Türk Tarım ve Doğa Bilimleri Dergisi 10(1): 68–75, 2023

https://doi.org/10.30910/turkjans.1196793

TÜRK
TARIM ve DOĞA BİLİMLERİ
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TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

www.dergipark.gov.tr/turkjans

Araştırma Makalesi

Adult Male Syrian Hamsters (*Mesocricetus auratus*) Exhibit Daily Oscillations in Their Serum Levels of Melatonin and Leptin As Well As in the Expression of the GnRH, GnIH, and Kisspeptin Genes[&]

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Geliş Tarihi: 31.10.2022 Düzeltme Geliş Tarihi: 28.12.2022 Kabul Tarihi: 10.01.2023

ABSTRACT

The complex neuronal and hormonal interaction between the brain and gonads controls the neuroendocrine reproductive axis. GnRH, GnIH, and kisspeptin are important neuropeptides in this relationship. Although seasonal variations of these neuropeptides have been demonstrated in photoperiodic animals, there is no clear evidence in their daily rhythms. Melatonin and leptin hormones are also two important hormones in reproductive regulation. In our study, the relationship of melatonin and leptin hormones with daily rhythm with GnIH, GnRH, and kisspeptin gene expressions and protein oscillations was examined. Adult male Syrian hamsters were exposed to the long photoperiod and at the end of the 30-day experimental period, blood and tissue samples from each group were collected at 04:00 h, 12:00 h, 20:00 h, and 00:00 h. Daily rhythms of melatonin and leptin hormones were determined by ELISA. Quantitative analysis of GnRH, GnIH, Kisspeptin, and β-actin genes was performed with the corresponding primers in Real-Time PCR. Protein expressions were determined by the Western Blot technique. Serum melatonin and leptin levels showed an inverse rhythmic relationship. Leptin level was found to be low while melatonin was high in the dark. Daily rhythms were observed in GnIH, GnRH, and kisspeptin mRNA expressions and protein oscillations. As a result, our findings could imply that all of the relationships between melatonin, leptin, GnIH, GnRH, and kisspeptin are not reproductive, but rather metabolic in natüre.

Key words: Gene expression, GnIH, GnRH, Kisspeptin, Leptin, Melatonin

Yetişkin Erkek Suriye Hamsterleri (*Mesocricetus auratus*), Serum Melatonin ve Leptin Seviyelerinde ve Ayrıca GnRH, GnIH ve Kisspeptin Genlerinin Ekspresyonunda Günlük Ritmin İncelenmesi

ÖZ

Nöroendokrin ve üreme sistemi beyin ve gonadlar arasındaki kompleks nöronal ve hormonal etkileşim ile kontrol edilmektedir. GnRH, GnIH ve Kisspeptin bu ilişkide önemli nöropeptidlerdir. Bu nöropeptitlerin mevsimsel olarak değişimleri fotoperiyodik hayvanlarda gösterilmiş olsa da, günlük ritimlerinde net bir bilgi bulunmamaktadır. Ayrıca, melatonin ve leptin hormonları da üremenin düzenlenmesinde önemli iki hormondur. Çalışmamızda günlük melatonin ve leptin ritimlerinin GnIH, GnRH ve kisspeptin gen ekspresyonları ve protein salınımları ile ilişkisi incelendi. Yetişkin erkek Suriye hamsterleri uzun fotoperiyotta tutuldular. 30 günlük deney süresinin sonunda her gruptan günün 04:00, 12:00, 20:00 ve 00:00 saatlerinde kan ve doku örnekleri alındı. Melatonin ve leptin hormonlarının günlük ritimleri ELISA ile belirlendi. Real Time PCR yöntemiyle GnRH, GnIH, kisspeptin ve β-aktin genlerine karşılık gelen primerler kullanılarak bu genlerin kantitatif analizi yapıldı. Protein ifadeleri Western Blot tekniği ile belirlendi. Serum melatonin ve leptin seviyeleri ters yönlü ritmik ilişki gösterdi. Karanlıkta leptin düzeyi düşük, melatonin düzeyi yüksek bulundu. GnIH, GnRH ve Kisspeptin mRNA ifadelerinde

ve protein salınımlarında günlük ritimler gözlendi. Sonuç olarak bulgularımız, melatonin, leptin, GnIH, GnRH ve kisspeptin arasındaki ilişkilerin üreme üzerinde olmayabileceği ancak metabolik olabileceğini düşündürmektedir.

Anahtar Kelimeler: Gen ekspresyonu, GnIH, GnRH, Kisspeptin, Leptin, Melatonin

INTRODUCTION

Photoperiod is the most important environmental signal regulating reproductive physiology in animals. The complex neuronal and hormonal interaction between the brain and gonads controls the neuroendocrine reproductive axis. The final common factor in the regulation of the reproductive system is the release of GnRH (Gonadotrophin releasing hormone) from neurons terminating in the median eminence (Bliss et al., 2010). The release of the GnRH peptide stimulates the gonads by causing the synthesis and secretion of pituitary gonadotropins LH (Luteinizing Hormone) and FSH (Follicle Stimulating Hormone) (Silverman, 1988; Guh et al., 2019). Many species exhibit seasonality in breeding as a way to prevent offspring from being born during the most challenging time of the year. Light is the environmental cue of this annual reproductive cycle, and it is controlled, sensed, and translated into a physiological signal via the pineal gland's nocturnal secretion of melatonin. Other cells in the brain that control the secretion of GnRH and/or gonadotropins detect the rhythmic secretion of the hormone melatonin (Johnston et al., 2003). The leptin hormone, which is produced mainly from adipose tissue, regulates food intake, controls body weight, and serves as a metabolic entry point to the reproductive system (Wade et al., 1996; Gündüz, 2014). The hormone leptin has been linked to reproduction in a variety of species. Obese ob/ob mice (endogenous leptin deficiency) are sterile, and leptin administration has been shown to restore fertility (Chehab et al., 1996). Photoperiodic changes, such as exposure to short photoperiods, have been shown to reduce leptin gene expression and hormone secretion in adipose tissues (Klingenspor et al., 1996, 2000). Leptin hormone, like melatonin, exhibits a rhythmic feature, and photoperiod influences its release (Gündüz, 2002). Anterior pituitary brain gonadotropin release that is triggered by GnRH is inhibited by the hypothalamic neuropeptide known as gonadotropin-inhibitory hormone (GnIH; The mammalian name is RFRP). The reproductive function of GnIH was first described in the quail brain (Tsutsui et al., 2000), and its similar functions were subsequently uncovered in many different species (reptiles, rodents, humans) (Kawano et al., 2006; Kriegsfeld et al., 2006; Ubuka etal., 2009). The Kiss1 gene produces the protein kisspeptin, whose secretion regulates the pulsatile release of GnRH and LH and is essential for regulating the timing of puberty and reproduction in both sexes (Navarro et al., 2009). Kisspeptin is the missing link between melatonin and the hypothalamic-pituitary-gonadal (HPG) axis. It has been established so far that melatonin affects a system different from the GnRH neurons. Understanding that photoperiod regulates KiSS-1 expression via melatonin and that kisspeptin transmits photoperiodic information to the hypothalamus-pituitary-gonad axis elevated the subject to a new level. The decreased kisspeptin signal in hamsters during the short photoperiod suggests that it may cause decreased reproductive activity in these animals.

There are no research that link melatonin and leptin rhythms with GnRH, GnIH, and kisspeptin daily rhythms since studies on these neurohormones are often independent of one another or studies involving two neurohormones. In Syrian hamsters with strong photoperiodic characteristics, a rhythmic relationship between melatonin and leptin has been demonstrated, but the relationship of this rhythmic cycle with GnRH, GnIH, and kisspeptin, which are associated with the reproductive system, has not been demonstrated. Daily GnRH, GnIH, and kisspeptin gene expressions and protein synthesis in adult male Syrian hamsters adapted to the long photoperiod were investigated in this study, and these rhythmic changes were associated with melatonin and leptin hormones.

MATERIALS AND METHODS

Animals

Adult male Syrian hamsters (*Mesocricetus auratus*) (3-4 months old) were obtained from the colony in the Hamster and Gerbil unit of Canakkale Onsekiz Mart University. Adult hamsters were exposed to 16L:8D (16 hours light, 8 hours dark; lights off from 20:00 to 04:00) photoperiod. The animals were housed in plastic cages. Lighting was provided by cool white fluorescent tubes controlled by automatic programmable timers. The temperature of the ventilated rooms was kept constant as 22 ± 2 ^{0}C. All animals had constant access to food and water throughout the experiment. The Experimental Animals Ethics Committee at Canakkale Onsekiz Mart University approved the procedures employed in this investigation with decision number 2019/ 06-09.

Experimental Procedures

Hamsters (n=24) were divided into 4 groups with similar body weights (100-110 g). At the end of the 30-day experiment period, blood and tissue samples from each group were collected at 04:00 (n=6), 12:00 (n=6), 20:00 (n=6) and 00:00 h (n=6) of the day.

Sample Preparations

At the end of the 30-day experiment period, the animals were decapitated at 04:00, 12:00, 20:00 and 00:00 h of the day and blood was collected for the analysis of melatonin and leptin hormones. Blood samples were collected in the dark phase (20:00 and 00:00 h) using a dim red light.

The brain was quickly removed, and the hypothalamus region was dissected under a dissecting microscope. Tissues were placed in Eppendorf tubes at and stored -86 °C until RNA isolation and western blotting.

Hormone Measurements

After the blood samples were taken, their serum was separated by centrifugation at 4000 rpm for 30 minutes. Aliquots of serum were aspirated and frozen at -20°C. Commercial ELISA kits (BioAssay-Technology Laboratory, Rat Melatonin, E0601 Ra; BioAssay-Technology Laboratory, Rat Leptin E0561 Ra) were used to measure hormones according to the manufacturer's instructions.

Real-Time qPCR

Total RNA was isolated from the frozen hypothalamus with PureLink RNA Mini Kit (Applied Biosystems™, Cat No: 2183018A) and cDNA synthesis was performed for all the samples with cDNA Synthesis Kit (A.B.T.™, Cat No: C03-01-05) according to the recommendation. 10 µl of Maxima SYBR Green/ROX qPCR Master Mix (2X) (Thermo Scientific™, Cat No: K0221), 2 µl of cDNA, 2 µl of primers, and 6 µl of nuclease-free water was prepared as PCR reaction mixture. In table 1, primer sequences for all the genes are listed. Quantitative analysis of GnRH, GnIH, Kisspeptin, and β-actin genes was performed with Real-Time PCR System (StepOne™, Cat no: 4376357). After 5 minutes of initial denaturation, 40 cycles took place as follows: 15 seconds at 95°C and 30 seconds at 60°C. β-actin was used as the endogenous gene and all the data were analyzed with the 2^{dCt} method.

Table 1. Real-Time PCR primers and their properties.

Gene	Forward Primer	Reverse Primer	Product
β-actin	ACAACCTTCTTGCAGCTCCTC	CTGACCCATACCCACCATCAC	186 bp
GnIH	ATGAGAAAAGAAGCCCCGCA	CATGACGTAGAGCAACTCGC	173 bp
GnRH	CCGGCATTCTACTGCTGACT	CCTCCTTGCCCATCTCTTGG	129 bp
Kiss-1	CTCTGTGTCGCCACCTATGG	AGGCTTGCTCTCTGCATACC	126 bp

Western-Blot

Protein purification from the frozen hypothalamus was performed with RIPA Lysis and Extraction Buffer (Thermo Scientific™, Cat No: K0221) according to the guidelines recommended by the manufacturer. After measuring protein concentrations, 20 µg of protein for each sample was loaded and separated on 10% SDS-PAGE gel electrophoresis. Protein samples were transferred onto a nitrocellulose membrane (iBlot™ 2 Transfer Stacks, Cat. No: IB23001) with a Dry Blotting System (iBlot™ 2 Cat. No: IB21001) before 3 hours of blocking with Flex Solution Kit (iBind™, Cat. No: SLF2020) according to their recommendations. All antibody assays were performed on the Flex Western device (iBind™, Cat. No: SLF2000) within 2.5 hours with followed primary polyclonal antibodies: β-Actin (sc-130656, Santa Cruz Biotechnology), Kisspeptin (bs-0749R-TR, Bioss Antibodies), GnRHR (bs-1464R-TR, Bioss Antibodies), NPFV (NBP1-86724, Novus Biologicals). Enhanced chemiluminescence (Thermo Scientific™, Cat. No: 32106) substrate was used for visualization of protein bands and ImageJ software (National Institute of Health, Washington, USA) was used to measure their relative density.

Statistics

Data were analyzed using SPSS 22 statistical software program. Data are given as mean ± standard error. Mann Whitney U analysis was used to compare the two time periods. Charts created in Sigma Plot 14.5

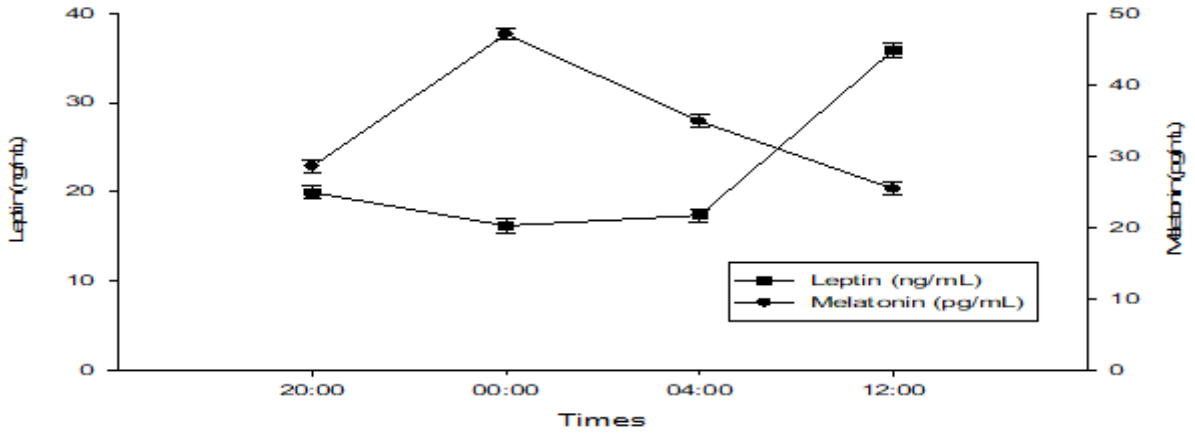

Figure 1. Leptin (ng/mL) and melatonin (pg/mL) values in male Syrian hamsters. The black bar indicates the period of darkness. Same letters indicate statistical similarity (mean+SEM, $p<0.05$ in melatonin values. Same * and letter indicate statistical similarity (mean + SEM, $p<0.05$) in leptin values.

RESULTS

Figure 1 displays the serum levels of leptin and melatonin. Melatonin began to rise after the lights went off at 20:00 h, but leptin started to fall ($p<0.05$). Melatonin peaked around midnight (00:00 h), whereas leptin hormone reached at its lowest level ($p<0.05$). Leptin, on the other hand, reached its highest level around 12:00 h, when melatonin was at its lowest.

Figure 2 displays the GnIH, GnRH, and kisspeptin mRNA expressions and protein concentrations. Four separate times (20:00, 00:00, 04:00 and 12:00 h) throughout the day were used to collect tissues. GnIH mRNA expression levels decreased during the dark period, reaching their lowest point at 04:00 h, just as the dark phase was about to terminate (Figure-2A). GnIH relative protein density peaked at 00:00 h ($p<0.01$), in the middle of the dark phase, and declined at 12:00 h, in the middle of the light phase (Figure-2B). At 00:00 h, the mRNA expressions of GnRH and Kiss-1 are at their lowest levels (Figure-2C, E) and highest levels ($p<0.05$) at 20:00 h just after lights out (Fig-2C, E). Their relative protein density, on the other hand, is highest at 20:00 and 00:00 h ($p<0.01$) (Figure-2D, F).

DISCUSSION

We measured the blood's serum levels of leptin and melatonin. They were inversely correlated, meaning that leptin levels were lower when melatonin levels were higher in the serum. A possible explanation for these results is that melatonin is produced and secreted by the pineal gland into blood circulation at night through circadian influences of SCN. Most of the studies revealed that the daily rhythm of leptin is under the influence of the suprachiasmatic nucleus (Kalsbeek et al., 2001, Karakas and Gündüz 2006). Moreover, the rhythmic profile of melatonin synchronizes metabolic and hormonal functions such as leptin secretion. Our previous results showed that the pineal gland has an inhibitory role in daily leptin rhythm (Gündüz, 2002). Given that rhythmic leptin secretion was blunted without circulating melatonin hormone demonstrates that melatonin has time-giver properties of rhythmic leptin secretion. Therefore, the inhibitory function of the pineal gland and the impact of SCN may be the cause of the inverse link between melatonin and leptin. Although leptin profiles do not show the same rhythmic release, melatonin profiles are similar in all vertebrates. Leptin levels are higher in the dark and lower in the light as a result of this rhythm in both rats and humans, in contrast to the hamster example given above (Ahima et al., 1998). In other species, this rhythmic relationship is not strong (Drazen et al., 2000). Leptin is known to control metabolism, whereas melatonin informs the organism about the time of day (Reiter, 1993). If leptin rhythmically affects food intake, it's critical to carefully examine the feeding and sleeping habits of the relevant organisms. Humans are diurnal, thus in this instance, they engage in their eating activities during the light period. As long as it remains in the circulation, the hormone leptin suppresses hunger. In this instance, leptin will be more strongly correlated with melatonin in the dark phase than in the day phase, which means that people will eat less when it is dark. Interestingly, hamsters experience the reverse. Although leptin levels are low during the dark phase (Gündüz, 2002), these animals are active at night and sleep during the day. In other words, their food consumption is higher in the dark phase when they are active. It is crucial to look at the actions of leptin receptors in order to understand what this rhythmic difference in leptin between animal groups.

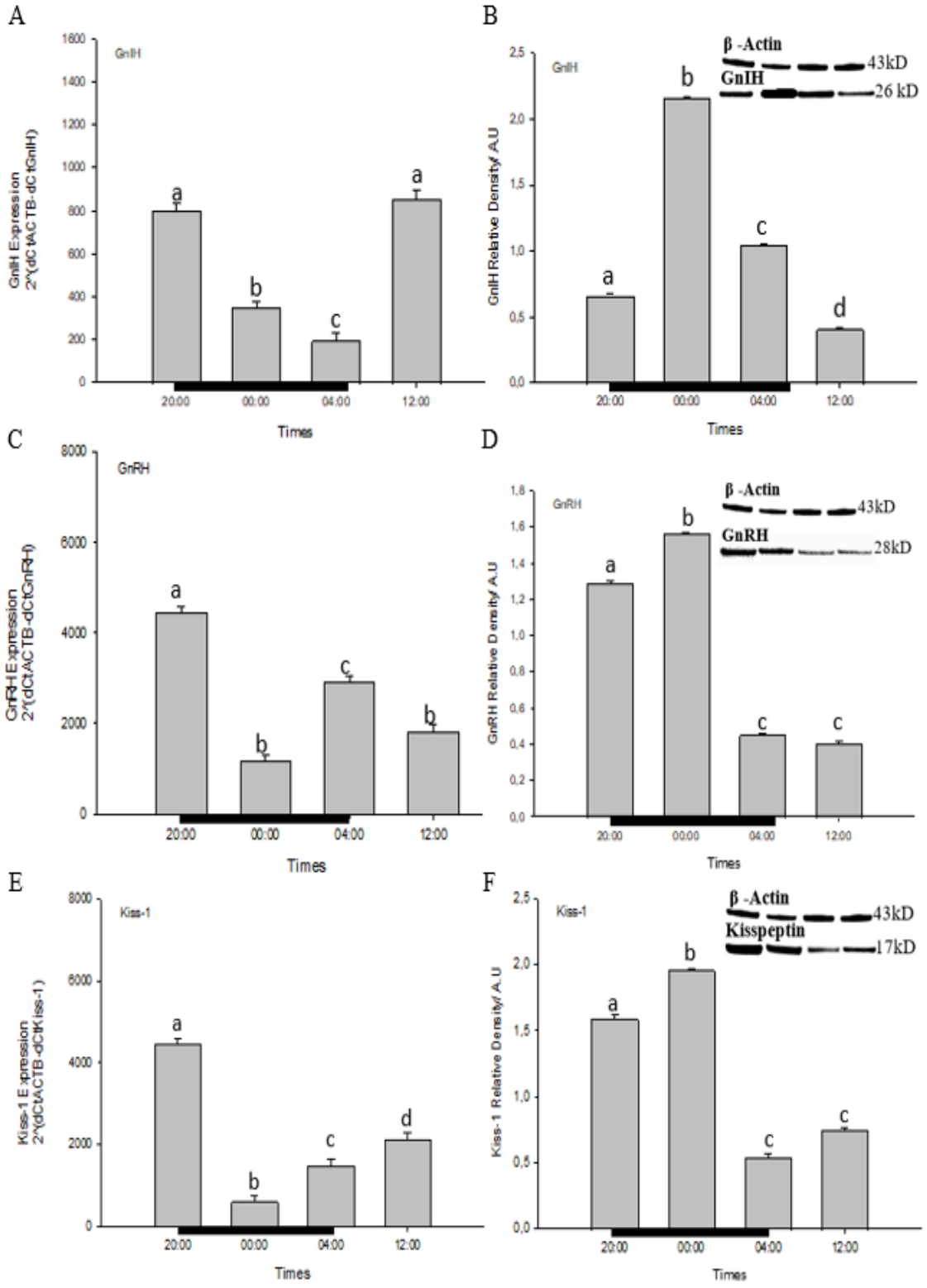

Figure 2. Quantification of mRNA expression and relative protein density changes at different times of day (20:00, 00:00, 04:00, and 12:00). Samples were extracted from the hypothalamus of Syrian hamsters (n=24). (A, C, E) mRNA expression of GnIH, GnRH, and Kiss-1. Different letters show significance levels. Different letters mean differences between bars.

It is widely known that nocturnal melatonin's annual/photoperiodic cycle is essential for synchronizing reproduction with the seasons (Hoffman and Reiter, 1965; Bartness et al., 1993). Actually, it is still unknown

which cellular sites melatonin acts on to regulate reproductive activity throughout the year. Numerous brain areas have been found to contain melatonin binding sites, however there are significant species variances (Masson-Pevet et al., 1994). According to studies, the medial basal part of the hypothalamus actively contributes to the effects of melatonin on reproduction (Maywood and Hastings, 1995). The major regulators of reproductive activity Kisspeptin and GnIH added new dimensions to the way melatonin affects the reproductive system. GnRH, kisspeptin, and GnIH show predictable changes in photoperiod or melatonin-induced gonadal quiescence across species, indicating that these neuropeptides play important roles in seasonal reproduction. The daily rhythmic variation of these peptides on melatonin and leptin has not been studied, despite investigations on the effects of photoperiod on these neuropeptides in other seasonally breeding animals.

We observed that GnIH (RFRP) mRNA levels in Syrian hamsters were found to be light-dependent, with lower expression during the dark phase. Information on the biological functions of GnRH peptides in mammals is still scarce. Both peptides might have feeding-modulating properties. The human RFRP gene is orthologous to the bird GnIH gene, which is associated with the GnIH peptide's inhibitory activity on gonadotropin secretion (Tsutsui et al., 2000; 2007). In our study, when we examine the levels of GnRH mRNA, we notice that they rise, particularly during light transitions (when the lights are turned on and off), whereas they fall at other times during both the light and dark phases. However, peptide releases are higher than other times, especially in the dark phase and at the time the lights are turned off. In mammals, GnIH may indirectly influence gonadotropin secretion through GnRH neurons. Few GnIH-immunoreactive fibers have been found in the outer layer of the median eminence in several rodent studies, including the Syrian hamster, compared to the number of GnRH cells that these fibers link (Johnson et al., 2007). On the other hand, it is unknown if GnIH influences LH secretion at the pituitary level and/or through GnRH release. One can speculate that it happens either directly through GnIH and GnRH neuronal connections or indirectly through links to kisspeptin neurons in the arcuate nucleus.

Our conclusion may seem surprising for a number of reasons, however we found that GnIH expression is decreased in dark phase at the mRNA level but not at the peptide level. First, if GnIH's primary function was to block the gonadotropic axis, we would anticipate that its expression would rise during the dark phase, increasing the negative urge for reproduction while simultaneously decreasing the positive drive represented by kisspeptin. One hypothesis is that GnIH regulates the activity of the hypothalamo-pituitary-gonadal axis in hamsters. This could happen directly on GnRH neurons or via kisspeptin neurons or other intermediaries. Given the great efficacy of kisspeptin as a secretagogue of GnRH release, this option would be particularly relevant. However, when we consider GnIH's role in both birds and hamsters, the results can be interpreted differently. While quails and Syrian hamsters are long-day both breeders, photoperiod has different effects on reproduction in the two species of animals. The decrease in GnIH expression during the dark phase also raises several issues about GnIH's function as a gonadotropin-inhibitory factor. The biological effects of GnIH peptides might go well beyond just regulating reproductive activity. Additionally, it is important to properly differentiate the regulation of GnIH mRNA level from the timing of its release as GnIH. Further research is necessary, however it implies that although melatonin may decrease GnIH gene expression, there may be a difference in how mRNA and peptides are regulated. Photoperiod causes changes in GnIH expression in studies on seasonally breeding animals. However, there is no publication that studies the daily rhythmic changes of GnIH and GnRH, as we did. However, there are variations between species. In contrast to hamsters, day length had no effect on GnIH expression in Wistar rats, which do not exhibit photoperiodic variation in testicular function. These findings imply that photoperiod exclusively modifies GnIH expression in seasonal species.

Finally, factors other than melatonin may influence GnRH expression. Leptin may be one of these factors. However, we did not observe any long-term effects of melatonin and leptin rhythmicity on GnIH, GnRH, or kisspeptin expression in our experiments. It is obvious that additional research should be done to see if and how additional factors may affect such expressions. Studies on animals that breed seasonally have shown that photoperiod alters the expression of GnIH over the long term. There is, however, no publication that examines the daily rhythmic fluctuations in GnIH and GnRH, just as there was in our work.

The finding that leptin induces kisspeptin gene expression supports the hypothesis that kisspeptin neurons may modulate energy balance (Smith et al., 2006; Hill et al., 2008). Kisspeptin gene expression is increased by leptin. However, it is unknown where melatonin acts to control kisspeptin expression because kisspeptin neurons lack melatonin receptors (Li et al., 2011). In our investigation, we found that during the dark phase, when leptin hormone is low, kisspeptin mRNA expression is low but peptide release is high. While this feature is present in Syrian hamster species, the linear relationship between kisspeptin and leptin is more visible in rat and mouse species. As a result, we cannot claim that our findings will affect similar mechanisms in all animals. For example, our findings could imply that all of the relationships between melatonin, leptin, GnIH,

GnRH, and kisspeptin are not reproductive, but rather metabolic in nature. Furthermore, daily regulation of kisspeptin expression, for example, appears to be complex and species-dependent.

As a result, the determination of the daily rhythms of GnIH, GnRH, and kisspeptin, as well as their association with melatonin and leptin hormones, is a first in its field, and further research is required to determine whether all of these rhythmic connections are strictly regulated by melatonin in the absence of the pineal gland.

ACKNOWLEDGEMENT

This study was funded by the Scientific Research Projects Coordination Unit at Çanakkale Onsekiz Mart University (FDK-2021-3831).

[&] This study was produced from doctorate thesis conducted by Emine İnci BALKAN

Conflict of Interest Statement: The authors declare no conflict of interest

Contribution Rate Statement Summary: The authors declare that they have contributed equally to the article

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REFERENCES

Ahima, R.S., Prabakaran, D. and Flier, J.S. 1998. Postnatal leptin range and regulation of circadian rhythm of leptin by feeding implications for energy homeostasis and neuroendocrine function. *Journal of Clinical Investigation*, 101 (5): 1020-1027.

Bartness, T.J., Powers, J.B., Hastings, M.H., Bittman, E.L., and Goldman, B.D. 1993. The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodiccontrol of seasonal responses? *Journal of Pineal Research*, 15: 161-190.

Bliss, S.P., Navratil, A.M., Xie, J. and Roberson, M.S. 2010. GnRH signaling, the gonadotrope and endocrine control of fertility. *Neuroendocrinology*, 31(3): 322-340.

Chehab, F.F., Lim, M. and Ronghua, L. 1996. Correction of sterility defect in omozygous obese female mice by treatment with the human recombinant leptin. *Nature Genetics*, 12 (3): 318–320.

Drazen, D.L., Kriegsfeld, L.J., Schneider, J.E. and Nelson, R.J. 2000. Leptin, but not immune function, is linked to reproductive responsiveness to photoperiod. *American Journal of Physiology*, 278 (6): 1401-1407.

Guh, Y. J., Tamai, T. K. and Yoshimura, T. 2019. The underlying mechanisms of vertebrate seasonal reproduction. *Proceedings of the Japan Academy, Series B*, 95 (7): 343-357.

Gündüz, B. 2002. Daily rhythm in serum melatonin and leptin levels in the Syrian hamster (*Mesocricetus auratus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 132 (2): 393-401.

Gündüz, B. 2014. Serum leptin profiles, food intake, and body weight in melatonin-implanted Syrian hamsters (*Mesocricetus auratus*) exposed to long and short photoperiods. *Turkish Journal of Biology*, 38 (2): 185-192.

Hill, J.W, Elmquist, J.K. and Elias, C.F. 2008. Hypothalamic pathways linking energy balance and reproduction. *American Journal Physiology Endocrinology Metabolism*, 294 (5): 827-832

Hoffman, R. A. and Reiter, R. J. 1965. Pineal gland: influence on gonads of male hamsters. *Science*, 148 (3677): 1609-1611.

Johnson, M.A., Tsutsui, K. and Fraley, G.S. 2007. Rat RFamide-related peptide-3 stimulates GH secretion, inhibits LH secretion, and has variable effects on sex behavior in the adult male rat. *Hormones* & *Behaviour* 51 (1): 171–180.

Johnston, J.D., Messager, S., Ebling, F.J.P., and Hazlerigg, D.G. 2003. Gonadotrophin-releasing hormone drives melatonin receptor down-regulation in the developing pituitary gland. *Proceedings of the National Academy of Sciences.* 100 (5): 2831-2835.

Kalsbeek, A., Fliers, E., Romijn, J. A., La Fleur, S. E., Wortel, J., Bakker, O. and Buijs, R. M. 2001. The suprachiasmatic nucleus generates the diurnal changes in plasma leptin levels. *Endocrinology*, 142 (6): 2677-2685.

Karakas, A. and Gündüz, B. 2006. Suprachiasmatic nuclei may regulate the rhythm of leptin hormone release in Syrian hamsters (*Mesocricetus auratus*). *Chronobiology international*, *23* (1-2): 225-236.

Kawano, E., Takahata, Y., Oishi, T., Ukena, K., Tsutsui, K. and Tamotsu, S. 2006. Neural interaction of gonadotropin-regulating hormone immunoreactive neurons and the suprachiasmatic nucleus with the paraventricular organ in the Japanese grass lizard (*Takydromus tachydromoides*). *Zoological Science*, 23 (3): 277-287.

Klingenspor, M., Dickopp, A., Heldmaier, G. and Klaus, S., 1996. Short photoperiod reduces leptin gene expression in white and brown adipose tissue of *Djungarian hamsters*. *FEBS Letters*, 399 (3): 290–294.

Klingenspor, M., Niggemann, H. and Heldmaier, G., 2000. Modulation of leptin sensitivity by short photoperiod acclimation in the *Djungarian hamster*, *Phodopus sungorus*. *Journal of Comparative Physiology B*, 170 (1): 37–43.

Kriegsfeld, L. J., Mei, D. F., Bentley, G. E., Ubuka, T., Mason, A. O., Inoue, K. and Silver, R. 2006. Identification and characterization of a gonadotropin-inhibitory system in the brains of mammals. *Proceedings of the National Academy of Sciences*, 103 (7): 2410-2415.

Li, Q., Rao, A., Pereira, A., Clarke, I. J. and Smith, J. T. 2011. Kisspeptin cells in the ovine arcuate nucleus express prolactin receptor but not melatonin receptor. *Journal of Neuroendocrinology*, 23 (10): 871-882.

Masson-Pévet, M., George, D., Kalsbeek, A., Saboureau, M., Lakhadar-Ghazal, N. and Pevet, P. 1994. An attempt to correlate brain areas containing melatonin-binding sites with rhythmic functions: a study in five hibernator species. *Cell and tissue research*, 278 (1): 97-106.

Maywood, E. S. and Hastings, M. H. 1995. Lesions of the iodomelatonin-binding sites of the mediobasal hypothalamus spare the lactotropic but block the gonadotropic response of male Syrian hamsters to short photoperiod and to melatonin. *Endocrinology*, 136(1): 144-153.

Navarro, V. M., Gottsch, M. L., Chavkin, C., Okamura, H., Clifton, D. K. and Steiner, R. A. 2009. Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. *Journal of Neuroscience,* 29(38): 11859-11866.

Reiter, R.J. 1993. The melatonin rhythm: both a clock and a calendar. *Experientia*, 49(8): 654–664.

Silverman, A. J. 1988. The gonadotropin-releasing hormone (GnRH) neuronal systems: immunocytochemistry and in situ hybridization in *The Physiology of Reproduction* (eds. Knobil, E and Neill, J.). Raven Press, New York, 1683-1709.

Smith, J. T., Clifton, D. K. and Steiner, R. A. 2006. Regulation of the neuroendocrine reproductive axis by kisspeptin-GPR54 signaling. *Reproduction*, *131* (4): 623-630.

Tsutsui, K., Bentley, G.E., Ubuka, T., Saigoh, E., Yin, H., Osugi, T., Inoue, K., Chowdhury, V.S., Ukena, K., Ciccone, N., Sharp, P.J. and Wingfield, J.C. 2007. The general and comparative biology of gonadotropin-inhibitory hormone (GnIH). *General and Comparative Endocrinology*, 153 (1-3): 365-370.

Tsutsui, K., Saigoh, E., Ukena, K., Teranishi, H., Fujisawa, Y., Kikuchi, M., Ishii, S. and Sharp, P.J. 2000. A novel avianhypothalamic peptide inhibiting gonadotropin release. *Biochemical and Biophysical Research Communications*, 275 (2): 661–667.

Ubuka, T., Morgan, K., Pawson, A. J., Osugi, T., Chowdhury, V. S., Minakata, H. and Bentley, G. E. 2009. Identification of human GnIH homologs, RFRP-1 and RFRP-3, and the cognate receptor, GPR147 in the human hypothalamic pituitary axis. *Plos One*, 4 (12): 8400.

Wade, G.N., Schneider, J.E. and Li H.Y. 1996. Control of fertility by metabolic cues. *American Journal of Physiology*, 270 (1): 1–19.