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Original Article

A study on effect of altered circadian rhythm in the development of obesity

Nilesh N. Kate *, M. Chandrasekhar **, Ambareesha Kondam ***, E. Kayalvizhi *, M.Suresh *** & U. Kavitha ****

Assistant Professor, Prof And HOD, Department of physiology, Meenakshi Medical College and research institute, Kanchipuram—631552, Tamilnadu, India

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ABSTRACT

Background: Most living things have a daily cycle that reflects the rising and setting of the sun. A variety of studies have demonstrated that retinal light exposure can increase alertness at night. The global increase in the prevalence of obesity and metabolic disorders coincides with the increase of exposure to light at night (LAN) and shift work. The circadian clock prepares individuals for predictable events such as food availability and sleep, and disruption of clock function causes circadian and metabolic disturbances. **Aim:** To determine whether a causal relationship exists between night time light exposure behavioral changes and obesity. **Methods:** In this experiment 18 Swiss-albino male mice were divided into three groups i.e. Continuous light exposure (CL), light at night (LAN), standard (LD) light/dark cycle (control) and the effect of altered circadian rhythm on development of obesity and behavioral changes is seen. The body mass was assessed at the end of eight weeks to find out whether there was any correlation between the three variants. **Results:** Mice housed in continuous light (CL) or LAN have significantly increased body mass and increased prevalence of day time eating and altered behavioral pattern than mice in a standard (LD) light/dark cycle. **Conclusion:** These results suggest that light at night disrupt the timing of food intake and other metabolic signals, leading to excess weight gain. Melatonin is vital to this process, mediating the seasonal photoperiodic information through the clock system. Disrupting the melatonin signal or increasing the duration of light leads to changes in metabolism and adiposity consistent with fat storage and insulin resistance. These data are relevant to the coincidence between increasing use of light at night and obesity in humans (night shift worker).

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1. Introduction

The wide-ranging circadian rhythms (circa = around; dies = one day) of behaviour, physiology and metabolism include the sleep-wake cycle, feeding behaviour, control of body temperature, as well as numerous aspects of cardiovascular, endocrine, gastrointestinal, hepatic and renal function. They are generated by an endogenous oscillator, which is composed of a central clock in the suprachiasmatic nucleus (SCN) of the hypothalamus, known as the 'master clock' or 'circadian pacemaker', and numerous peripheral clocks in peripheral tissues. [1, 2] The central clock consists of multiple, autonomous, self sustaining, single cell circadian oscillators which are linked to produce a co-ordinated, in-phase, circadian signal.[1,3,4] The SCN oscillations

are not exactly 24 hours and so they are synchronized and 'entrained' every day by the external light/dark signal transmitted through the retino hypothalamic tract. [1, 4]. Thus, light is the primary environmental 'Zeitgeber' or time-giver. This gives information not only about the 24- hour day but also about the change in the relative proportion of light/dark (the photoperiod) and the amplitude of light intensity that tracks the seasons [5].

There has been an increasing awareness of the importance of the biological clock in man's physiology and concern over disruption of its rhythms. [7, 10-14] Man's body clock is tightly regulated to keep his physiology and metabolism in time with day length. Glucose, lipids, insulin, adiponectin, leptin, plasminogen activator inhibitor-1 (PAI-1) and blood pressure normally exhibit diurnal variation in man. [13] To determine whether a causal relationship exists between nighttime light exposure and obesity, the effects of LAN on body mass in male mice was examined. Because multiple studies have linked disruption of the molecular

* Corresponding Author : Dr. Nilesh N. Kate,
Dept. of Physiology, Meenakshi Medical College & R.I,
Kancheepuram 631552, Tamilnadu, INDIA.
E-mail: nileshkate79@gmail.com
Cell Phone: 09444568609/09444644586
Office Phone: 044-27264337/8

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circadian clock and metabolic disorders [20,21,22], It is hypothesized that LAN exposure alters circadian organization and affects metabolic parameters. The possibility of a direct link between altered light cycles and metabolic disorder by housing mice in a standard light/dark cycle (LD); a light at night cycle (LAN) 16 h light at ~150 lx/8 h light at ~5 lx, or 24 h of continuous lighting (CL; constant ~150 lx) is investigated and metabolic parameters were assessed.

Though many studies have linked disruption of the molecular circadian clock and metabolic disorders, very few studies only explained that LAN exposure alters circadian organization and development of obesity. This study has been undertaken to relate the light exposure at night and shifting of food intake in the development of obesity.

1.1. Aim & Objective

To determine whether a causal relationship exists between nighttime light exposure, behavioral changes and obesity.

- To examine the effects of LAN (nighttime light exposure) on body mass in male mice.
- To relate the time of food intake with weight gain.
- To explain the night time light exposure stress on behavioral changes.

2. Materials and Methods

The following study was conducted in the Department of Physiology Meenakshi Medical College and Research Institute, Kancheepuram. All experimental procedures were approved by The 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.

2.1. Animals and housing

Swiss-albino male mice weighing 20–30 g (5–6 wk old) at the beginning of the experiment were housed in a sound proof monitoring room in individual transparent acrylic cages (40 × 50 × 20 cm) placed in isolated lockers housing two animals each. Upon arrival, all mice were maintained under a 16:8 light/dark cycle for 1 wk to allow them to entrain to local conditions.

A 16:8 light/dark cycle rather than a 12:12 cycle was used to avoid providing a seasonally ambiguous signal. After the habituation period, mice were assigned a number pseudo randomly and were assigned to one of three groups housed in Controls (C) i.e. light dark cycle, continuous light (CL), or light at night (LAN) conditions.

Mice were weighed during group assignment to establish that all groups had a similar baseline body mass; mice weighing >35 g were excluded from the experiments. As multiple studies have linked disruption of the molecular circadian clock and metabolic disorders (20, 21, 22), it is hypothesized that Light at night (LAN) exposure alters circadian organization and affects metabolic parameters. The possibility of a direct link between altered light cycles and metabolic disorder by housing mice in a standard

light/dark cycle i.e. controls (n=6) (LD; 16 h day light /8 h dark at ~0 lx), a light at night cycle (LAN; 16 h day light /8 h light at ~40 lx), or 24 h of continuous lighting (LL 40 lx) and metabolic parameters were assessed. We included a light at night (LAN) group in addition to continuous light (CL) is because mice in constant lighting have no temporal cue to distinguish time of day, and their biological clocks free-run. By including the continuous light (CL) group, this study focused not only on the effects of light pollution but also on the effects of a desynchronized circadian system on metabolism.

2.2. Experimental design

2.2.1. Experiment 1.

For this study, a total of 18 Swiss-Webster mice were used. Mice were randomly assigned to one of three groups: control (C) or light at night (LAN) and continuous light (CL). Control mice were housed in individual cages in the monitoring system and were left undisturbed during the baseline and 7 additional weeks, corresponding to the scheduled manipulations. Case mice were monitored for 8–10 d for a baseline and were then submitted to the altered scheduled protocol of 8 h daily from Monday to Friday for 7 wk.

2.2.2. Altered Scheduled Protocol

After group assignment, the CL mice were placed in a constant-light room where they were exposed to ~150 lx of continuous light. The LAN mice were placed in a room with a 16:8 light/dim light cycle; during the light period they were exposed to ~150 lx of light, and during the night period they were exposed to ~5 lx of light at cage level. Following parameters were assessed.

1. Body mass.
2. Food intake.
3. Weight gain.

Experiments were approved by the committee for ethical evaluation in strict accordance with the norms for animal handling. All efforts were made to minimize the number of animals and their suffering.

2.3. Experiment 2.

Because altered timing of food consumption may mediate changes in body mass in the DM group, an additional experiment is performed in which mice housed in either day & night or LAN conditions had continuous access to food (FA) or had food access limited to either the light (FL) or dark (FD) phase.

2.4. Open Field Behaviour

To observe the effect of altered circadian rhythm on behaviour pattern, an open field behavior study was done which includes the following components, Peripheral ambulation, Central ambulation, Rearing, Grooming, Immobilization, Defecation, and Urination.

2.5. Statistical Analysis.

All the information is statistically analyzed using SPSS software (17.0 version). Obtained data was analyzed using two way analysis of variance (ANOVA). $P < 0.005$ had taken as statistically significant.

3.Result

3.1.Body Mass

Body mass was affected differentially by light condition over the 8 experimental weeks [$P < 0.0001$] A significant increase in body mass among mice in the CL and LAN groups, relative to the LD control group, was evident beginning 1 wk after onset of light treatment and continuing throughout the 8-wk study ($P \leq 0.01$). The elevated body mass among the CL and LAN groups resulted from increased gain in body mass [$P < 0.05$] rather than from initial differences in body mass among groups

Table 1:- Shows comparison of Mean values of change in the body mass in all the three groups.

DURATION (WKS)	CONTROL (GMS)	LAN (GMS)	CL (GMS)
1	22.6±1.87	22±1.13	22.5±1.08
2	23.8±1.69	26.4±1.13	26.9±1.03
3	25.3±1.522	27.4±1.57	27.8±1.53
4	26.3±1.352	28.6±1.98	29±1.80
5	26.8±1.522	29.5±1.85	30.7±1.96
6	27.2±1.51	30.8±1.56	31.5±1.99
7	27.6±1.78	31.2±1.64	32.6±1.75
8	28.1±1.90	31.7±1.41	32.8±1.84

GRAPH 1:- Change in the body mass in all the three groups

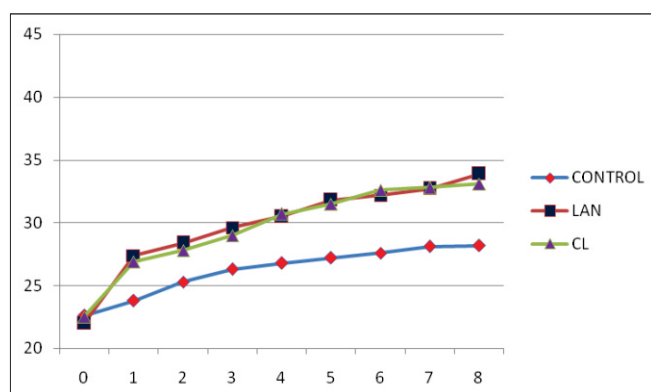


Table 2 :- Shows comparison of total weight gain in all the three groups over a period of 8 weeks.

Weight gain	CONTROL	LAN	CL
Gms	5.6	10	8

GRAPH 2 :- Total weight gain in all the three groups over a period of 8 weeks

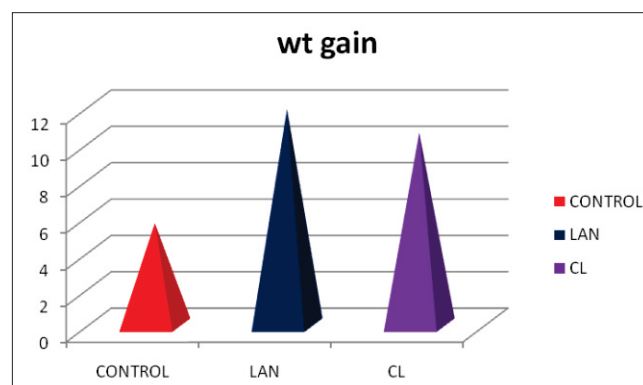
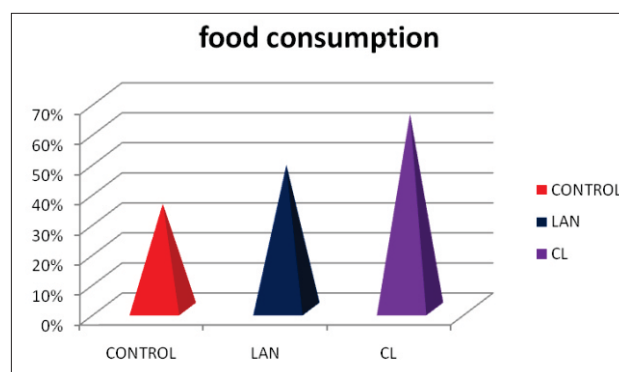


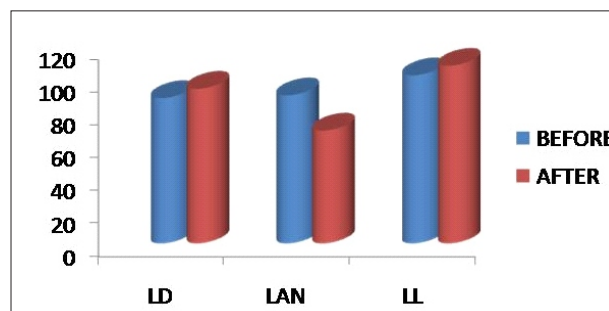
Table 3 :- Shows comparison of percentage (%) day time food consumption in all the three groups.

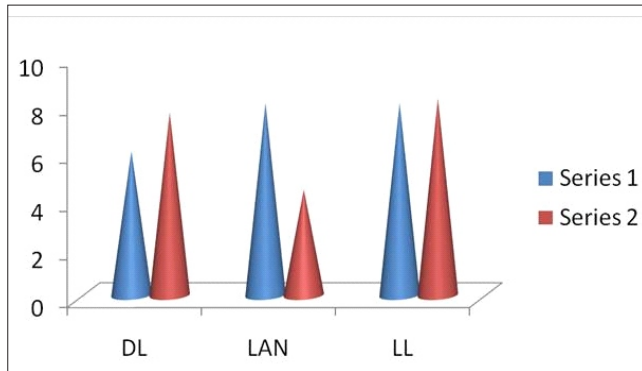
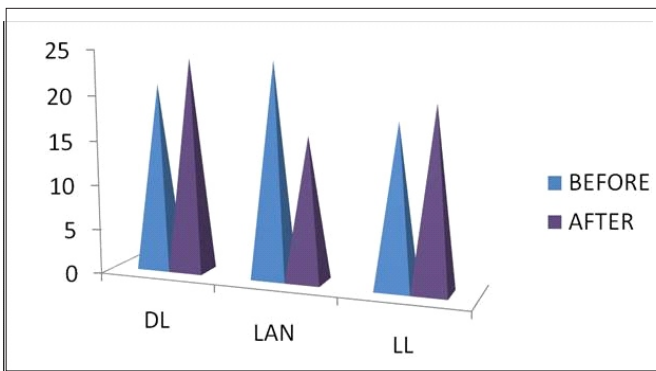
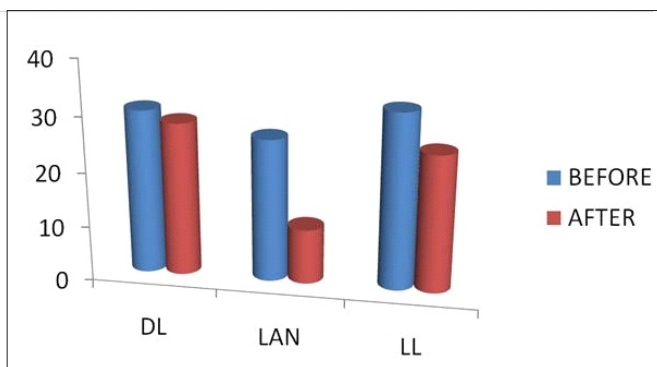
Day time food consumption.	CONTROL	LAN	CL	CONTROL
% food consumption	35%	48%	65%	35%

GRAPH 3 :- Comparison of percentage (%) day time food consumption in all the three groups.



Graph 4 :- Open Field Behavior-Periperal Ambulation



Graph 5:- Central Ambulation**Graph 6:- REARING****Graph 7:- GROOMING**

4. Discussion

The amount of sleep that we have each night has been declining in the westernized world. On average, we sleep 6.8 hours a night, which is 1.5 hours less than we did a century ago, with a third of adults sleeping less than six hours a night. [23] Numerous population-based studies have shown a relationship between short sleep duration, increasing obesity and T2DM, but it is only with prospective studies that the cause and effect is becoming clearer. [24]

The extent to which light influences the endogenous clock and metabolism is well illustrated in seasonal mammals. Their clock is synchronized to the day/night cycle but also to relative lengths of day/night (photoperiod), allowing coordination of physiology and metabolism over 24 hours and in response to seasonal change. [5] During long summer days the seasonal mammal gains weight and by the autumn it becomes insulin- and leptin-resistant. [25-28] In response to the short day lengths of autumn/winter it spontaneously reduces its food intake, irrespective of adequate supply, and enters hibernation during which its body weight declines.

By the time spring arrives with longer day lengths, the animal is insulin and leptin-sensitive again. [25-28,30-33] It is clear that melatonin is vital to this process, mediating the seasonal photoperiodic information through the clock system. [29-37] Disrupting the melatonin signal or increasing the duration of light leads to changes in metabolism and adiposity consistent with the summer/autumn phenotype, with fat storage and insulin resistance. [32,33,38] Persistent phase shifting of the light/dark cycle in other animals disrupts sleep/wake cycles and eating patterns, mimicking shift work. [39,40] Not only has this been shown to disrupt peripheral clock gene expression [39] but it is associated with weight gain [40] and premature death. [41]

These observations are physiologically relevant to man as it has been demonstrated that man has conservation of photoperiodic mechanisms able to influence circadian rhythms but these seasonally changing photoperiodic responses have been suppressed by modern artificial lighting. [42,43] Indeed, it has been proposed that humans live in a state of constant 'summer', due to artificial lighting, with a metabolism that reflects this and encourages obesity. [11,44]

Our present data indicate the importance of the moment of food intake for the maintaining of circadian internal synchrony or the induction of desynchrony, body weight gain in a rodent model of altered circadian rhythm.

Swiss-albino mice were housed in an LD cycle, in CL conditions, or in a LAN cycle. A significant increase in body mass among mice in the CL and LAN groups, relative to the LD control group, was evident beginning 1 wk after onset of light treatment and continued throughout the 8-wk study. Increased body mass is indicative of a prediabetic-like state [45] Thus, as little as 5 lx of light exposure during the typical dark period is sufficient to increase body mass and compromise glucose regulation.

Although no differences in total daily food consumption were detected among groups, feeding behavior was altered in the LAN group. The mice in the LAN group consumed 55.5% of their food during the light phase, as compared with 36.5% by mice in the LD group, indicating that mice exposed to LAN ate more food during the day than at night. CL mice were not considered in this comparison, because they had no temporal signal to distinguish the light and dark phases. Again, total 24-h consumption did not differ among groups, but food intake is substantially higher at night

among nocturnal rodents [46], and altered timing of food consumption has been associated with metabolic syndrome in other animal models [20,47]. Correlation analyses confirmed that percentage of daytime food consumption was positively related to final body mass.

To establish whether the altered timing of food intake contributed to the increased weight gain, we performed an additional study with a timed feeding schedule. Because mice housed in LL probably would entrain to the time of food access, they were omitted from the food access iteration (48). Mice were housed in either LD or LAN with FA, FL, or FD feeding. FD feeding prevented weight and fat gain among mice in the Control group. The weight and fat gain in control-FD mice was equivalent to that in control-FD mice and control-FA mice. These results further suggest that altered timing of food consumption in the LAN mice leads to increased body mass gain. As previously reported for rats and mice, FL mice increased body mass; this increase was not dependent on the light/dark cycle and control- and LAN-FL mice had comparable weight gain. Mice with limited access to food displayed an initial spike in consumption that decreased over time. With FL feeding, mice increased their food consumption during week one; however, FD-fed mice consumed more food in subsequent weeks. Again, overall food intake for the study was comparable among all groups, suggesting that the timing of food intake is a critical factor mediating increased weight gain.

These results establish that nighttime illumination at a level as low as 5 lx is sufficient to uncouple the timing of food consumption resulting in metabolic abnormalities. LAN mice display desynchrony between internal metabolic activity and food intake, as demonstrated by the altered timing of food consumption; this desynchrony may be the primary factor leading to increased weight gain. Mice exposed to LAN may have disrupted melatonin signaling leading to a misalignment of food intake and activity and resulting in altered fuel metabolism. Melatonin concentrations have been relatively unexplored in Swiss-Webster mice, but in one previous study [49] retinal melatonin levels were undetectable, in common with melatonin values in common strains of laboratory mice such as C57BL/6. Although previous research failed to detect melatonin in many strains of mice [50], more recent studies report attenuated but rhythmic melatonin expression in strains such as C57BL/6, which previously were thought void of melatonin [51]. Melatonin rhythmicity, rather than absolute quantities of nightly melatonin secretion, plays a crucial role in metabolic function [52]. For example, blunted nighttime melatonin rhythms caused by CL conditions increased visceral adiposity in rats [53], and daily administration of melatonin suppressed abdominal fat and plasma leptin levels [54]. Furthermore, melatonin influences clock gene expression in peripheral tissues such as the heart [55] and similarly may modulate clock gene expression in the peripheral tissue involved in metabolism.

Mice exposed to LAN also may have disrupted clock expression leading to altered metabolism. The SCN are the primary pacemakers at the top of a hierarchy of temporal regulatory systems wherein multiple peripheral tissues contain molecular machinery necessary for self-sustaining circadian oscillation [56]

In addition to becoming obese, mice fed a high-fat diet have disrupted Clock gene expression in the liver [57], and mice with mutations in clock genes have altered energy homeostasis [20]. Moreover, mice with mutations in either Clock or Brain and muscle Arnt-like protein-1 (Bmal1) show impaired glucose tolerance, reduced insulin secretion, and defects in the proliferation and size of pancreatic islets [58].

Several models of obesity have reported attenuated amplitude of circadian clock gene expression, and changes in the phase and daily rhythm of clock genes may cause obesity [59]. Metabolism and the circadian clock are intrinsically related [60], with desynchrony of feeding and activity causing metabolic alterations [47,61]. In humans even brief circadian misalignment results in adverse metabolic and cardiovascular consequences [62].

The decrease in rearing, grooming and ambulation in acute exposure to stress might be due to fatigue and altered excitability of nervous system. [63]

5. Conclusion

The seemingly innocuous manipulation of environmental light used in this study that changed feeding behavior and resulted in obesity may have important implications for humans. Patients with night-eating syndrome are obese and appear to display circadian rhythm disruption [64,65]. More generally, prolonged computer use and television viewing have been identified as risk factors for obesity, diabetes, and metabolic disorders [66]. For the most part, researchers considering this correlation have focused on the lack of physical activity associated with television and computer use; however, the results from the current study suggest that exposure to nighttime lighting and the resulting changes in the daily pattern of food intake and activity also may be contributing factors.

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