

Dim Light at Night Exaggerates Weight Gain and Inflammation Associated With a High-Fat Diet in Male Mice

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Elevated nighttime light exposure is associated with symptoms of metabolic syndrome. In industrialized societies, high-fat diet (HFD) and exposure to light at night (LAN) often cooccur and may contribute to the increasing obesity epidemic. Thus, we hypothesized that dim LAN (dLAN) would provoke additional and sustained body mass gain in mice on a HFD. Male mice were housed in either a standard light/dark cycle or dLAN and fed either chow or HFD. Exposure to dLAN and HFD increase weight gain, reduce glucose tolerance, and alter insulin secretion as compared with light/dark cycle and chow, respectively. The effects of dLAN and HFD appear additive, because mice exposed to dLAN that were fed HFD display the greatest increases in body mass. Exposure to both dLAN and HFD also change the timing of food intake and increase *TNF α* and *MAC1* gene expression in white adipose tissue after 4 experimental weeks. Changes in *MAC1* gene expression occur more rapidly due to HFD as compared with dLAN; after 5 days of experimental conditions, mice fed HFD already increase *MAC1* gene expression in white adipose tissue. HFD also elevates microglia activation in the arcuate nucleus of the hypothalamus and hypothalamic *TNF α* , *IL-6*, and *Ikbkb* gene expression. Microglia activation is increased by dLAN, but only among chow-fed mice and dLAN does not affect inflammatory gene expression. These results suggest that dLAN exaggerates weight gain and peripheral inflammation associated with HFD. (*Endocrinology* 154: 3817–3825, 2013)

The prevalence of obesity is increasing on a global scale with obesity-associated issues replacing concerns such as undernutrition and infectious disease as the most significant contributors to global health problems (1). Although well-documented factors such as dietary choices and lethargy are known to contribute to the prevalence of obesity and metabolic disorders, additional environmental factors are now considered critical in the development and maintenance of these conditions (2). The worldwide increase in obesity correlates with increased exposure to artificial light at night (LAN) during the 20th century (3). Artificial lighting allows people to extend daytime activities into the night and engage in countercyclical nighttime shift work.

This type of aberrant light exposure can disrupt the circadian system, because light is the most potent entrain-

ing signal for the mammalian biological clock (5). Many homeostatic processes are regulated by the circadian system, including metabolism (6). For example, there are 24-hour variations in the expression of genes involved in gluconeogenesis, lipogenesis, and lipid catabolism, among others (7, 8). Disruption of both primary and secondary clock genes causes profound changes in metabolism (9–12). Furthermore, we have previously demonstrated that exposing mice to dim, as opposed to dark, nights significantly increases body mass and reduces glucose processing independent of changes in caloric intake or total daily activity (13, 14).

In addition to altering metabolism and increasing the risk of diabetes and metabolic syndromes (15, 16), disruption of circadian clock through activities such as shift work increases risk for cancer (17), heart disease (18), and

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Abbreviations: dLAN, dim LAN; GTT, glucose tolerance test; HFD, high-fat diet; LAN, light at night; LD, light/dark cycle; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; WAT, white adipose tissue; ZT, Zeitgeber.

stroke (19). A feature common to these pathologies is inflammation. Inflammation is integrally associated with obesity, likely contributing to both the development and maintenance of metabolic syndrome (20). Elevated fat mass leads to widespread chronic low-grade inflammation in adipose tissue, liver, and skeletal muscle (21–23). This inflammatory response is characterized by increased levels of circulating proinflammatory cytokines, as well as infiltration of the tissue by immune cells such as macrophages, neutrophils, and eosinophils (24, 25). Peripheral inflammation disrupts insulin signaling propagating fat accumulation.

Hypothalamic inflammation resulting from a high-fat diet (HFD) may occur before and contribute to the development of obesity (26). Changes in hypothalamic inflammation occur within hours of consuming high-fat food (27). Acute glucose overload significantly increases nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) activity in the hypothalamus but not peripheral tissue (28). Furthermore, hypothalamic insulin resistance occurs before insulin resistance in peripheral tissue (29).

In developed and developing countries, exposure to LAN and HFDs often coincide and may contribute to the increasing obesity epidemic. Thus, we hypothesized that dim LAN (dLAN) would exaggerate metabolic dysfunction produced by a HFD in mice. Because dLAN is associated with changes in immune function (30, 31), we also investigated whether dLAN works through a similar mechanism as HFD producing additional increases in peripheral and hypothalamic inflammation.

Materials and Methods

Animals

All mice were housed individually in polypropylene cages at an ambient temperature of $22 \pm 2^\circ\text{C}$ and provided with food 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.

Experiment 1

Ninety male Swiss-Webster mice (~8 wk of age) from Charles River Laboratories were housed in a standard light/dark cycle (LD) (14-h light [150 lux]/10-h dark [0 lux]) and provided basic rodent diet (chow) (Harlan Teklad 8640) for 1 week after arrival at our facility. After this, mice were randomly placed in 1 of 4 groups; mice were housed in either LD or dLAN (14-h light [150 lux]/10-h dim [5 lux]) and received either a HFD (Research Diets

D12451, New Brunswick) or chow. On day 1 of the experiment, mice were weighed and transferred to either an LD or dLAN light cycle and maintained on chow or switched to HFD for the duration of the study. After 4 weeks in lighting conditions, mice either underwent a glucose tolerance test (GTT) ($n = 9$ per group) or tissue was collected for quantitative PCR ($n = 6$ –7 per group) or immunohistochemical analyses ($n = 6$ –7 per group). Among the mice designated for quantitative PCR and immunohistochemical analyses, body mass was measured weekly during the experiment, and timing of food intake was established by weighing food twice daily at the onset of the light and dark phase for 4 consecutive days during the final experimental week. A terminal blood sample was collected from mice used for the GTT, 2 weeks after the GTT at either Zeitgeber (ZT)8 or ZT14 for analysis of serum insulin concentrations.

Experiment 2

Forty male Swiss-Webster mice (~8 wk of age) from Charles River Laboratories were housed in LD and provided chow for 3 weeks after arrival at our facility. After this, mice were randomly placed in 1 of the 4 groups listed above; mice were housed in either LD or dLAN and received either HFD or chow. Food intake was measured twice daily at the onset and end of the light phase, and mice were weighed daily at the onset of the dark phase. Both body and food measurements were initiated 3 days before placing mice into lighting and dietary conditions in order to acclimate the mice to the stress of handling. Tissue was collected 5 nights after placement in lighting conditions.

Quantitative PCR

Between ZT8 and ZT10, mice were anesthetized with isoflurane vapors, a blood sample was collected, and mice were rapidly decapitated. Brains were removed, placed in RNAlater overnight, and the hypothalamus (from anterior preoptic area to the posterior portion of the mammillary bodies) was dissected out. Epididymal fat pads and liver were removed, weighed, and flash frozen. Total RNA was extracted from the tissues using a homogenizer (Ultra-Turrax T8; IKAWorks) and an RNeasy Mini kit (QIAGEN) according to manufacturer instructions. An additional chloroform separation step was added for extracting RNA from fat before using the kit. RNA was reverse transcribed into cDNA with M-MLV Reverse Transcriptase enzyme (Invitrogen). Gene expression for *MAC1*, *TNF α* , *IL1 β* , *IL6*, *Ikk β* , and *POMC* was determined using inventoried primer and probe assays (Applied Biosystems) on an ABI 7500 Fast Real-Time PCR System using TaqMan Universal PCR Master Mix. The universal 2-step RT-PCR cycling conditions used were: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Relative gene expression of samples run in duplicate was calculated by comparison with a pooled standard curve that ranged from 1 to 0.0001 and standardized by comparison with 18S rRNA signal.

Immunohistochemistry

Between ZT8 and ZT10, mice were given a lethal dose of sodium pentobarbital and perfused transcardially with ice-cold

0.1M PBS followed by 4% paraformaldehyde. Brains were removed, postfixed overnight, cyroprotected in 30% sucrose, and frozen with dry ice. Brains were sectioned on a cryostat at 40 μ m into cryoprotectant and stored at -20°C . Brain sections were treated with primary rabbit-anti-Iba1 (Wako Chemicals) (see Table 1), which is a 17-kDa EF hand protein that is specifically expressed in microglia/macrophages and is up-regulated during the activation of these cells. The primary antibody was diluted 1:1000 and was followed by treatment with biotinylated goat-antirabbit diluted 1:1000 (Vector Laboratories) as described in Ref. 32. Sections were developed in diaminobenzidine for 2 minutes (D4168; Sigma), mounted on gel-coated slides, dehydrated, and coverslipped with Permount. Images were captured on a Nikon E800 microscope at $\times 20$ (Nikon) and analyzed using ImageJ software (National Institutes of Health) to determine immunoreactive regions. Both sides of bilateral structures were counted in duplicate per animal.

GTT and insulin measures

After 4 weeks in experimental conditions, mice were fasted for 18 hours and then were administered a 1.5-g/kg body mass ip glucose bolus at ZT8. Blood samples of approximately 5 μ L were collected via submandibular bleed before injection and at 15, 30, 60, 90, and 120 minutes after injection. Blood glucose was immediately measured with the Contour blood glucose monitoring system and corresponding test strips (Bayer HealthCare, Mishawaka, Indiana). After 6 weeks in experimental conditions, a terminal blood sample was collected from the same mice at either ZT8 or ZT14. Total serum insulin concentration was determined in duplicate using electrochemiluminescence by the Clinical Research Center Core (The Ohio State University).

Statistical analyses

Comparisons between groups were conducted using a 2-way ANOVA with lighting condition and diet as the between subject factors. Change in body mass over time was analyzed using a repeated measures ANOVA. After a significant F score, multiple comparisons were conducted with Tukey's honestly significant difference test. The above statistical analyses were conducted with StatView software (version 5.0.1). In all cases, differences between group means were considered statistically significant if $P \leq .05$.

Results

Experiment 1

Somatic measures

Average body mass was comparable between all groups at the start of the study ($P > .05$), but within 1 week of experimental onset, both dLAN and HFD elevated body mass as compared with LD and chow (light: $F_{1,49} = 17.250$, diet: $F_{1,49} = 50.218$; $P < .0001$) (Figure 1A). Overall, relative body mass gain increased among both dLAN and HFD groups (light: $F_{1,49} = 15.779$, diet: $F_{1,49} = 136.447$; $P < .001$) (Figure 1B). Elevated body mass likely reflects increases in fat depots, because both dLAN and HFD increased relative epididymal fat pad mass (light: $F_{1,21} = 9.785$, diet: $F_{1,21} = 103.282$; $P < .01$) (Figure 1C).

GTT and serum insulin concentrations

Blood glucose concentrations measured after an 18-hour fast at the onset of the GTT were significantly elevated by HFD ($F_{1,31} = 14.631$; $P < .001$) (Figure 2A) but unaffected by lighting condition ($P > .05$). There was a main effect of both lighting and dietary condition on blood glucose levels in the GTT (light: $F_{1,155} = 4.787$, diet: $F_{1,155} = 8.107$; $P < .05$) (Figure 2B), such that mice exposed to dLAN as compared with dark nights elevated blood glucose concentrations 60 minutes after the glucose injection ($F_{1,31} = 6.223$) and mice fed HFD elevated blood glucose concentrations at 15 minutes ($F_{1,31} = 4.628$), 30 minutes ($F_{1,31} = 9.9649$), 90 minutes ($F_{1,31} = 4.655$), and 120 minutes ($F_{1,31} = 6.929$) after injection ($P < .05$ in all cases).

Two weeks after the GTT, a terminal blood sample was collected to evaluate serum insulin concentrations either during the middle of the light phase (ZT8) or 2 hours after lights off (ZT14). There was an interaction between time of day and lighting condition on serum insulin concentrations ($F_{1,27} = 5.205$, $P < .05$) (Figure 2C). Although mice exposed to dark nights showed el-

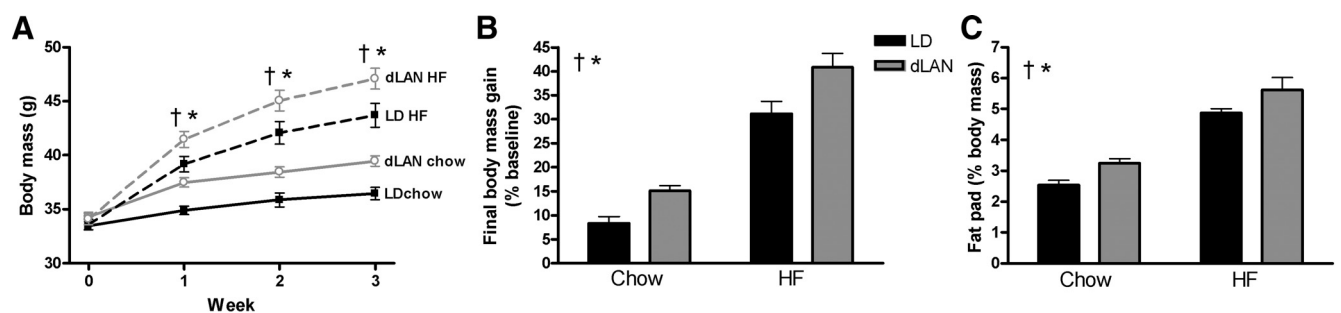


Figure 1. Body mass and fat pad mass were elevated by dLAN and HFD. A, Body mass throughout the experiment. B, Final body mass gain expressed as a percentage from baseline body mass. C) Epididymal fat pad mass corrected for final body mass. Data are presented as mean \pm SEM; *, $P < .05$ between lighting conditions; †, $P < .05$ between dietary conditions.

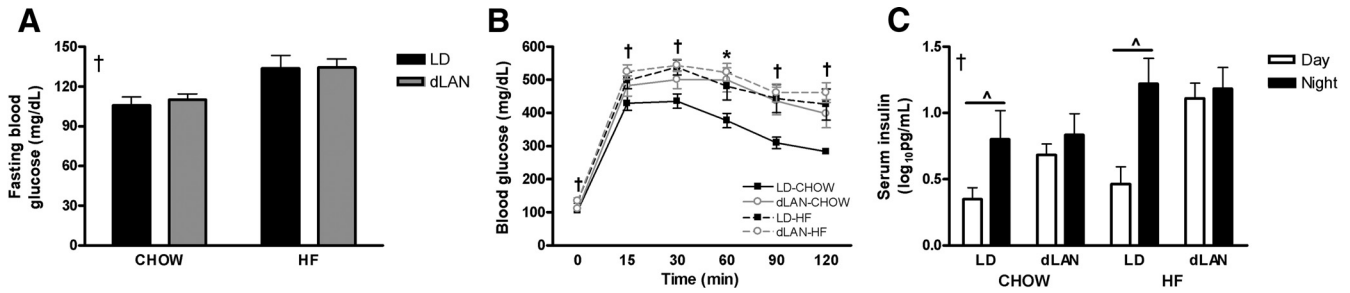


Figure 2. Exposure to LAN and HFD altered insulin concentrations and glucose regulation. A, Fasted blood glucose concentrations. B, After 4 experimental weeks, mice underwent a GTT. C, Insulin concentrations during the light phase and dark phase. Data are presented as mean \pm SEM; *, $P < .05$ between lighting conditions; †, $P < .05$ between dietary conditions; $\hat{}$, $P < .05$ between time points.

evated insulin concentrations during the dark phase as compared with the light phase ($F_{1,13} = 12.535$; $P < .005$), mice exposed to dLAN had comparable insulin concentrations during the light and dark phase ($F_{1,14} = 0.747$; $P > .05$). Insulin concentrations were also elevated among mice fed HFD as compared with standard chow ($F_{1,27} = 9.356$; $P < .01$).

Food intake

Despite increases in body mass among mice exposed to dLAN, there were no differences in total daily food intake between lighting conditions ($P > .05$) (Figure 2A). Mice fed a HFD increased caloric intake compared with the mice fed standard chow ($F_{1,49} = 23.83$; $P < .0001$) (Figure 3A). Furthermore, dLAN and HFD both altered timing of food intake. dLAN mice increased daytime food intake as compared with mice exposed to dark nights (light, $F_{1,49} = 42.649$; $P < .0001$) (Figure 3B). HFD also caused a slight shift toward daytime food intake (diet, $F_{1,49} = 5.509$; $P < .05$).

Peripheral inflammation

dLAN and HFD both elevated gene expression of *MAC1* (light: $F_{1,20} = 9.304$, diet: $F_{1,20} = 25.442$; $P < .01$) (Figure 4) and *TNF α* in white adipose tissue (WAT) (light: $F_{1,20} = 4.649$, diet: $F_{1,20} = 4.979$; $P < .05$) (Figure 4A). HFD also elevated *IL6* expression in WAT

($F_{1,20} = 7.064$; $P < .05$), but there was no effect of lighting condition ($P > .05$). There were no differences in gene expression in the liver (data not shown). Lack of differences in liver gene expression is consistent with previous research, because it is common for changes in peripheral inflammation to take more than 4 weeks to develop in response to HFD (33).

Hypothalamic inflammation

Consistent with previous reports (27, 28, 34), HFD elevated hypothalamic *TNF α* , *IL6*, and *Ikkkb* expression relative to standard chow (*TNF α* : $F_{1,21} = 4.433$, *IL6*: $F_{1,20} = 4.622$, *Ikkkb*: $F_{1,20} = 5.848$; $P < .05$) (Figure 5A). No differences in *MAC1* or *IL1 β* expression were apparent between light or dietary conditions ($P > .05$). The lack of difference in *MAC1* expression may reflect the distribution of hypothalamic microglia; with immunohistochemistry, we established that the concentration of Iba1 positive cells was elevated in the arcuate nucleus of the hypothalamus in mice fed a HFD ($F_{1,22} = 9.612$; $P < .01$) (Figure 5, B and D–G) but not other hypothalamic nuclei such as the dorsomedial hypothalamus ($P > .05$) (Figure 5C). There was no main effect of dLAN or interaction between lighting condition and diet with respect to Iba1 immunoreactivity. However, there was a simple main effect of lighting condition within mice fed chow diet, such that dLAN

increased the number of Iba1 positive cells ($F_{1,12} = 4.855$; $P < .05$). Neither diet nor lighting condition affected *POMC* gene expression (data not shown, $P > .05$).

Experiment 2

Body mass gain

Because changes in body mass in mice exposed to dimly lit, compared with dark, nights appeared to occur relatively early on and then level off

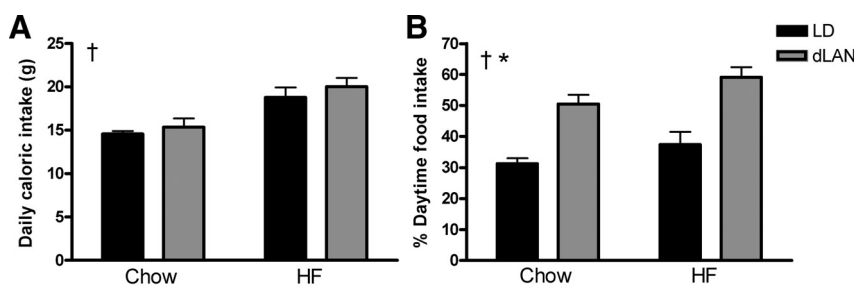


Figure 3. Total daily caloric intake was comparable between lighting conditions. However, dLAN mice consumed a higher percentage of food during the light phase. A, Total daily caloric intake. B, Relative daytime food intake. Data are presented as mean \pm SEM; *, $P < .05$ between lighting conditions; †, $P < .05$ between dietary conditions.

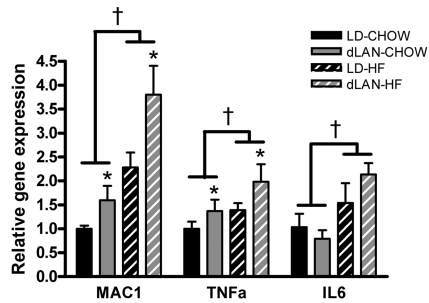


Figure 4. Both dLAN and HFD increased inflammatory gene expression in WAT. Quantification of mRNA encoding *MAC1*, *Il6*, and *TNFα*. Values are expressed as relative abundance (mean \pm SEM) after normalization to *18S* and presented as fold change relative to chow-fed dark night controls. *, $P < .05$ between lighting conditions; †, $P < .05$ between dietary conditions.

over time, we performed an additional experiment where we examined changes in the timing of food intake and body weight in the first 5 days after placement in experimental conditions. Over the course of the experiment, mice increased body weight ($F_{5,175} = 363.298$; $P < .0001$) (Figure 6A). Both HFD and exposure to dLAN increased body mass over time as compared with the standard chow diet and exposure to dark nights (diet: $F_{5,175} = 75.069$, light: $F_{5,175} = 8.523$; $P < .0001$). There were no differences in baseline body mass ($P > .05$). However, within 1 day ($F_{1,35} = 5.531$; $P < .05$) of beginning the dietary manipulation and continuing throughout the 5 experimental nights, mice fed HFD elevated body mass compared with mice fed standard chow. Beginning 4 days after placement in lighting conditions, mice exposed to dLAN elevated weight gain as compared with mice exposed to dark nights ($F_{1,35} = 4.216$; $P < .05$).

Food intake

Mice fed HFD significantly increased total daily caloric intake ($F_{5,175} = 50.472$; $P < .0001$) (Supplemental Figure 1, published on The Endocrine Society's Journals Online web site at <http://endo.endojournals.org>). In contrast, there was no effect of lighting condition on total caloric intake ($P > .05$). Mice fed HFD and/or exposed to dimly lit nights showed changes in timing of food intake. Mice fed HFD significantly increase daytime food intake 2 days after beginning the diet ($F_{1,35} = 4.748$; $P < .05$) and continuing through days 3–5. After 3 nights in lighting conditions, mice exposed to dLAN significantly increase daytime food intake compared with mice exposed to dark nights ($F_{1,35} = 4.956$; $P < .05$).

Peripheral inflammation

MAC1 and *IL6* gene expression were elevated in WAT 5 nights after beginning a HFD as compared with

chow diet (*MAC1*: $F_{1,36} = 4.395$, *IL6*: $F_{1,36} = 4.239$; $P < .05$). *TNFα* expression did not differ in the WAT due to either diet or lighting condition ($P > .05$). There was also no effect of light exposure on *MAC1* or *IL6* expression.

Hypothalamic inflammation

Five nights of exposure to dLAN or HFD did not significantly affect hypothalamic *MAC1*, *IL-1β*, *IL6*, or *Ikbkb* gene expression. In contrast, 1 week of HFD decreased hypothalamic *TNFα* expression ($F_{1,36} = 7.623$; $P < .01$).

Discussion

We hypothesized that housing mice in dLAN would exaggerate metabolic changes induced by a HFD. Moreover, we predicted that dLAN and HFD would elevate peripheral and hypothalamic inflammation, indicating the manipulations worked through similar mechanisms to increase body mass. Although dLAN and HFD increased weight gain and peripheral inflammation, results in the central nervous system were less clear. *TNFα* expression and microglia staining were increased in the arcuate nucleus of mice fed HFD. In contrast, dLAN did not affect hypothalamic *TNFα* expression, and microglia staining was only increased in dLAN mice fed the chow diet. Furthermore, changes in peripheral inflammation occurred more rapidly due to HFD than exposure to LAN; mice fed HFD for 5 days elevated *MAC1* and *IL6* gene expression in WAT, but there was no affect of nighttime light exposure at this earlier time point.

Body mass was elevated by dLAN and HFD within the first week of experimental conditions. Mice fed HFD increased weight gain within 24 hours of initiating the diet, and mice exposed to dimly lit, compared with dark, nights increased body mass after 4 days in lighting conditions. Among HFD mice, dLAN potentiated increases in body and fat pad mass compared with LD, demonstrating that changes in environmental lighting can exacerbate the adverse affects of a HFD. HFD and dLAN also altered timing of food intake. As previously reported, mice exposed to dimly lit nights increased the percentage of food consumed during the day (13). The shift in timing of food intake directly preceded significant increases in body mass among dLAN mice; the shift in the timing of food intake occurred after 3 days in lighting conditions, and significant increases in body weight were apparent on experimental day 4. Daytime food intake is atypical for nocturnal rodents and is associated with weight gain (35). Mice

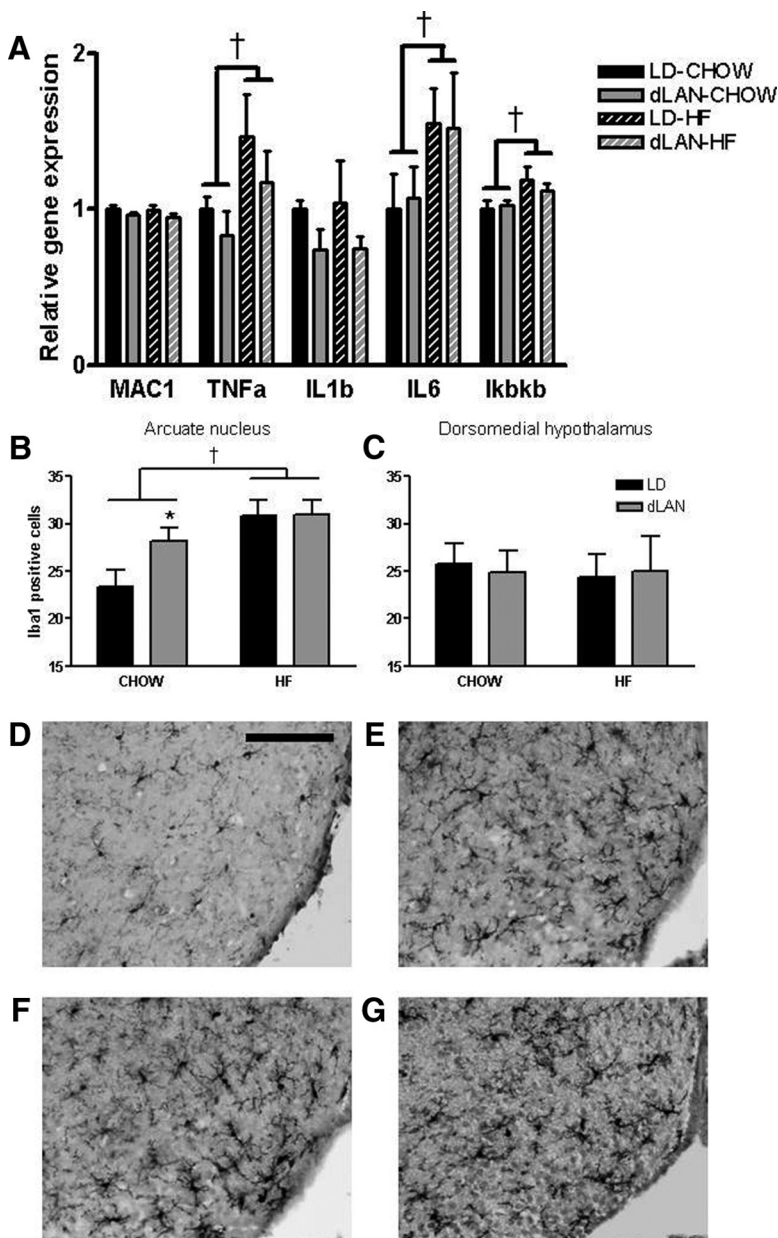


Figure 5. Hypothalamic inflammation is elevated by HFD but not dLAN. (A) Quantification of mRNA encoding a marker for macrophages (*MAC1*), proinflammatory cytokines (*Il1b*, *Il6*, and *TNF α*), and an NF- κ B pathway gene (*Ikkbb*). Values are expressed as relative abundance (mean \pm SEM) after normalization to *18S* and presented as fold change relative to chow-fed dark night controls. Iba1 staining in the (B) arcuate and (C) dorsomedial nuclei of the hypothalamus. Representative photomicrographs captured at $\times 20$ from the arcuate nucleus of (D) an LD-chow mouse, (E) a dLAN-chow mouse, (F) an LD-HFD mouse, and (G) a dLAN-HFD mouse. Scale bar, 100 μ m; *, $P < .05$ between lighting conditions; †, $P < .05$ between dietary conditions.

fed HFD increased total daily caloric intake and percentage of food consumed during the day (36). Changes in timing of food intake followed weight gain in mice fed a HFD rather than preceding changes in body mass. Previous work demonstrates that blocking access to food during the light phase can prevent weight gain due to HFD or dLAN (13, 37–39). This suggests that weight gain due to HFD and dLAN may partially result from the shift in timing of food intake.

and is well established in models of obesity (24). Macrophage infiltration can increase proinflammatory cytokine release and result in widespread, chronic, low-grade peripheral inflammation (21, 22). The development of peripheral inflammation generally follows the onset of obesity, although it promotes obesity by disrupting insulin and leptin signaling (33). Additionally, changes in peripheral inflammation may affect circadian rhythms. For ex-

In addition to shifting the timing of food intake, mice exposed to dLAN altered daily insulin secretion. Mice exposed to dark nights had higher insulin concentrations in the dark phase as compared with the light phase. In contrast, mice exposed to dimly lit nights had comparable insulin concentrations during the light and the dark phase. These results do not establish whether the change in insulin secretion is a cause or consequence of the shift in timing of food intake. However, elevated insulin concentrations during the light phase may induce insulin resistance (40). Indeed, lowering hyperinsulinemia can prevent high-fat feeding-induced obesity by increasing energy expenditure (41). Both exposure to LAN and HFD resulted in reductions in glucose processing. dLAN and HFD mice displayed prolonged elevation of blood glucose concentrations in the GTT. Glucose tolerance is used as a key diagnostic criteria for human diabetes and is considered an overall indicator of metabolic efficiency (42).

LAN is associated with changes in immune function in rodents (30, 31). However, the effects of LAN on metabolic inflammation have not been characterized. Here, we report that dLAN and HFD up-regulate *TNF α* and *MAC1* gene expression in WAT. Exposure to HFD results in more rapid changes in gene expression than nighttime light exposure, because mice fed HFD increase *MAC1* and *IL6* gene expression after 5 experimental nights. Elevated *MAC1* expression indicates increased macrophage infiltration into fat tissue

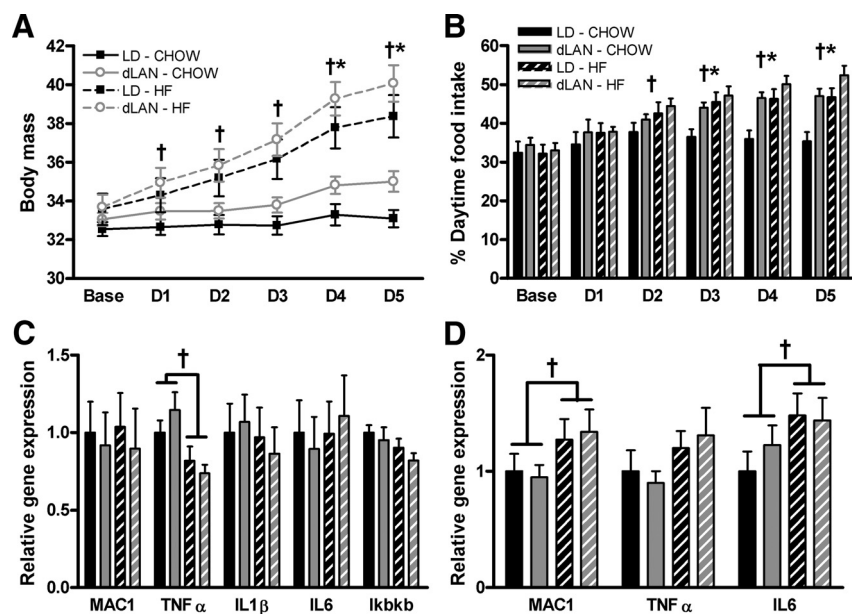


Figure 6. The effect of short duration exposure to dLAN and HFD on body weight gain, timing of food intake, and gene expression in the hypothalamus and adipose tissue. (A) Body mass gain and (B) timing of food intake over the first 5 days of experimental conditions. (C) Quantification of mRNA encoding *Il1b*, *Il6*, *Tnf α* , *Ikbkb*, and *Mac1* in the hypothalamus of mice exposed to dLAN and HFD for 5 days. (D) Quantification of mRNA encoding *Il6*, *Tnf α* , and *Mac1* in WAT collected at the same time point. Values are expressed as relative abundance (mean \pm SEM) after normalization to *18S* and presented as fold change relative to chow-fed dark night controls. *, $P < .05$ between lighting conditions; †, $P < .05$ between dietary conditions.

ample, inducing an inflammatory response with endotoxin treatment phase shifts the circadian system (43). This suggests that low-grade inflammation characteristic of obesity may alter circadian rhythms and be a potential mechanism through which HFD causes shifts in feeding rhythms and clock gene expression (36). However, we did not address this issue with our study, and the level of inflammation that occurs after stimulation with an inflammogen is different from obesity-induced inflammation.

In contrast, hypothalamic inflammation may precede and contribute to the development of obesity, because changes in the hypothalamic milieu are apparent within 24 hours of the induction of high-fat feeding (27). Therefore, we hypothesized that hypothalamic inflammation may contribute to changes in weight gain in dLAN mice. Mice fed HFD for 4 weeks increased hypothalamic *Tnf α* , *Il6*, and *Ikbkb* gene expression. In contrast, 5 days of HFD decreased hypothalamic *Tnf α* expression and did not affect *Mac1*, *Il1 β* , *Il6*, or *Ikbkb* gene expression. This is in agreement with previous findings that demonstrate that hypothalamic inflammation is elevated directly after initiating a HFD (1–3 d) and then shows a transient reduction after a week of high-fat feeding before increasing again by 1 month (27). Neither 5 days nor 4 weeks of nighttime light exposure affected hypothalamic cytokine gene ex-

pression. Moreover, no differences in *Mac1* expression were apparent between light or dietary conditions. Previous work indicates that increases in microglia activation are apparent in the arcuate nucleus as early as 3 days after initiation of a HFD (27). Lack of significant changes in hypothalamic expression of *Mac1* may be due to the specificity of the changes in hypothalamic inflammation. For example, there is a regionally specific enrichment of *Ikbkb* and *NF- κ B* in the mediobasal hypothalamus (includes the arcuate) that becomes potently up-regulated with HFD (28). This suggests that elevated inflammation in the arcuate nucleus may be masked by the lack of change in inflammation in other hypothalamic nuclei. In support of this hypothesis, the concentration of Iba1 positive cells was elevated in the arcuate of mice fed a HFD but not in the dorsal medial hypothalamus. Although dLAN did not affect Iba1 positive cells among HFD mice, dLAN increased the number of Iba1 positive cells within the chow group. It is possible that HFD maxes out the microglia response characteristic of this low grade metabolic inflammation. However, because dLAN-HFD mice show additional increases in weight gain relative to LD-HFD mice without increasing hypothalamic microglia, these results suggest that hypothalamic inflammation is not essential for weight gain in dLAN. Continued elevation of central nervous system inflammatory responses combined with a long-term HFD leads to gliosis and damage to proopiomelanocortin neurons (4). However, neither diet nor lighting condition affected *POMC* gene expression.

These results indicate that dLAN exacerbates weight gain and peripheral inflammation associated with HFD. Lack of elevated hypothalamic inflammation among dLAN mice suggests that central inflammation may not be the primary mechanism for light-induced weight gain. Our results do not preclude the possibility that LAN may cause more subtle shifts in inflammation in specific hypothalamic nuclei. Further understanding of the mechanisms through which LAN contributes to inflammation and obesity is important for characterizing and treating metabolic disorders.

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