-exposure to dark nights ameliorates metabolic disruption caused by dim light at night. dLAN appears as an innocuous environmental manipulation, which may be why it was overlooked as a significant risk factor for health and disease for many years. However, because of the profound impact light has on the circadian system and upon downstream outputs such as hormone secretion, dLAN likely exerts a significant effect on many physiological processes. The circadian clock and metabolic pathways are intrinsically linked (15,35) with desynchrony of feeding and activity causing metabolic alterations (23,36). In humans even brief circadian misalignment results in adverse metabolic and cardiovascular consequences (8). Our study suggests that exposure to nighttime lighting and the resulting changes in the daily pattern of food intake may be contributing factors in the current obesity epidemic. If these results apply to humans, then humans who experience weight gain in response to exposure to dim light at night may be able to help manage body weight by adjusting when they eat or by using low cost light blocking interventions such as sleep masks. **O**

Acknowledgments

We thank Natalie Hood and James Walton for technical assistance.

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