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Reversed Light-Dark Cycle and Restricted Feeding Regime Affect the Circadian Rhythm of Insulin and Glucose in Male Rats

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Abstract

Circadian clock is entrained by external time cues and influences nearly all aspects of physiology. This work aimed to study the effect of reversed light- dark cycle and restricted feeding regime for two weeks on the circadian rhythm of glucose and insulin in male rats. Serum glucose rhythm was advanced after exposing rats to a reversed light-dark cycle and there was an increase in its level. In contrast, insulin concentration was lower than that of the control group. Its rhythm was shifted only after two weeks. In restricted feeding group, glucose level remained higher at night. High level of insulin was recorded after 4 hours from the meal after two weeks. Its secretion showed no association with glucose levels. From this study we concluded, that restricted feeding and reversed light-dark cycle could shift the phase of the circadian rhythm of glucose and insulin either after one or two weeks.

Keywords: Circadian rhythm; Glucose; Insulin; Light-dark cycle; Rat; Restricted feeding**.**

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1. Introduction

One of the most dramatic features of the world in which we live is the cycle of day and night. Correspondingly, almost all species exhibit daily changes in their behavior and/or physiology. These daily rhythms arise from a timekeeping system or biological "clock," within the organism [1]. In mammals, the central circadian clock located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus in the brain [2, 3, 4]. It is composed of multiple single-cell circadian oscillators. When synchronized, generate coordinated circadian outputs that regulate overt rhythms [5]. Similar clock oscillators have been found in peripheral tissues, such as the liver, intestine, and retina [6, 7, 8, 9, 10].

The SCN clock is reset mainly by the external light signal via the retino hypothalamic tract [2, 11, 12] whereas the peripheral clocks seem to be reset mainly by food cues rather than the light cue in mammals. It has been shown that a forced daytime restricted feeding, limiting the time and duration of food availability with no calorie reduction [13, 14, 15], can completely synchronize peripheral clocks of nocturnal animals, independent on the master clock and the environmental light-dark (LD) cycle [4, 16, 17]. Thus, the circadian clocks presented in various tissues may be entrained differently by external time cues, including light and food [18, 19, 20]

Insulin is a peptide hormone, produced by beta cells of pancreas and is the center of regulating carbohydrate and fat metabolism in the body. Beta cells in the islets of Langerhans release insulin in two phases. The first phase release is rapidly triggered in response to increased blood glucose levels. The second phase is a sustained, slow release of newly formed vesicles triggered independently of sugar [21]. Although the main physiological stimulus for insulin secretion in humans and animals is food intake, the size of the insulin response after food intake may also depend on the time of day [22, 23]. However, previous studies indicated that the fasting period preceding a meal was different for morning and evening meals. Because it is known that even short-term fasts may influence insulin responses [24] it is not clear whether the differences observed in previous studies are due to the unequal distribution of feeding activity or reflect a true circadian modulation [25]. So the present work is designed to study the effect of reversed light-dark cycle and restricted feeding regime on the circadian rhythm of insulin and glucose in adult male rats.

2. Material and methods

2.1. Animals

Adult male rats (*Rattus norvegicus*) weighing (100-120 g), were purchased from the breeding unit of the Animal House of National Research Center (Dokky- Giza, Egypt). Rats were maintained for one week under the normal environmental conditions of temperature and relative humidity before the start of the experiment for accommodation on the environment. They were kept under a normal light-dark cycle with 12 h of light and 12 h of darkness per day. They were fed standard commercial rodent pellet diet and water *ad libitum*.

2.2. Experimental design

Rats were divided into 4 groups, control group (36 rats) and three experimental groups (72 rat / each) maintained for two weeks under the different experimental conditions.

Group 1 (control group):

Rats were maintained under normal light-dark cycle (LD 12:12) where lights on from 0700h till 1900h; and fed *ad libitum*.

Group 2: (reversed light-dark cycle group):

Rats were exposed to 12h delay of one light period, so that the light was switched on at 1900h instead of being switched on at 0700h. Rats were maintained under a reversed light-dark cycle with lights on from 1900h in the evening till 0700h and fed *ad libitum*.

Group (3): (restricted feeding group)

Rats were maintained under normal LD 12:12 with lights on from 0700h to 1900h; fed only from 0900h to 1100h**.**

Group (4): (reversed light-dark cycle and restricted feeding group)

Rats were maintained under a reversed light-dark cycle with lights on from 1900h in the evening till 0700h; fed only from 0900h to 1100h.

2.3. Blood sampling:

After one and two weeks (control group after one week only) at a four hour-interval throughout the 24 h cycle (at 0700h, 1100h, 1500h, 1900h, 2300h and 0300h) 6 male rats from each group were anaesthetized with diethyl ether and dissected. Blood was collected from the heart puncture with 10-ml vein syringe and then transferred to labeled centrifuge tubes for serum separation, centrifuged for 10 min at 5000 rpm then serum was rapidly stored at -70 ˚C until analysis.

2.4. Insulin and glucose analysis:

2.4.1- Insulin analysis:

Insulin levels were determined by immunoradiometric assay [26] using [^{125}I] IRMA KIT (obtained from ɣ - trade company)**.** Radioimmunoassay was performed in Radioisotopes Department, Nuclear Research Center, Atomic Energy Authority, Egypt.

2.4.2. ***Determination of serum glucose:***

Glucose was determined according to Enzymatic Colorimetric Method [27].

2.5. *Statistical analyses of the data:*

The data obtained in the present study were represented as mean ± S.E. The statistical analyses were carried out using one way analysis of variance (ANOVA). **Duncan's test** [28] was performed between the means of control and different groups, as well as between different time intervals of the same group using the Statistical Package for the Social Science (SPSS) version 8.

3. Results

3.1. The effect of light-dark cycle and different feeding regime on the circadian rhythm of serum glucose levels at different time intervals after one week (Fig.1).

It was clear that in control group (group 1) the serum glucose levels showed gradual and significant increase started after 0700h, recording its maximum elevation (peak) at 2300h. However, the minimum decline was observed at 0300h compared to different time intervals of the same group at $P < 0.05$.

Comparing the glucose levels in reversed light-dark cycle group (group 2) to its corresponding control (group 1); the serum glucose levels increased significantly at all time intervals in group (2). The maximum peaks of glucose level within group2 were observed at 0700h and 2300h, whereas minimum decline was observed at 0300 h compared to different time intervals of the same group at $P < 0.05$ (Fig. 1A).

The serum glucose levels in rats exposed to normal lightening and maintained under restricted feeding regime (group 3) showed a significant decrease throughout experimental intervals at 0700h, 1100h, 1500h, 1900h comparing to control group. In contrary there were a significant increase at 2300h and 0300h. However, within group 3 serum glucose levels showed a gradual and significant increase started after 1100h; recording its maximum peak at 2300h. A sharp decline was observed thereafter showing a minimum decline at 1100h compared to different time intervals of the same group at $P < 0.05$ (Fig.1B).

The group of rats that kept for one week under reversed light-dark cycle and restricted feeding regime (group 4) showed an increase in the serum glucose level at 0700h, 1100h and 1500h (the light phase of the cycle), and significant decreases at 1900h, 2300h and 0300h, compared to its corresponding control. By comparing serum glucose levels at different time intervals within group 4, there were significant increases at 0700h, 1100h and 1500h indicating a peak at 1500h; significant decreases were observed thereafter. Minimum decline was recorded at 0300h ($p<0.05$) (Fig.1C).

3.2. The effect of light-dark cycle and different feeding regime on the circadian rhythm of serum glucose levels at different time intervals after two weeks (Fig.2).

Comparing the serum glucose level in reversed light dark-cycle group (group 2) to control group there were significant increases all over the experimental period at all-time intervals at 0700h, 1100h, 1500h, 1900h, 2300h and 0300h. By comparing serum glucose levels at different time intervals of group 2 there were

significant increases at 1100h, 1900h and 0300h, its maximum value was recorded at 1100h. Minimum decrease was recorded at 1500h compared to different time intervals of the same group at $p<0.05$ (Fig.2A).

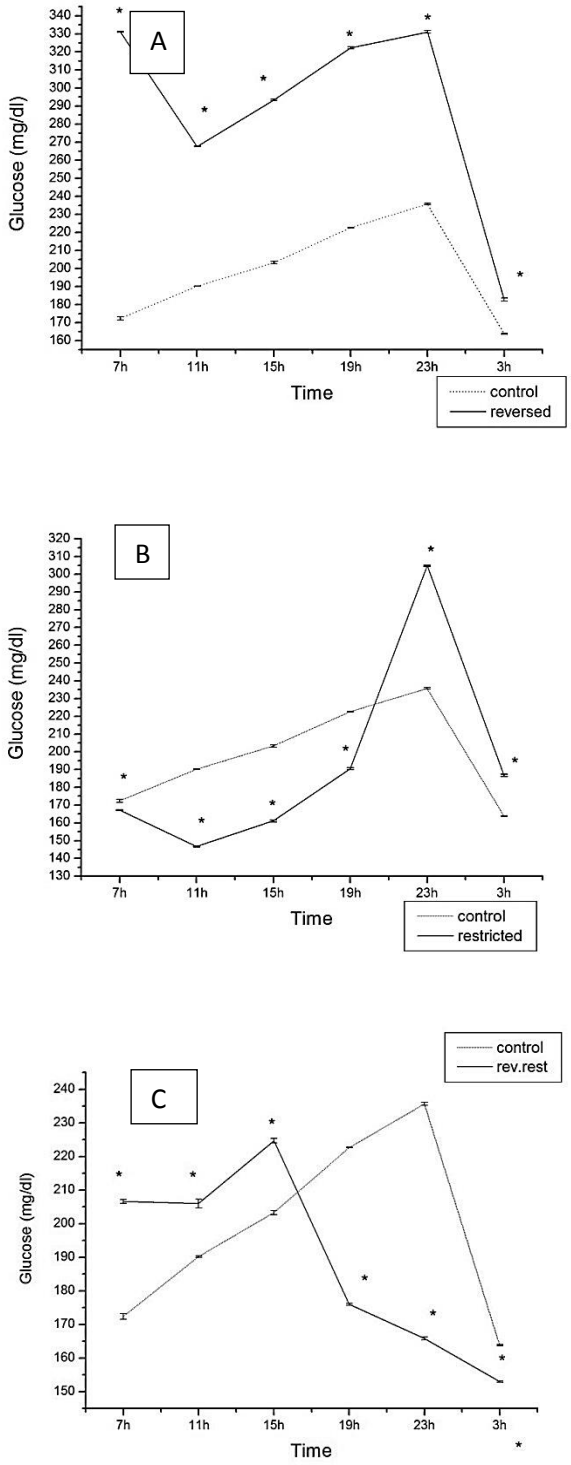

Figure 1: Daily profile of serum glucose levels in rats kept for one week under reversed light-dark cycle (A), restricted feeding regime (B), and reversed light-dark cycle as well as restricted feeding regime (C).

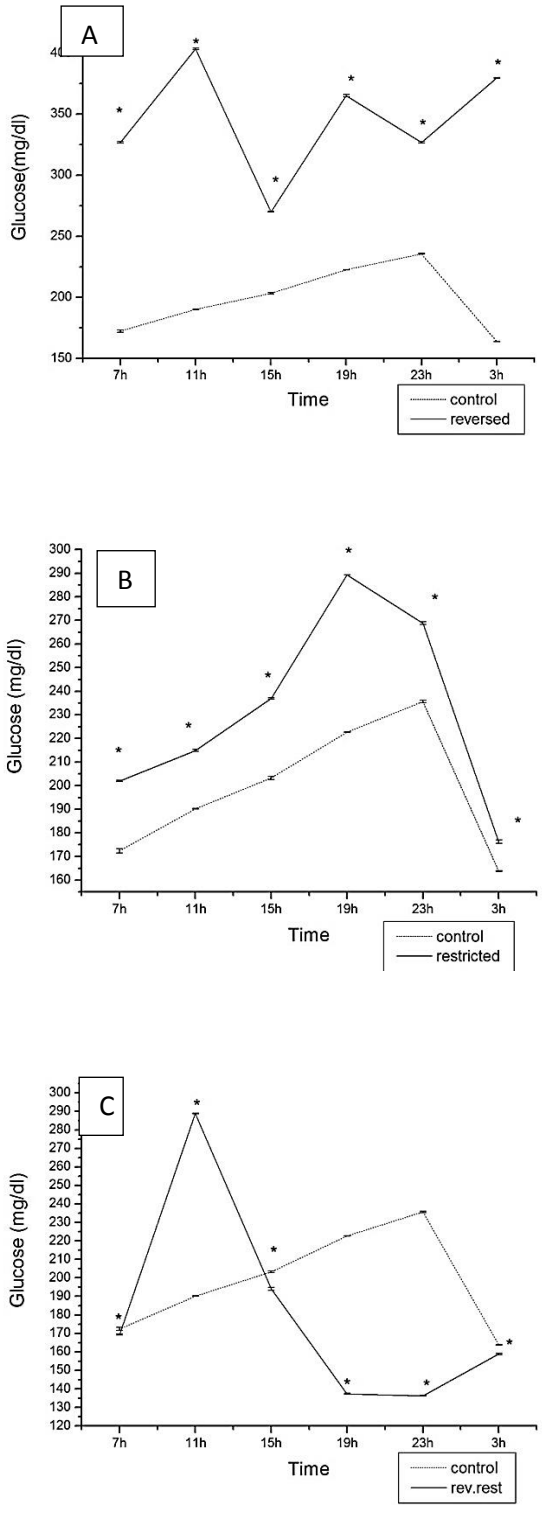

Figure 2: Daily profile of serum glucose levels in rats kept for two weeks under reversed light-dark cycle (A), restricted feeding regime (B), and reversed light-dark cycle as well as restricted feeding regime (C)

Moreover, the serum glucose levels recorded a significant increment in restricted feeding group (group 3) compared to that in the control group (group 1) at all-time intervals at 0700h, 1100h, 1500h, 1900h, 2300h and 0300h ($P < 0.05$). By comparing glucose levels at different time intervals in restricted feeding group, there were gradual and significant

increases observed after 0700h having a peak at 1900h while significant decreases were observed after 1900h recording a minimum decline at 0300h (Fig. 2B).

Comparing serum glucose levels of animals maintained under reversed light-dark cycle and restricted feeding regime (group 4) to that of control group (group1) there was a significant increase at 1100h. However, at 1900h and 2300h there were significant decreases ($P < 0.05$). The serum glucose levels within group 4 showed a maximum peak at 1100h. However, minimum declines were observed at 1900h and 2300 h compared to different time intervals of the same group at $P < 0.05$(fig. 2C).

3.3. The effect of light-dark cycle and different feeding regime on the circadian rhythm of serum insulin level at different time intervals after one week (Fig.3).

Serum insulin levels in control group showed the highest value (the highest peak) at 1900h. In contrast, the lowest value was shown at 2300h ($P < 0.05$).

In the reversed light-dark cycle group (group 2), the serum insulin levels were significantly increased comparing to its corresponding control (group 1) at 0700h and 1500h ($P< 0.05$); whereas there were significant decreases at 1100h, 1900h and 0300h. Within the same group, the maximum values of serum insulin levels were observed at 0700h, 1500h and 1900h showing a peak at 1900h whereas a minimum decrease was recorded at 1100h compared to different time intervals of the same group ($P <0.05$) Fig.3A.

Comparing the serum insulin levels of restricted feeding group (group 3) to its corresponding control, there were a significant increase in group 3 at 0700 h, 2300 h and 0300 h. However at 1100h, 1500h and 1900h there were significant decreases ($P <0.05$). Significant differences were observed in insulin levels compared to different time intervals of the same group (group 3) showing the lowest value at 1100h. However, highest values observed at 0700h, 1900h and 0300h. Noticing that the maximum level was recorded at 0300h ($P <0.05$) (Fig.3B).

The serum insulin levels in reversed light-dark cycle and restricted feeding group (group 4) were significantly decrease at all time intervals (0700 h, 1100, 1500 h, 1900 h, 2300 h and 0300 h ($P < 0.05$) comparing to control group (group 1) . Rats in group 4 showed a significant increase in the serum insulin levels at 1500 h and 0300h compared to different time intervals of the same group at $P < 0.05$(Fig. 3C).

3.4. The effect of light-dark cycle and different feeding regime on the circadian rhythm of serum insulin levels at different time intervals after two weeks (Fig. 4).

In rats exposed to reversed light-dark cycle (group 2), the serum insulin levels showed a slight increase at 0700h where at 1500 h and 2300 h, there were a significant increase, comparing to its corresponding control. The serum insulin levels within group 2 showed a gradual and significant increase throughout 0700h, 1100h and 1500h at $p<0.05\%$ compared to different time intervals of the same group .The highest peak was observed at 2300h (Fig.4A).

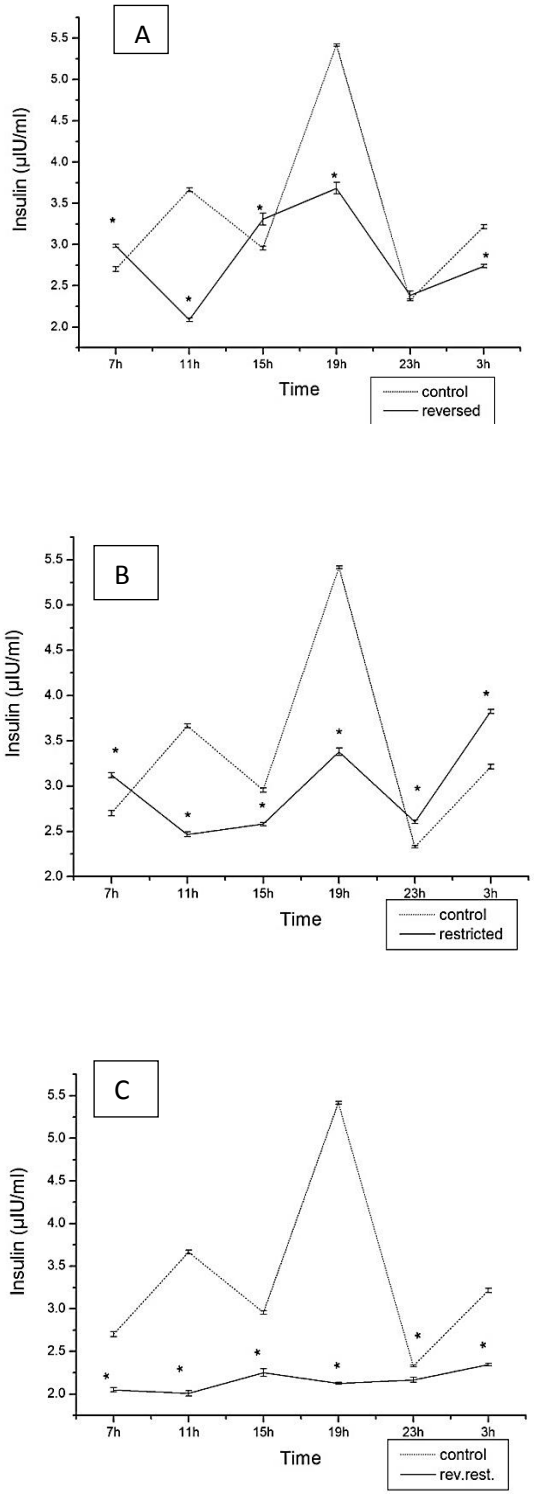

Figure 3: Daily profile of serum insulin levels in rats kept for one week under reversed light-dark cycle (A), restricted feeding regime (B), and reversed light-dark cycle as well as restricted feeding regime (C)

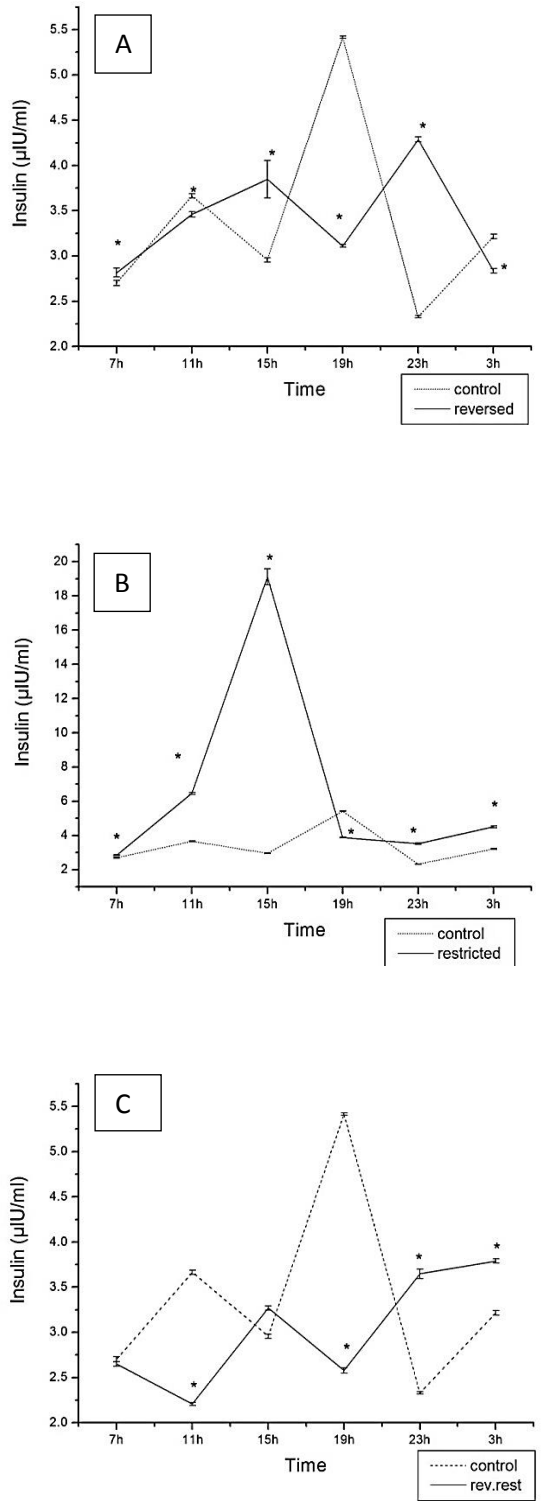

Figure 4: Daily profile of serum insulin levels in rats kept for two weeks under reversed light-dark cycle (A), restricted feeding regime (B), and reversed light-dark cycle as well as restricted feeding regime (C)

A sudden significant increment observed in the serum insulin levels in rats exposed to restricted feeding regime but normal light-dark cycle (group 3) at 1500h compared to its corresponding control. Insulin level

decreased only at 1900h compared to its corresponding control ($p<0.05\%$) . By comparing the different time intervals within group3, serum insulin levels showed gradual and significant increase throughout 0700h, 1100h and 1500h with the highest peak at 1500h.There were a sharp decrease thereafter showing the lowest value at 0700h ($p<0.05\%$) (Fig.4B).

The serum insulin levels in rats kept under reversed light-dark cycle and restricted feeding regime (group 4) for two weeks significantly increased ($P < 0.05$) at 1500h, 2300h and 0300h, compared to its corresponding control (group 1).The serum insulin levels within group (4) showed the maximum values at 2300h and 0300h; while the minimum values observed at 0700h, 1100h and 1900h compared to different time intervals of the same group ($p<0.05\%$) (Fig.4C).

4. Discussion

Extensive studies indicated that circadian rhythms of several biological and behavioral processes can be entrained to multiple oscillators [29, 30]. Light-dark cycle and feeding time have been proposed to be important synchronizers of physiological rhythms [31, 32].

Daily variations in plasma glucose levels have been widely reported [23]. The present study revealed significant variations in the serum glucose levels in rats. In control group, the peak of serum glucose was observed around mid-night. These observations agreed with [33] who demonstrated that there were significant variations in the levels of plasma glucose during both 24-h cycles with a significant progressive increase during the scotophase and a decrease during the photophase.

After one week of keeping rats under reversed light-dark cycle, serum glucose levels peaked twice. The first and second peaks were recorded at the onset and the offset of the dark phase of the reversed light-dark cycle respectively.

A phase advance in the time of maximum value of glucose rhythm was observed after one week of exposing rats to reversed light-dark cycle which may be due to a transient down-regulation of period 1 (Per1) and period 2 (Per2) expression in the SCN [34]. These findings means that glucose rhythm entrained by light-dark cycle which in agreement with [33] who postulated that the plasma glucose levels seem to be under photo periodic control entrained by the light- dark cycle. While after two weeks of the present study, the serum glucose levels peaked only at the dark phase of the reversed light-dark cycle. It was clear that serum glucose levels after two weeks were higher than that of the control group. This may be due to the increase of cortisol levels which was observed in the previous study [35]. Increased HPA activation by stress has been implicated in the impact of stress on the pathogenesis of the metabolic syndrome [36] and elevated cortisol levels has been shown to play a role in mediating the association between depressive symptoms and elevated blood glucose levels [37]. It is known that cortisol intervenes in energy release via libration of glucose and inhibition of insulin [38].

In the present result, serum insulin levels of rats exposed to reversed light-dark cycle for one week exhibit nearly the same pattern of the circadian rhythm of the control group, both of them peaked at 1900h. It is clear that insulin secretion levels wasn`t affected by the reversal of the light-dark cycle. This finding seems to be in

agreement with the results of [33] who found that the daily patterns of insulin in sea bass seem to be mainly influenced by feeding time; however, an effect of photoperiod cannot be excluded.

In contrast to glucose, the peak of the serum insulin levels was observed during the light phase of the reversed light-dark cycle. The rhythm was delayed by 4 hours compared to the control group after two weeks of the present study. Serum insulin rhythm may be responded lately to the reversal of light dark cycle and its concentration is lower than that of control group. The effect of nocturnal life in groups of students was studied by [39] and they found that the association between glucose and insulin was damaged in nocturnal life style group. It was suggested that insulin secretion tended to be relatively inadequate for the response to glucose at most of time intervals. This failure of the compensatory rise in plasma insulin in response to the raised glucose was reported to be partially due to a diminished sensitivity of the pancreatic B cells to glucose [40] and also to an increase in the rate of insulin clearance from the plasma at night [41]. The impairment of the insulin response to plasma glucose has a trend to increase the risk of diabetes [39]. In the past few years, melatonin receptors have been identified in pancreatic islets, suggesting a possible direct role of melatonin in the regulation of insulin secretion so, there is a potential link between melatonin receptors with hyperglycemia and impaired insulin secretion [42].

Apart from light, circadian rhythms can also be reset by temporally restricted access to food. Restriction of food availability to only the resting part of the day is a potent entraining cue for the peripheral clocks, while the SCN clock remains dominantly synchronized with the LD cycle [14, 16, 17, 43, 44]. However, under specific conditions, when the signal is of significant relevance and affects the motivational state such as palatable food reward [45], malnutrition, or caloric restriction, the SCN-driven behavioral rhythms can be entrained by food availability [46, 47, 48, 49].

Glucose levels after one and two weeks of the present study remained higher at night in restricted feeding group. It was suggested that daily rhythm with 24 hours period of glucose plasma levels seem to be independent of feeding time in restricted feeding regime [33]. Moreover, the glucose levels after one and two weeks of restricted feeding were higher than that of the control group at all-time intervals. This result is in agreement with [50] who studied the restricted feeding regime on pigs and found that restricted feeding pigs had a lower lipolytic rate and enhanced generation of glucose from non-carbohydrate carbon substrates (gluconeogenesis) compared with free feeding pigs. The present finding suggested that glucose rhythm affected by the restricted feeding only after two weeks where there was 4 hours advance in the peak at night. Insulin is secreted postprandially to stimulate the uptake of feed-derived glucose and amino acids from blood into tissues. It is hypothesized that the plasma insulin concentration is not only in proportion to the consumed meal, but also in proportion to counteract the catabolic pressure of cortisol on metabolism [51].

In the present study insulin showed no association with the glucose levels secretion after one as well as two weeks of keeping rats under restricted feeding regime. After one week, the high level of insulin was observed at night not after meal. On the other hand, after two weeks, the high level of insulin was recorded after 4 hours from the meal. According to [50], insulin and glucose concentrations were greater after 1.5 h from feeding compared with concentrations after 18 h of fasting.

This study revealed that reversed light-dark cycle and restricted feeding regime together resulted in new environmental condition which affect the circadian rhythm of glucose and insulin. There was an advance in the time of maximum levels of glucose by 8 and 12 hours after one and two weeks respectively after exposure of rats to reversed light-dark cycle and restricted feeding regime. This result is similar to that observed in the reversed light-dark cycle group and indicated that glucose entrained mainly by the light-dark cycle but not affected by the food restriction. Also, there was an increase in insulin secretion after 4 hours from feeding, its peak was delayed compared to the control group. This result similar to that obtained in restricted feeding and also in reversed light-dark cycle groups after two weeks of the experiment. This result is in agreement with [52] who postulated that, the effect of the light cue in the food-induced re-entrainment of peripheral clocks should not be overlooked, as the combination of the RF shift with the LD shift can markedly together facilitate an adjustment of the peripheral clock system to new environmental conditions.

5. Conclusion

From the present results it could be concluded that restricted feeding regime and reversed light-dark cycle are able to shift the phase of the rhythm of glucose and insulin either after one or two weeks, which may cause the impairment of the insulin response to plasma glucose. This impairment could increase the risk of diabetes and obesity. Further works should be done to study the effect of the nocturnal lifestyle associated with circadian disorders on increasing the risk of metabolic syndromes, and diabetes.

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Conflict of interest

None to declare.

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