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PHASE SHIFT IN BMAL1 MRNA CIRCADIAN RHYTHM INCREASES CHOLESTEROL AND TRIGLYCERIDES IN MALE RATS MAINTAINED UNDER ALTERED LIGHT-DARK CYCLE

Rehab E. El-Hennamy* and Mona Abdel-Rahman

Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt.

***Author for Correspondence: Dr. Rehab E. El-Hennamy**
Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt.

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ABSTRACT
Advances in technology and modern life style cause a production of 24h society, that accompanied by increased exposure to light at night. This phenomenon may have harmful effects on human health that result from disruption of the body circadian rhythm. The present study aimed to examine the effect of altered light-dark cycle on the circadian rhythm of Bmal 1 gene expression in liver as well as the changes in serum cholesterol, and triglycerides concentrations and their daily profiles. Rats were divided into two groups, a control group kept under normal 12-12 light –dark cycle and a group exposed to 12 hours phase delay of the light-dark cycle followed by altered light-dark cycle for one week. The daily profiles of cholesterol, triglycerides and Bmal1 mRNA after one week of the study, were shifted in the altered light-dark cycle group as compared with control. Cholesterol and triglycerides levels were increased significantly in the altered light-dark cycle group than control. It is concluded from the present study that the shift in the clock gene Bmal1 expression in liver cause alteration in the daily profile of cholesterol and triglycerides and increase in their concentration which may be due to its action on the metabolic genes.

KEYWORDS: circadian rhythm, light- dark cycle, cholesterol, triglycerides, Bmal1 gene.

INTRODUCTION
Circadian rhythm (Latin, circa: "approximate"; dies: "day") is a cyclical biological processes that repeats every 24h approximately.[1] Circadian rhythms are generated by the master clock in the suprachiasmatic nucleus (SCN) of the hypothalamus that synchronize peripheral oscillators in almost all cell types and tissues.[2] Daily light/dark cycles are the main entraining agent, or Zeitgeber (time giver) that synchronize the SCN clock to the external environment. Lights pass from the eyes to the SCN through the retinohypothalamic tract.[3] Non-photic cues such as food, social interaction, or exercise can also entrain the SCN clock.[4] The SCN then processes the information given by these Zeitgebers and, through complex neurological pathways, affects hormonal, behavioral and biochemical outputs that synchronize peripheral tissues to central timing.[5]

The circadian clock consists of a group of genes that are transcribed rhythmically, and which are interconnected in transcriptional, translational, and post-translational loops.[6] Protein products of the core clock genes Clock (circadian locomotor output cycles kaput) and Bmal1 (brain and muscle ARNT-like 1), that represent the positive limb, heterodimerize, then translocate to the nucleus, and bind to E-box promoter sequences of the clock genes Per1 and 2 (Period) and Cry1 and 2 (Cryptochrome) to initiate their transcription. The translated PER and CRY proteins, the negative limb, translocate to the nucleus and interact with CLOCK/BMAL1 to inhibit their own transcription. The PER/CRY complex then degraded via phosphorylation by casein kinase, causing the release of CLOCK/BMAL1 from suppression; this feedback loop takes approximately 24 h to complete. The nuclear receptors retinoid-related orphan receptor α (RORα) and REV-ERBα, an additional regulatory loop, compete for the ROR response element (RORE) binding site in the Bmal1 promoter to activate or inhibit its transcription, respectively.[7]

Various metabolites involved in carbohydrate, amino acid, lipid, and nucleotide metabolic pathways, oscillate in liver and controlled by circadian clock.[8] Fatty acid oxidation, fatty acid and cholesterol synthesis and bile acid biosynthesis are also under circadian control in the liver.[9, 10] This circadian control make the body able to form lipids, emulsify fats, or transport and oxidize lipids at the right time according to the eating cycle.[11]

Shift work and long-term exposure to light at night cause a circadian disruption that are linked with cardiovascular disorders[12], metabolic syndrome and obesity.[13] It has been also accompanied with increased risk of breast cancer.[14] The present study aimed to investigate the influence of altered light-dark cycle on the circadian rhythm of the clock gene Bmal 1 expression in liver, as well as the changes in serum cholesterol, and triglycerides concentrations and their daily profiles. An animal model of shift work was designed by exposing rats to 12 hours of phase delay followed by altered light-dark cycle for one week.

MATERIAL AND METHODS

Animals

Adult male rats (*Rattus norvegicus*) weighing (100-120 g), were purchased from the breeding unit of the Animal House of National Research Center (Giza, Egypt) and maintained for one week under the normal environmental conditions of temperature and humidity for accommodation on the environment. They were kept under the normal light-dark cycle with 12 h of light and 12 h of darkness (LD12:12) per day with free access of food and water. The ethics of animal use and care was followed in this study.

Experimental design

36 Male rats were divided into 2 groups. The first group of rats were maintained under normal light-dark cycle (LD 12:12) with lights on from 0700h till 1900h (control group). The second group of rats were exposed to 12h delay of one light period, so that the light was switched on at 1900h instead of being switched on 0700h. After the 12 h delay, rats were maintained under altered light-dark cycle with lights on from 1900h in the evening till 0700h in the morning for one week. The food was introduced to all rats *ad libitum.*

Blood and tissue sampling

After one week at a four-hour- interval throughout 24 h cycle (at 0700h, 1100h, 1500h, 1900h, 2300h and 0300h), rats from each group were anaesthetized with diethyl ether then dissected. Blood was collected, then centrifuged for 10 min at 5000 rpm. Serum was then stored at -70 ˚C until analysis. The liver was rapidly removed and quickly frozen at -70 ˚C till used.

Cholesterol and triglycerides determination

Cholesterol[15] and triglycerides[16] were estimated by the enzymatic colorimetric method according to the manufacturer instruction and purchased from Biodiagnostic, Cairo, Egypt.

RNA isolation and RT-PCR.

RNA isolation and RT-PCR (Real-time reverse transcription followed by polymerase chain reaction) were performed in Sigma company according to the method of Sládek et al.[17] Total RNA was isolated from 20–50 mg of homogenized liver tissue. 1 µg of RNA was reverse transcribed to cDNA. The cDNA reaction was diluted then amplified in a 20 µl PCR reaction containing commercial SYBR Green and Hot Start Taq polymerase mix (QuantiTect SYBR Green PCR kit) with specific primers for Bmal1 or housekeeping gene (β-2 microglobulin). Primer sequences used were as follows: Bmal1 (GenBank accession no. AB012600) forward: 5´-CAATGCGATGTCCCGGAAGTTAGA-3´; reverse.

5´-TCCCTCGGTCACATCCCTGAGAAT-3. B-2-m (GenBank accession no. NM_012512) forward: 5´-CGCTCGGTGACCGTGATCTTTCTG- 3´; reverse: 5´-CTGAGGTGGGTGGAACTGAGACACG- 3´. Real-time PCR reactions were performed on a Light Cycler system (PR0241500615, version PCR 2.3.2, RT 1.1.12.23) with the following thermo profile: initial denaturation at 95°C for 15 min, 55 cycles with 15-s denaturation at 94°C, 20-s annealing at 55–62°C (primer-specific temperature), and 10-s elongation at 72°C. At the end of each run, melting curve analysis was performed to ascertain the presence of a single amplicon. The expression of Bmal1 clock gene was normalized to expression of β-2-microglobulin.

Statistical analysis.

The data obtained in the present study were represented as mean ± S.E and were analyzed using Graph pad prism version 6. Data of different time points in the same group were analyzed by one-way ANOVA and Tukey's multiple comparison test. Significant differences between the 2 groups were determined by 2-way ANOVA followed by Sidak´s multiple comparison test. Changes in body weight among the 2 groups were evaluated by unpaired t- test. Results with $P < 0.05$ were considered significant.

RESULTS

Body weight of rats exposed to 12 hours phase delay followed by altered light-dark cycle for one week increased significantly ($P < 0.05$) as compared with control group (Fig. 1). Liver weight of the control group rose significantly at 7h,15h, 23h,3h as compared with 19h ($P < 0.05$). Liver of rats maintained under altered light-dark cycle has minimum weight at 11h, then began to increase significantly at 15h until reach maximum value at 23h. Two –way ANOVA for comparison of the 2 groups reveal significant effect of group ($F = 103.3$, $P < 0.0001$), a significant effect of time ($F = 21.08$ $P < 0.0001$) and a significant interaction effect ($F = 12.24$, $P < 0.0001$). The liver weight increased significantly in the altered light-dark cycle group at 19h, 23h, 3h compared with control group (Fig.2).

Cholesterol levels in rats exposed to normal light-dark cycle (control group) showed significant differences during different time points as revealed by one way ANOVA. Cholesterol levels were increased significantly ($P < 0.05$) at 03h as compared with all-time points as revealed by Tukey's multiple comparison test. After 12 hours of phase delay and exposing rats to altered light-dark cycle for one week, the cholesterol levels increased

significantly ($P < 0.05$) at 11h compared with 07h and 19h. To compare the differences between the two groups, two way ANOVA showed a highly significant effect of group ($F= 12.04$, $P < 0.0001$), significant effect of time ($F = 3.601$, $P = 0.0143$) and significant interaction effect ($F = 4.805$, $P = 0.0035$). Sidak's multiple comparisons test revealed a significant increase in the cholesterol levels in the altered light-dark cycle group at 11h, 15h ($P < 0.05$) as compared with the control group (Fig. 3).

Similarly, triglycerides levels of the control group increased significantly at 3 h compared with 7h, 11h, 15h ($P < 0.05$). In the altered light-dark cycle group, the triglyceride level increased at 19h as compared with 15h, and 3h. Two way ANOVA revealed a highly significant effect of group ($F= 18.9$, $P = 0.0002$), significant effect of time ($F = 3.755$, $P = 0.0119$) and interaction effect ($F= 4.355$, $P = 0.0058$). A significant increase ($P < 0.05$) in triglycerides levels were observed in rats exposed to altered light-dark cycle at 19 h, as compared with control group (Fig.4).

Bmal1 gene expression in rats exposed to normal light-dark cycle were increased significantly ($P < 0.05$) at 7h compared with 15h, 19h, 23h, 3h and at 11h as compared with 19h, 23h, 3h. The minimum expression was at 19h. After one week of altered light-dark cycle, the Bmal1 gene expression began to increase significantly at 15h, the maximum increase was at 23h and 3h as compared with 7h ($P < 0.05$). For group comparison, two way ANOVA showed a highly significant interaction effect ($F=29.75$, $P < 0.0001$). Bmal1 gene expression decreased significantly at 7h, 11h and increased significantly ($P < 0.05$) at 19h, 23h, 3h in the altered light-dark cycle group as compared with control group (Fig.5).

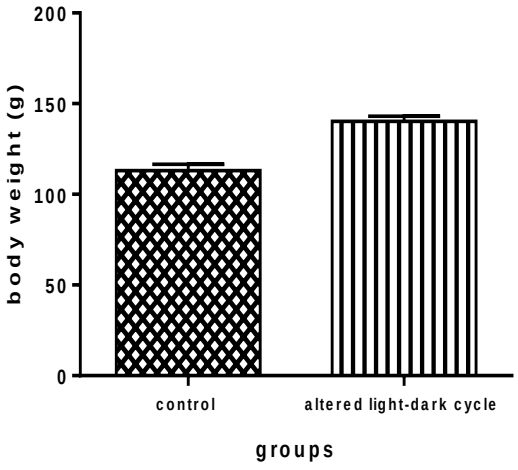

Fig.1: Body weight of rats kept under altered light-dark cycle for one week.

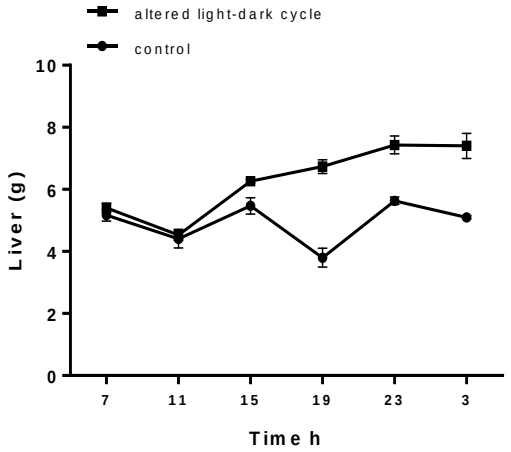

Fig. 2: Daily profile of liver weight of rats kept under altered light-dark cycle for one week.

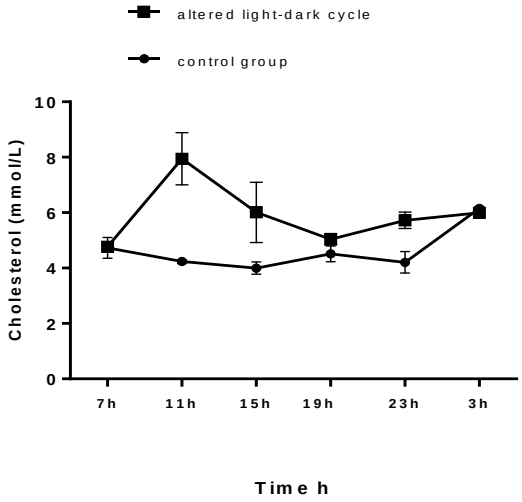

Fig. 3: Daily profile of serum cholesterol level of rats kept under altered light-dark cycle for one week

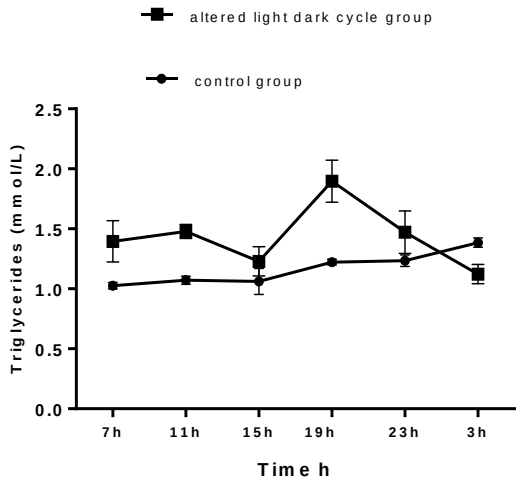

Fig. 4: Daily profile of serum triglyceride level of rats kept under altered light-dark cycle for one week

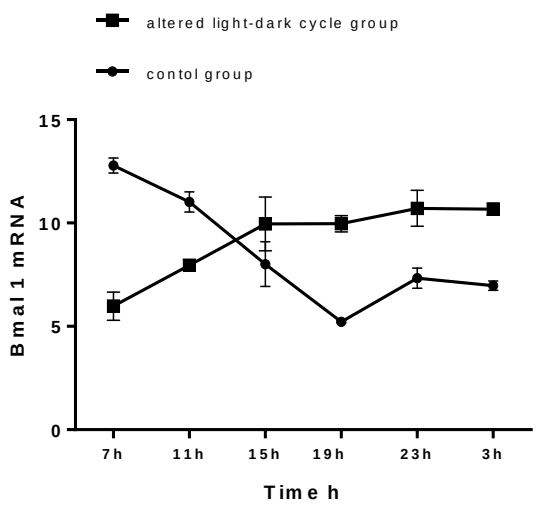

Fig. 5: Daily profile of Bmal 1 gene expression of rats kept under altered light-dark cycle for one week. The gene expression levels of (Bmal1), were estimated by RT-PCR. The expression of clock gene was normalized to expression of β-2-microglobulin. Each point represents the mean of 3 animals +/- SE.

DISCUSSION

The circadian clock has a major role in the regulation of plasma and tissue lipids, including triglycerides, cholesterol and free fatty acids.[18] Circadian disruption, sleep deprivation, and shift work are associated with hyperphagia, hyperinsulinemia, weight gain, and hypertriglyceridemia.[19] Several studies attributed metabolic disorders and weight gain caused by circadian disruption to changes in hormonal pathways and conflicting physiological signals that regulate metabolism.[5] Sleep restriction in healthy humans results in altered circulating levels of leptin[20], and it is thought that loss of neurohormonal control of appetite and energy balance could participate in the weight gain (though only partially due to overeating) associated with circadian disruption and shift work.[21] Leptin expression in fat cells regulated by melatonin[22] and cortisol[23] in the presence of insulin. These hormones are shifted due to shift work[24] and reversed light-dark cycle.[25, 26]

In the present study, cholesterol and triglycerides from control group rose normally at the dark phase of the light-dark cycle. After exposing rats to 12h phase delay followed by altered light-dark cycle, cholesterol and triglycerides levels were increased accompanied with body weight gain. On the other hand, cholesterol circadian rhythm was delayed by 8 hours and rose during day time that corresponds to the dark phase of the altered light-dark cycle. Likewise, triglyceride daily profile of the altered light-dark cycle was shifted toward the beginning of the illumination period. In the same way, Stanhope et al.[27] revealed an increase in lipogenesis after meals in both humans and nocturnal rodents. In nocturnal rodents, lipogenesis rises during dark periods (active phases) and declines during light periods (resting phases). In another study, wild type mice subjected to constant bright or dim light had increased body weight, decreased glucose tolerance, and ate more food during the daytime compared with those maintained under a normal light/dark cycle.[28] In addition, liver weight in this study was increased during the dark phase that correspond to the day of the altered light-dark cycle as compared with control. The fluctuation of liver weight may be due to changes in triglycerides and cholesterol levels at different time points as indicated by Yoshida et al.[29] who found rhythmic changes in the liver weight of mice subjected to different feeding regimes that correlated with increase in hepatic triglycerides and cholesterol.

Taken together these studies and the present result, it is obvious that constant light or altered light dark cycle cause a shift in meal time and consequently shift the lipogenesis rhythm and increase body weight.

The circadian clock has been reported to control metabolism and energy homeostasis in peripheral tissues[30] by mediating the expression and/or activity of certain metabolic enzymes and transport systems.[31] Furthermore, a large number of nuclear receptors implicated in glucose and lipid metabolism[32] as well as hormones involved in metabolic processes, has been found to exhibit circadian rhythmicity.[33]

Most genes regulating processes such as mitochondrial oxidative phosphorylation, carbohydrate metabolism and transport, cholesterol synthesis and degradation, and lipid biosynthesis, can oscillate in expression depending on the environment.[34, 35] CLOCK:BMAL1, the positive limb of the molecular clock, has several metabolic target genes which include peroxisome proliferator-activated receptor alpha (Ppara), nicotinamide phosphoribosyltransferase (Nampt), Dec1, Dec2, estrogen-related receptor alpha (Erra) and proper homeobox 1 (Prox1). BMAL1 is an upstream regulator of Ppara gene expression, producing a regulatory feedback loop for peripheral clocks. Another gene, Nampt[36, 37] is the rate-limiting enzyme that converts Nicotinamide (NAM) to Nicotinamide Mononucleotide (NMN), which is a key reaction required for the intracellular save of Nicotinamide Adenine Dinucleotide (NADþ) a key molecule in metabolism.[38]

Bmal1 gene expression in liver of control group in this study reached to the maximum value at the beginning of the light phase then declined until reached its minimum value at the beginning of the dark phase. A phase shift in the circadian rhythm of Bmal1 gene expression was observed in the altered light-dark cycle group. The increase in Bmal l mRNA was shifted toward the dark phase that corresponds to the illumination period of the altered light-dark cycle (2300h, 0300h). The phase shift in the circadian rhythm of Bmal1 mRNA caused by altered light-dark cycle was accompanied with weight gain, a shift in the daily profile of cholesterol and

triglyceride as well as an increase in their levels. Likewise, Barclay et al.[39] exposed mice to sleep restriction for two weeks, that simulating shift work and found a suppression of core clock mRNA rhythms that followed by metabolic disruption. Molecular studies have proven the association of BMAL1 activity in the control of adipogenesis and lipid metabolism in mature adipocytes. Loss of BMAL1 expression cause a significant decrease in gene expression of several key adipogenic/lipogenic factors and adipogenesis. In addition, over-expression of BMAL1 in adipocytes cause an increase in lipid synthesis.[40] Correspondingly, studies on Bmal1−/− mice show impaired glucose tolerance, gluconeogenesis, altered triglyceride rhythms and increased body fat.[41, 42] The impairment of the adipose clock and Bmal1 depletion, led to alteration in circadian energy intake and obesity in mice.[43]

Phase shift in liver Bmal1 gene expression, cholesterol and triglycerides associated with the increase of cholesterol and triglycerides levels that caused by altered light-dark cycle in the present study may be due to desynchronization between the molecular clock in SCN and liver resulted from altered environmental conditions. As indicated by Roberto et al.[44] who stated that, feeding and forced activity during the rest phase desynchronizes the rhythm between the SCN and the liver and disturbs internal molecular rhythms within the liver, particularlly the relationship between the clock genes and the metabolic genes.

CONCLUSION

From the present study, it could be concluded that altered light –dark cycles as night shift work cause an increase in serum triglycerides and cholesterol levels and a shift in their circadian rhythms as a result of a phase shift in the circadian rhythm of the clock gene, Bmal1, expression. Bmal1 clock gene exerts its effect through down regulating metabolic genes.

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Conflict of interest

None to declare.

REFERENCES

1. Aschoff J. Circadian rhythms in man. Science, 1965; 148: 1427–32.
2. Karatsoreos IN, Bhagat S, Bloss EB, Morrison JH, McEwen BS. Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. Proc Natl Acad Sci U S A, 2011; 108: 1657–1662.
3. Moore RY, Card JP. Visual pathways and the entrainment of circadian rhythms. Ann NY Acad Sci, 1985; 453: 123–33.
4. Mrosovsky N. Locomotor activity and non-photic influences on circadian clocks. Biol Rev, 1996; 71: 343–72.
5. Ferrell J.M, Chiang J.Y. Circadian rhythms in liver metabolism and disease. Acta Pharmaceutica Sinica, B 2015; 5(2): 113–122.
6. Froy O. Metabolism and circadian rhythms—implications for obesity. Endocr Rev, 2010; 31: 1–24.
7. Guillaumond F, Dardente H, Giguère V, Cermakian N. Differential control of Bmal1 circadian transcription by REV-ERB and ROR nuclear receptors. J Biol Rhythms, 2005; 20: 391–403.
8. Eckel-Mahan, K.L., Patel, V.R., Mohney, R.P., Vignola, K.S., Baldi, P., Sassone- Corsi, P., 2012. Coordination of the transcriptome and metabolome by the circadian clock, 2012; 109(14): 5541-6.
9. Gilardi, F., Migliavacca, E., Naldi, A., Baruchet, M., Canella, D., Le Martelot, G., et al. Genome-wide analysis of SREBP1 activity around the clock reveals its combined dependency on nutrient and circadian signals. PLoS Genetics, 2014; 10(3): e1004155.
10. Gooley, J.J., Chua, E.C.-P. Diurnal regulation of lipid metabolism and applications of circadian lipidomics. Journal of Genetics and Genomics ¼ Yi Chuan Xue Bao, 2014; 41(5): 231e250.
11. Salgado-Delgado, R., Angeles-Castellanos, M., Saderi, N., Buijs, R.M., Escobar, C. Food intake during the normal activity phase prevents obesity and circadian desynchrony in a rat model of night work. Endocrinology, 2010; 151(3): 1019e1029.
12. Puttonen S, Härmä M, Hublin C. Shift work and cardiovascular disease pathways from circadian stress to morbidity. Scand J Work Environ Health, 2010; 36: 96–108.
13. Shi SQ, Ansari TS, McGuinness OP, Wasserman DH, Johnson CH. Circadian disruption leads to insulin resistance and obesity. Curr Biol, 2013; 23: 372–381
14. Grundy A, Richardson H, Burstyn I, Lohrisch C, SenGupta SK, Lai AS, et al. Increased risk of breast cancer associated with long-term shift work in Canada. Occup Environ Med, 2013; 70: 831–8.
15. Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res, 1970; 11: 583.
16. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem, 1982; 28(10): 2077-80.
17. Sládek M., Jindráková Z., Bendová Z., and Sumová A.´Postnatal ontogenesis of the circadian clock within the rat liver. Am J Physiol Regul Integr Comp Physiol, 2007; 292: R1224–R1229.
18. Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshu C, Kobayashi Y, et al. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. Cell Metab, 2007; 6: 414–21.

19. Gale JE, Cox HI, Qian J, Block GD, Colwell CS, Matveyenko AV. Disruption of circadian rhythms accelerates development of diabetes through pancreatic beta-cell loss and dysfunction. J Biol Rhythms, 2011; 26: 423–33.
20. Omisade A, Buxton OM, Rusak B. Impact of acute sleep restriction on cortisol and leptin levels in young women. Physiol Behav, 2010; 99: 651–6.
21. Crispim CA, Waterhouse J, Dâmaso AR, Zimberg IZ, Padilha HG, Oyama LM, et al. Hormonal appetite control is altered by shift work: a preliminary study. Metabolism, 2011; 60: 1726–35.
22. Alonso-Vale MIC, Andreotti S, Peres SB, Anhê GF, das Neves Borges-Silva C, Neto JC, et al. Melatonin enhances leptin expression by rat adipocytes in the presence of insulin. Am J Physiol Endocrinol Metab, 2005; 288: E805–12.
23. Wabitsch M, Bo Jensen P, Blum WF, Christoffersen CT, Englaro P, Heinze E, et al. Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes, 1996; 45: 1435–8.
24. Mirick DK, Bhatti P, Chen C, Nordt F, Stanczyk FZ, Davis S. Night shift work and levels of 6-sulfatoxymelatonin and cortisol in men. Cancer Epidemiol Biomarkers Prev, 2013; 22(6): 1079-87.
25. Abdel-Rahman M, Abdel-Kader S, El-Masry H, and El-Hennamy R. The effect of reversed light-dark cycle and restricted feeding regime on the circadian rhythm of cortisol and serotonin in male rats. Isotope Rad. Res, 2013; 45: 679-699.
26. Abdel-Kader S, Abdel-Rahman M, El-Masry H, Kassab R, and El-Hennamy R. Reversed light-dark cycle and restricted feeding regime affect the circadian rhythm of insulin and glucose in male rats. IJSBAR, 2015; 19: 1-16.
27. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, The circadian clock. Proceedings of the National Academy of Sciences of the United States of America, 2009; 109(14): 5541e5546.
28. Fonken LK, Workman JL, Walton JC, Weil ZM, Morris JS, Haim A, et al. Light at night increases body mass by shifting the time of food intake. Proc Natl Acad Sci USA, 2010; 107: 18664–9.
29. Yoshida C, Shikata N, Seki S, Koyama N and Noguchi y. Early nocturnal meal skipping alters the peripheral clock and increases lipogenesis in mice. Nutrition & Metabolism, 2012; 9: 78.
30. Green CB, Takahashi JS, Bass J. The meter of metabolism. Cell, 2008; 134: 728-742.
31. Kohsaka A, Bass J. A sense of time: how molecular clocks organize metabolism. Trends Endocrinol Metab, 2007; 18: 4-11.
32. Yang X, Downes M, Yu RT, Bookout AL, He W, Straume M, Mangelsdorf DJ, Evans RM. Nuclear receptor expression links the circadian clock to metabolism. Cell, 2006; 126: 801-810.
33. Bodosi B, Gardi J, Hajdu I, Szentirmai E, Obal F, Jr., Krueger JM. Rhythms of ghrelin, leptin, and sleep in rats: effects of the normal diurnal cycle, restricted feeding, and sleep deprivation. Am J Physiol Regul Integr Comp Physiol, 2004; 287: R1071-R1079.
34. Patel, V.R., Ceglia, N., Zeller, M., Eckel-Mahan, K., Sassone-Corsi, P., Baldi, P.The pervasiveness and plasticity of circadian oscillations: the coupled, circadian-oscillators framework. Bioinformatics (Oxford, England), 2015; 31(19): 3181e3188.
35. Ribas-Latre A, Eckel-Mahan K. Interdependence of nutrient metabolism and the circadian clock system: Importance for metabolic health MOLECULAR METABOLISM, 2016; 133e152.
36. Asher, G., Gatfield, D., Stratmann, M., Reinke, H., Dibner, C., Kreppel, F., et al.,. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell, 2008; 134(2): 317e328.
37. Nakahata, Y., Kaluzova, M., Grimaldi, B., Sahar, S., Hirayama, J., Chen, D., et al. The NADþ-dependent deacetylase SIRT1 modulates CLOCK mediated chromatin remodeling and circadian control. Cell, 2008; 134(2): 329e 340.
38. Magni, G., Amici, A., Emanuelli, M., Raffaelli, N., Ruggieri, S. Enzymology of NADþ synthesis. Advances in Enzymology and Related Areas of Molecular Biology, 1999; 73: 135e182 xi.
39. Barclay JL, Husse J, Bode B, Naujokat N, Meyer-Kovac J, Schmid SM, et al. Circadian desynchrony promotes metabolic disruption in a mouse model of shiftwork. PLoS One, 2012; 7: e37150.
40. Shimba S, Ishii N, Ohta Y, Ohno T, Watabe Y, Hayashi M, Wada T, Aoyagi T, Tezuka M: Brain and muscle Arnt-like protein-1 (BMAL1), a component of the molecular clock, regulates adipogenesis. Proc Natl Acad Sci U S A, 2005; 102: 12071–12076.
41. Rudic R, McNamara P, Curtis A, Boston R, Panda S, et al. BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. PLoS Biol, 2004; 2: e377.
42. Lamia KA, Storch KF, Weitz CJ: Physiological significance of a peripheral tissue circadian clock. Proc Natl Acad Sci U S A, 2008; 105: 15172–15177.
43. Paschos, G.K., Ibrahim, S., Song, W.-L., Kunieda, T., Grant, G., Reyes, T.M., et al. Obesity in mice with adipocyte-specific deletion of clock component Arntl. Nature Medicine, 2012; 18(12): 1768e1777.
44. Roberto C. Salgado-Delgado, Nadia Saderi, Marı´a del Carmen Basualdo, Natali N. Guerrero-Vargas1, Carolina Escobar, Ruud M. Buijs. Shift Work or Food Intake during the Rest Phase Promotes Metabolic Disruption and Desynchrony of Liver Genes in Male Rats. PLOS ONE, 2013; 8: e60052.