ISSN (electrónico): 1699-5198 - ISSN (papel): 0212-1611 - CODEN NUHOEQ S.V.R. 318

ARÁN

Nutrición Hospitalaria

Trabajo Original

Obesidad y síndrome metabólico

Light-dark cycle inversion effect on food intake and body weight in rats

Efecto de la inversión del ciclo de luz-oscuridad sobre el consumo de alimentos y el peso corporal en ratas

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Abstract

Background: most organisms inhabiting this planet have rhythmic functions in cycles that approximate 24 hours as a result of evolutionary adaptation. Disruption of these rhythms causes disruption in many bodily functions, including energy expenditure and consumption, and lipid and glucose metabolism, in addition to altering several biochemical parameters.

Objective: the aim of this study was to determine the effect of altering the light-dark cycle on diurnal and nocturnal food consumption and body weight in rats.

Material and methods: three experiments were carried out with an experimental group and a control group in each one. The groups included six males with an age of four months at the beginning of the experiment. Each experiment was 30 days long, starting with a baseline of 10 days and then inverting the light-dark cycle for another 20 days. In the first experiment the inversion took place at the end of the baseline period; in the second, the inversion was performed on days 10 and 20; in the third experiment inversions occurred every five days following the initial 10 days of baseline.

Results: our results show a lower body weight gain in the experimental groups when compared to the control groups.

Conclusions: significant differences in total consumption of food were not found, but were seen in the patterns of day and night consumption, along with a tendency to develop alterations characteristic of metabolic syndrome, which increased with the frequency of light-dark cycle inversion.

Keywords:

Light. Dark. Circadian cycle. Food intake. Body weight.

Resumen

Introducción: la mayoría de los organismos que habitan este planeta tienen funciones rítmicas que siguen ciclos cercanos a las 24 horas, resultado de la adaptación evolutiva. La alteración de estos ritmos provoca disrupción en funciones como el gasto y el consumo de energía, y el metabolismo de los lípidos y la glucosa, además de alterar varios parámetros bioquímicos.

Objetivo: el objetivo de este trabajo fue determinar el efecto de la alteración del ciclo luz-oscuridad sobre el consumo diurno y nocturno de alimento y el peso corporal en ratas.

Material y métodos: se llevaron a cabo tres experimentos con un grupo experimental y uno de control en cada uno de ellos. Los grupos estuvieron compuestos de seis machos de cuatro meses de edad cada uno. Cada experimento tuvo una duración de 30 días, comenzando con una línea base de 10 días y realizando inversiones del ciclo luz-oscuridad durante los otros 20 días. En el primer experimento se realizó una inversión al término de la línea base; en el experimento dos se realizó en los días 10 y 20; en el tercero, las inversiones se realizaron cada cinco días, tras los 10 días de la línea base.

Resultados: los resultados muestran una ganancia de peso corporal menor en los grupos experimentales en relación con los grupos de control.

Conclusión: no se encontró ninguna diferencia significativa en el consumo de alimento total pero sí en los patrones de consumo diurno y nocturno, que se intensificaron con el aumento de la frecuencia de la inversión del ciclo luz-oscuridad.

Palabras clave:

Luz. Oscuridad. Ciclo circadiano. Consumo de alimento. Peso corporal.

Received: 13/10/2020 • Accepted: 07/02/2021

Conflict of interest: the authors declare no conflict of interest.

Espinoza-Gallardo AC, López-Espinoza A, Vázquez-Cisneros LC, Zepeda-Salvador AP, Santillano-Herrera D. Light-dark cycle inversion effect on food intake and body weight in rats. Nutr Hosp 2021;38(3):495-501

DOI: http://dx.doi.org/10.20960/nh.03392

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INTRODUCTION

Organisms on earth have developed the ability to predict light-dark cycles of around 24 hours, called circadian cycles, which occur with the rotation of the earth on its axis, and this has allowed them to develop endogenous structures with the ability to synchronize almost all physiological and behavioral aspects to lighting conditions (body temperature, activity of the endocrine system, liver metabolism, wakefulness and sleep cycles, among others), whether they have evolved to be diurnal or nocturnal (1). These endogenous structures have been given the name of circadian clocks, and they are classified into three groups: peripheral, central, and homeostatic (2-4).

Evidence has shown that circadian cycles play an important role in maintaining health (5). In the case of nutrition, the relationship that circadian rhythms have is with the development of obesity, lipid metabolism (6), the development of metabolic syndrome, and feeding schedules (7). Diet, body weight, and circadian cycles are closely related (8). There are experiments that suggest that this relationship is bidirectional, since changes in nutritional status may cause changes in the balance of day and night activities, while alterations in the light-dark cycles can alter both the energy balance and the hours of food consumption (8-12).

Additionally, the results of some research suggest that food consumption and body mass may be influenced by fluctuations or alterations in the presence of light or darkness (8-12).

Experimental evidence indicates that homeostatic mechanisms can regulate the functions and behaviors of an organism to achieve internal balance. However, there are also studies that indicate that an alteration in circadian cycles may have repercussions on the functioning of various systems within an organism, resulting in alteration of food consumption, metabolism, storage, and body weight (8). However, the evidence is controversial, and there are investigations such as those carried out by De Assis et al. (13), who reported that an alteration of circadian cycle did not affect food consumption by subjects. The studies that found that changes in circadian cycles did not affect food consumption (14) indicated that the mechanisms by which they could affect it were uncertain.

However, the effects of frequency of exposure to such changes in the light-dark cycle that may have consequences, or the time limit of an event that allows the organism to return to normal conditions, as well as the exact effects of variations in lighting that are now constant and present in any modern civilization, are issues that remain poorly understood (15-24). Therefore, the aim of this research was to determine the effect of inversion of the light-dark cycle on body weight, and on diurnal and nocturnal food consumption in rats.

METHODS

A total of 3 experiments, lasting 30 days each, were designed utilizing 12 experimentally naïve male albino rats each. It was decided to use male subjects exclusively due to some morphological and hormonal differences that could potentially confound results (25). Rats with an age of four months at the beginning of the experiment, and initial weights between 310 and 360 grams (g) were assigned by convenience to the experimental or the control group, six for the control group and six for the experimental group, for each experiment. They were obtained at the vivarium of the *Instituto de Investigaciones en Comportamiento Alimentario y Nutrición* (IICAN). The subjects of this study were managed according to the criteria established by the Official Mexican Standard NOM-062-ZOO-1999 technical specifications for the production, care, and use of laboratory animals (26). In all experiments 5001 Rodent Chow from Purina was used as diet with unlimited access, and records of food intake and body weight were obtained in 12-hour intervals (6:00-8:00 am and 6:00-8:00 pm). To carry out the control of the light-dark cycles, the rats were placed in a laboratory isolated from sunlight using a model TE-102, IPSA brand timer. In all experiments control groups were exposed to a light-dark (L-D) cycle with light presence from 8:00 am to 8:00 pm. In all 3 experiments, the experimental group shared this condition during the first 10 days. In experiment 1 there was a L-D cycle inversion on day 10, with light from 8:00 pm to 8:00 am for the next 20 days of the experiment. This inversion in lightening conditions was performed at day 10 of experiment 2, going back to the original conditions at day 20. Meanwhile, experiment 3 had the same baseline conditions for the first 10 days, which was followed by lighting cycle inversions every 5 days (on days 10, 15, 20, and 25). The statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS), version 21.

RESULTS

In control groups and over the baseline period of experimental groups food was predominantly eaten during the dark phase, with 67.4 % to 76.4 % of ingestions occurring during this 12-hour period as expected for a nocturnal species. However, in experimental groups, following the L-D cycle inversions, the intake pattern was modified as may be observed in figures 1, 2, and 3, representing the experimental group's intake patterns for the three experiments; in all graphs the color black represents the data obtained in the 12-hour period from 8:00 pm to 8:00 am, independently of illumination conditions, whereas white represents the remaining 12-hour period (8:00 am to 8:00 pm). Figure 1 shows the data obtained for the experimental group in the first experiment as diurnal and nocturnal intake percentages; here, it may be observed that after the L-D cycle inversion, there is a corresponding modification of the intake pattern, that becomes predominantly diurnal, thus keeping the main intake during the dark period. However, as the frequency of L-D inversions increases, the ability to display this behavioral adaptation decreases, as can be observed in figures 2 and 3. In the second experiment we may see an intake inversion around 5 days after the L-D cycle modification; however, during experiment 3 this ability seems to get lost as the frequency increases, until in the last phase an equally distributed intake between the light

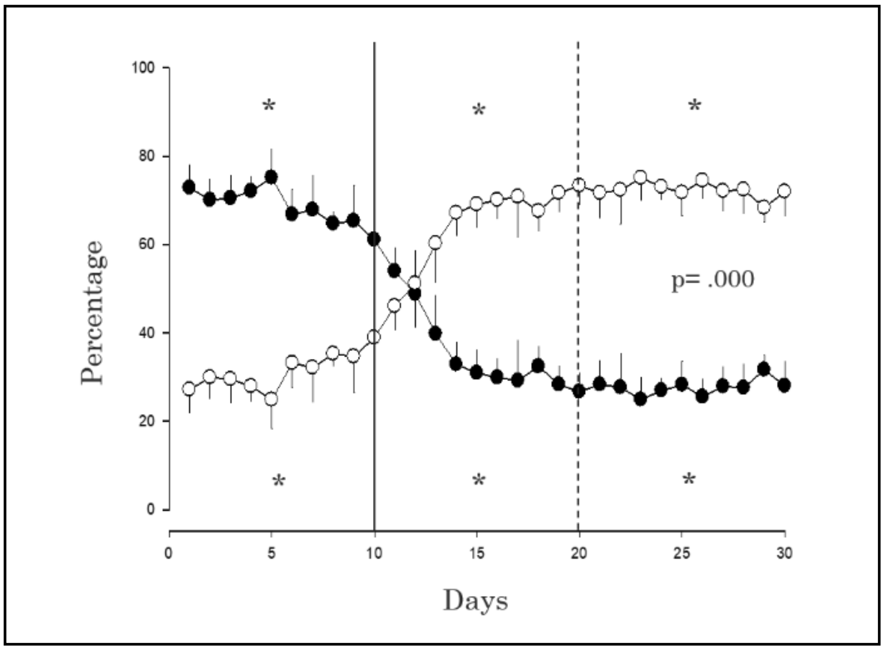

Figure 1.

Experimental group, first experiment. Diurnal and nocturnal food intake percentages.

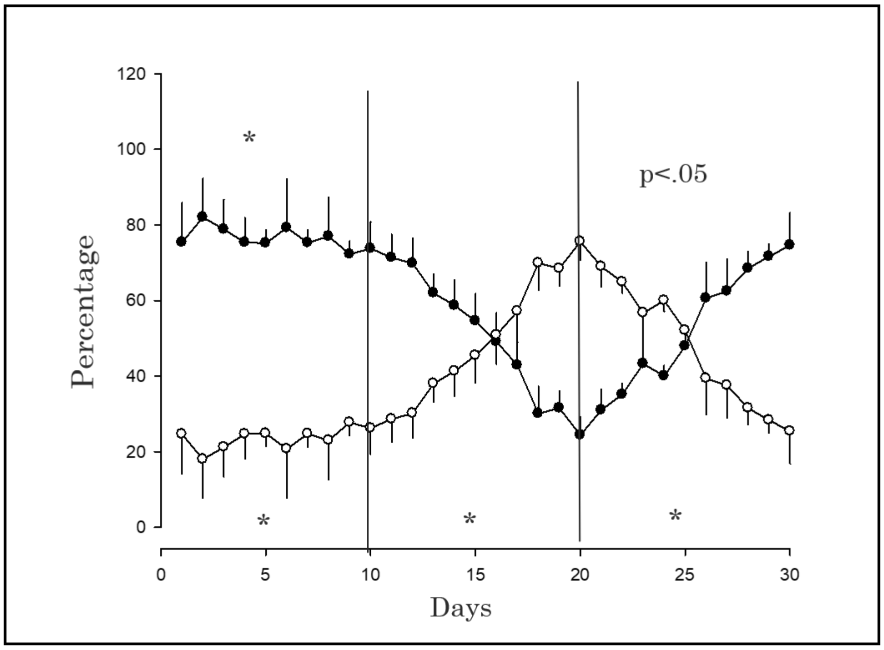

Figure 2.

Experimental group, second experiment. Diurnal and nocturnal food intake percentages.

and dark periods may be observed. An ANOVA and Student's t test were performed to compare groups and phases. Our findings include statistical differences between the experimental and control groups for diurnal intake (control, 6.16 g; experimental, 12.5 g) and nocturnal (control, 16.3 g; experimental, 9.7 g) in the first experiment, as well as in the second experiment (diurnal intake: control, 5.5 g, 6.0 g, and 6.2 g; experimental 4.7 g, 8.7 g, and 10.4 g, $p < 0.05$; nocturnal intake: control, 14.1 g, 14.6 g, and 14.9 g; experimental, 15.1g, 10.2 g, and 10.4 g, $p < 0.05$). Regarding the third experiment, we found statistically significant differences

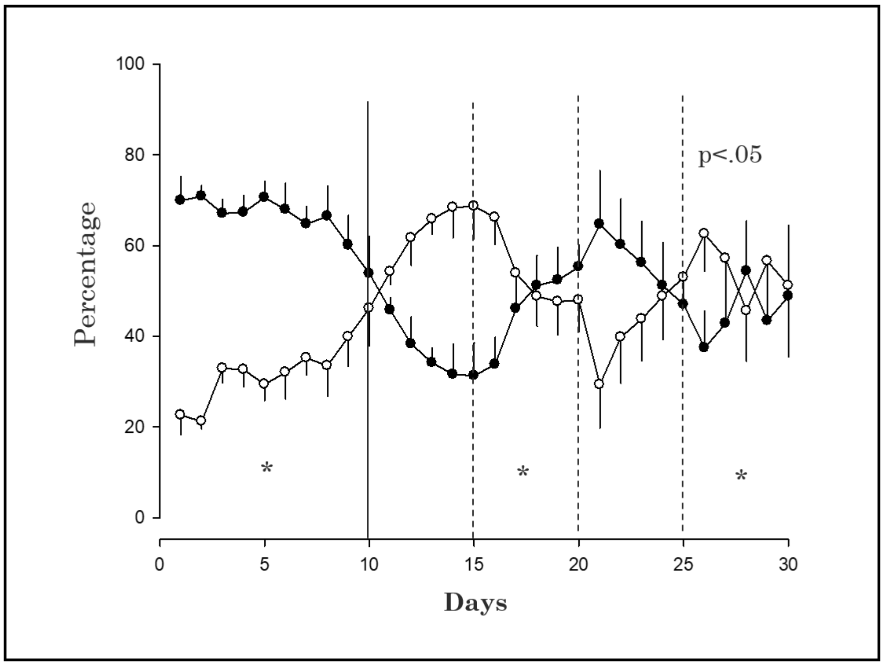

Figure 3.

Experimental group, third experiment. Diurnal and nocturnal food intake percentages.

in diurnal and nocturnal intake between both groups for all experimental phases, and similar diurnal intakes between phases 3 and 5, as well as phases 2 and 5; nocturnal phases 3 and 5 were also similar ($p < 0.05$). Regarding total food ingestion there were no statistical differences between the experimental and control groups or between phases during experiment 1 (control group intake per phase was 21.5 g, 22.3 g, and 22.6 g; experimental group intake was 22.2 g, 22.5 g, and 22.8 g, respectively). In experiment 2 there were no differences between groups or phases in the case of the control subjects; however, there was a statistically significant difference between phases 2 and 3 in the experimental group, as may be observed in figure 2 ($p < 0.05$). No statistically significant differences were found in total food intake between phases or groups in experiment 3. Regarding body weight, however, the average weight gain per day was 1.03 g for the control group and 0.18 g for the experimental group, which resulted in statistically significant differences ($p < 0.05$) between the average body weight seen in the control group (417 g) versus that seen in the experimental group (406 g). This trend was repeated in the third experiment (control, 350.9 g; experimental, 342.1 g, $p = 0.001$) but not in the second one, which presented statistical differences but with higher values for the experimental group (control, 342 g; experimental, 350.9 g, $p = 0.001$).

DISCUSSION

The main findings of this research include: 1) patterns of food consumption that adapted to the lighting conditions to which they were exposed; 2) this adaptation of consumption patterns disappeared when L-D cycle inversion frequency increased; 3) the energy balance of experimental subjects was maintained, exhibiting consumption values similar to those shown by the control group, regardless of inversions in the light-dark cycle and their frequency; 4) there were statistically significant differences in body weight between groups; however, a clear tendency was not found. Most scientific evidence reports an increase in body weight, mass gain, or development of overweight or obesity in subjects undergoing chronobiological alterations. According to Borniger et al. (16), body weight gain occurs, even in the absence of increased food consumption or decreased total caloric expenditure, due to the modification of food intake schedules. Since there is evidence that sleep quality and duration are not affected by the presence of dim light, and this is not interrupted by lighting of these characteristics during the rest period, but has the ability to alter the body weight balance of organisms, it has been concluded that the main oscillator, the suprachiasmatic nucleus (SQN), when synchronized by light signals is altered in the presence of dim light, but not so the peripheral oscillators that respond to the body's food intake signals. This lack of synchrony between the central and peripheral oscillators is the tentative cause of altered body weight control as seen in exposed subjects. Following the above reasoning we may conclude that, possibly, the reason why the subjects in our experiments did not show a clear tendency to increase body weight, to develop overweight or obesity, is the presence of food in free access conditions, and the fact that the behavioral and physiological adaptation to the new lighting conditions in all cases was carried out simultaneously.

The research carried out by Zucker (27) gives us the basis for another possible explanation. In the case of the subjects used in his experiment, which were subjected to constant light while at an early age, and therefore during a growth period, with the natural dislike that nocturnal rodents have for light, there was a loss of body weight. Taking this into account, another logical conclusion would be that the subjects used for these experiments were in the final stages of growth, and that the alteration of the light-dark cycle to which they were exposed had a reducing effect on their growth, causing the rats in the control group to maintain the normal weight gain for the species, whereas the experimental group remained stagnant, keeping the weight they had before the inversion.

Another common characteristic seen in the experiments that report body weight gain in animal models, and in the living conditions of people with night-shift jobs or in those who suffer from jet lag, is the shifting of phases for periods of less or more than 12 hours. However, when researching human phenomena it is important to acknowledge the personal, cultural, and social complexity that may contribute to these results. But whether conditions of continuous light, continuous darkness, advance or delay in the cycle are present, 12:12 behaviors are seldom maintained. The presence of a light-dark cycle lasting 24 hours could be part of the reason that prevented body weight gain from occurring in the subjects of these experiments, as has been reported in other experiments (15,16,22).

The diurnal and nocturnal consumption of food presented by the subjects in the experimental groups was modified according to the frequency of light-dark cycle inversions. During the first experiment an adaptation may be seen in the food consumption pattern that, after a period of about five days, goes from being predominantly nocturnal to diurnal, adjusting itself to the lighting conditions to which the subjects were exposed, similarly to the subjects in the experiment carried out by Humlová and Illnerová (28).

In this case, among the variables analyzed, consumption patterns were not found, but locomotor activity was, the subjects showed behavioral adjustments to the new lighting conditions; considering eating as part of the waking period activities, we could assume that consumption patterns also adjusted to the new lighting conditions. Additionally, the authors reported adaptation rate differences to the new lighting conditions for subjects, depending on their characteristics, when the light-dark cycle progressed for 8 hours, the subjects were able to adapt to the new surrounding conditions two days earlier than when it was delayed.

Despite the clear trend to nocturnal food consumption by rats (24), both in continuous light conditions and when kept in a 12:12 light-dark cycle, there are studies that have experimentally manipulated the duration, continuity, or absence of light periods due to the primary role that the SQN plays in the temporary control of organic functions, and because light has, through photoreception and phototransduction mechanisms, the ability to alter its functioning, the periods of wakefulness and rest of an organism, and with this, their feeding patterns (29,30).

Secondary oscillators can alter the wake-sleep patterns of an organism when the availability of food is limited, leading to the development of activity patterns that anticipate the availability of food (FAA). In the development of these experiments there were no limitations in food availability, therefore these patterns did not appear (8). The consumption patterns over the baseline period of each experiment, as well as those presented by the control groups, follow the consumption trend present in any nocturnal species.

During the first experiment, the control group presented an adaptation in their eating patterns at about 5 days after reversal of the light-dark cycle, moving to a predominantly diurnal food and water consumption pattern, following the lighting conditions to carry out most of their consumption in the dark period. During experiment two, after the first inversion, subjects were able to adapt to the new light-dark conditions to which they were exposed, although without reaching the average consumption presented during the baseline period. After the second exposure, the reversal of the food and water consumption pattern began almost immediately after the reversal of the lighting cycle, without the phase being long enough to reach the average presented in the first phase.

For the third experiment, it is possible to observe that the first inversion of the light-dark cycle is followed by an adaptation trend to the new lighting conditions like that presented in the previous experiments. Subsequent inversions, however, present lower quality in the lighting condition in which food is consumed predominantly; the response to lighting changes on the 20th and 25th, especially, was followed by little variation in the diet pattern, which at this point presented a consumption of food and water independent of the dark-light conditions, with a trend of 50-50 %.

There are studies that report responses like those presented by the above experimental subjects. Adaptation to different lighting conditions has been observed in several experiments, whether subjects were exposed to conditions of continuous light, continuous darkness, lengthening or shortening of light or darkness periods, or simply cycle delay or advance. Rosenwasser (24) used both continuous light conditions, under which the consumption pattern was maintained, with a cycle of around 24 hours, and conditions of 12 hours of light and 12 of darkness, to study the characteristics of food consumption by the subjects.

Cambras et al. (17) used cycle changes, lengthening their duration (25 hours), decreasing it (23 hours), and using conditions of continuous darkness, obtaining experimental evidence of the ability of subjects to adapt their behavior to the extant lighting conditions and their duration. These behavioral variations are present even in diurnal mammals (18). Whether they are subjected to conditions of continuous light, continuous darkness, phase advance or delay, subjects showed their ability to make behavioral adaptations, especially in their activity patterns in relation to new lighting conditions.

This same ability is similarly present in other species such as humans. Subjects exposed to repeated conditions of time zone change present alterations known as jet lag, which include behavioral, physiological, and performance alterations; however, after a few days of exposure to the new conditions, they manage to adapt to the new pattern of lighting, as did the subjects of experiments one and two (24).

This adaptation occurred within a period of time similar to that reported by Nagano et al. (23), of around 6 to 13 days, depending on the characteristics of the cycle change, although in the case of the present experiments, these characteristics were different. While the period between a light-dark cycle inversion and the next was around 10 days or more, the ability to adapt to new lighting conditions was clear. However, in experiment three, when the frequency of changes increased, taking place every five days, the ability of the subjects to adapt to the new conditions decreased notably.

In this case, the reaction of the subjects to the first inversion of the light-dark cycle clearly presented a trend similar to that presented by the subjects in the experimental group of the first two experiments after the first modification of lighting conditions, with a clear trend to a reversal of the predominantly nocturnal cycle at baseline, eating the greater portion of food during the daytime period but in accordance to the darkness present in that period.

In the second inversion of the cycle, and by not allowing the subjects to make a behavioral adjustment that would allow them to return to conditions similar to those presented at baseline, consuming most of the food during the dark period, a second inversion is made to experimental day 15. After this alteration, the ability of the subjects to adapt to light conditions seems to decrease with each new intervention (those carried out on experimental days 20 and 25), losing their consumption trend when observed in relation to temporality (day or night) or to lighting conditions (absence or presence of light), to end up with a distribution of around 50 % of consumption for each 12-hour period with no apparent trend.

It has been reported that the presence of light during the night period has effects on metabolism, the circadian rhythm of core temperature and body weight, all signals related to food consumption (16). The experiment carried out by Salgado-Delgado et al. (31) reported similar results under different experimental conditions. In this case, the alteration occurred in the activity of the subjects; however, as we have indicated, as long as food is not restricted, changes in consumption patterns can be closely related to those of physical activity. In shift-work animal models, the subjects also experience a dysregulation that leads them to lose their rhythm of food consumption with up to 46 % activity in daytime, suggesting that repeated alteration of the circadian cycle leads to loss of consumption patterns in subjects. All of the above occurs without altering the energy balance of the subjects, unlike in several studies that reported changes in energy balance and body weight, and development of obesity, metabolic syndrome, and high food consumption (1,5,8,32-36).

Total food consumption did not exhibit significant differences in any of the groups, in any of the experiments. Consumption in all phases was also similar, which suggests that the ability to maintain intake balance was not affected by the changes in light-dark conditions to which subjects were exposed. Maintaining the conditions of 12 hours of light and 12 hours of darkness even when reversing the original cycle could be part of the reason why energy balance was not affected, since we are evolutionarily adapted as organisms to inhabit this planet with changes in lighting conditions every 12 hours (1).

The presence of light during the night period has the ability to metabolically alter an organism, affecting its body weight and core temperature; however, in this case it did not disturb total food consumption in these subjects, even when the energy balance was different from that observed in the control group, whose weight gain was greater in the three experiments (16).

One relationship important to consider is that of weight gain and development of obesity as a secondary effect of food consumption during an organism's rest schedule. In the study carried out in rodents by Fonken et al. (37) a possible relationship between exposure to light during the night period and increased body mass is suggested, due to the change in food consumption patterns, with modification of eating times. In this study, in addition, variations were made in the intensity of the light used with three groups of mice (continuous bright light, continuous dim light, and light-dark cycle 12-12), and consumption patterns increased up to 55.5 % during the light phase in the group with the presence of dim light during the dark period. Additionally, a greater gain in body weight was reported both for the group with dim light and for those exposed to bright light during the night period. However, there were no significant differences in total food consumption between the groups, hence they concluded that changes in consumption patterns, as well as in schedules, may be responsible for the variation in body weight among subjects. The results of this research, the increased prevalence of obesity worldwide, and the ever increasing areas with light pollution make the study of the relationship between light-dark conditions and the development of metabolic diseases and obesity an area of opportunity that could provide answers helpful both both to understand this relationship and to develop strategies useful to address both problems, and to develop interventions and treatments that reduce the impact of light pollution both on the health of organisms and in the environment, and on biological diversity.

CONCLUSIONS

Our conclusions are as follows:

- Body weight was maintained in the experimental groups while controls exhibited a species-specific growth curve.
- Experimental groups showed eating patterns that adapted themselves to lighting conditions.
- This adaptation disappeared when the frequency of light-dark cycle inversions was increased.
- Water intake displayed variations closely related to food intake variations.
- Energy balance was maintained in the experimental subjects, showing similar variations, independently of light-dark cycle inversions.

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