Impact of Maternal Chronodisruption on Female Offspring Puberty. A Role of Kisspeptin and Irisin

Ana Alonso (alonsoana@uniovi.es)
University of Oviedo
Beatriz Medina
University of Oviedo
Celestino González
University of Oviedo
Elena Díaz
University of Oviedo

Research Article

Keywords: Maternal chronodisruption, melatonin, offspring, Kisspeptin, irisin, puberty, vaginal opening

Posted Date: January 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-152366/v1

Abstract

The neuroendocrine mechanisms regulating the onset of puberty are unclear. Kisspeptin is a potent gatekeeper of pubertal onset, but it has also been proposed that the time of puberty is dictated by kisspeptin independent mechanism. In this way, the myokine irisin could be involved in the puberty onset process serving as a metabolic trigger. Puberty is influenced by environmental factors like photoperiod. Maternal photoperiod influences postnatal development; thus alterations in maternal melatonin rhythm may affect the offspring's reproductive axis development, especially at puberty.

There is no research regarding the influence of maternal photoperiod on kisspeptin and irisin profile in offspring close to puberty. We investigated the influence of maternal photoperiod on serum kisspeptin and irisin levels during sexual development in female rat offspring.

The study was carried out on female offspring Wistar rats at 25 and 30 days of age and in the vaginal opening day. Pregnant mother rats were included in three groups; control (12L/12 D), other exposed to continuous light during pregnancy (24L/0D), and the third exposed to continuous light as well as receiving daily melatonin during pregnancy. Serum kisspeptin and irisin were determined and vaginal opening day were registered.

The results demonstrated that both continuous light exposure and melatonin treatment during intrauterine life affects kisspeptin and irisin secretion in the offspring during sexual maturation; these point out to an important role of the cytokine irisin in the onset of puberty.

Highlights

- Exposure to light at night exposure during intrauterine life affects postnatal kisspeptin and irisin levels at puberty.
- Maternal melatonin treatment may restore the night light effect only for serum irisin levels.

Introduction

Puberty results from the awakening of a complex neuroendocrine machinery in whose the primary mechanism is still unclear. In the last years, there is an extensive research suggesting changes in pubertal time for girls and boys. In this sense, in European countries and in United States, it has been reported a 4 years advanced in female pubertal time between 1890 and 1960 [1]. The advance in puberty onset has been related to an improvement in social, economic and nutritional status.

Although, there is a great heterogeneity related to ethnic populations or geographical localization, more recent studies, are still supporting a persistent decrease in puberty onset [2]. Moreover, several studies show and increase in central precocious puberty that seems to be more relevant in girls than in boys [3,4,5]. The main reason for this changes remains unknown, it has been proposed that exposure to several environmental factors, such as light-darkness cycle may play an important role [6].

Pubertal onset is a critical event in animal development including a reactivation of the hypothalamic hypophysis gonadal axis. It can be influenced by signals involving neurotransmitters and neuropeptides that originate in the hypothalamus in addition to peripheral or gonadal signals [6].

In this sense, strong evidences accumulated in the last years, showed that kisspeptin serves as a gatekeeper of pubertal onset [7]. The identification of kisspeptin system has been recognized as a major event in reproductive endocrinology, kisspeptin are potent gonadotropin secretagogue and inactivation of kisspeptin gene expression cause absence of puberty onset and hypogonadotropic hypogonadism in humans [8]. There are two populations of hypotalamic kisspeptin neurons, one localizated in the ARC and the other localized in the anteroventral periventricular nucleus and periventricular nucleus continuum in rodents (AVPV-PeN). The first one, neurons in the ARC have a role in GnRH pulse generation while neurons in AVPV-PeN are related to estrogen positive feedback to induce GnRH –LH surge in rodents [9]. In this sense, a recent work showed that photoperiod influences the sexual maturation of female mice via changes in the kisspeptin system. Short photoperiod (SP) determined a delay in time of puberty in female mice and a decrease in hypothalamic Kiss1 expression mainly depended on a reduced number of Kiss1-expressing neurons in the AVPV/PeN [10].

Related to peripheral signals, the classic idea that claimed the need to reach a certain threshold of body (fat) mass as a physiological requirement for puberty and fertility, becomes real in 1994 when the adipose hormone leptin was identified [11]. Initial pharmacological studies in female rodents suggested that leptin may act as a trigger of puberty. Later studies supported a permissive role for leptin to puberty onset. It is to say, there is needed a minimal amount of leptin to acquire and maintain reproductive function [12].

In this sense, is has been proposed that irisin, a recently discovered myo and adipokine could be also involved in puberty onset. It has been proposed the hypothesis that irisin could serve as a metabolic trigger for the onset of puberty [13]. There are several studies suggesting a role of plasma irisin levels during puberty [14,15]. Irisin is sex-specifically expressed in the primate hypothalamic–pituitary–gonadal axis and exert a stimulatory effect on GnRH expression and release in mouse hypothalamic cells [16].

In mammals, the uterine environment during foetal development exerts long-term influences on offspring phenotype [17]. Photoperiod duration is one of the most potent factors responsible for the synchronizing biological functions, including reproductive system [18].

Irisin is a hopeful molecule that could answer several reproductive questions that remains unknown, however more research is needed to understand its function. In this sense, to our knowledge, until date, there is no research about the influence of the maternal photoperiod on kisspeptin and irisin profile in offspring. Present study was designated to investigate the role of maternal photoperiod on kisspeptin and irisin plasma levels during sexual development in female Wistar rat.

Methods

Animals and treatments

All experimental procedures were performed according to the European Communities Council Directive of September 22, 2010 (2010/63/UE) and the Spanish legislation (R.D. 53/2013). The study has been approved by the Ethics Committee of the Principality of Asturias (PROAE 08/2016).

Experimental groups

Female Wistar rats (n = 24), weighting 200–250 g were obtained from from the Central Biotery of the University of Oviedo and maintained on rat chow and tap water ad libitum. They were kept under standard conditions of temperature (23°C ± 2°C), relative humidity (65% ± 5%), and on an artificial light/dark cycle of 12 hours (lights on at 8:00 AM. Mating pairs were kept in polypropylene cages, one male with two females. The presence of vaginal plugs of sperm was considered indicative of pregnancy and designated as gestational day 0. Pregnant rats were immediately isolated and kept one per cage.

Pregnancy was achieved in 18 rats, male rats and non-pregnant female rats were removed from the study. Pregnant rats were randomly divided into three groups: control (C) was exposed to a normal photoperiod (12L:12D), LL group was exposed to continuous light (24L:0D) and LL-Mel group was exposed to continuous light and treated with melatonin. Considering previous findings (Díaz et al 1999), 250mg Melatonin /100 g.B.W. were used in present study. Mel (Sigma Chemical, CO) was dissolved in a small volume of absolute ethanol and diluted in saline solution (NaCl 0,9%) to reach the dose. C and LL mothers received ethanol/saline alone. All the treatments were administered between 18:00 and 18:30 pm.

After delivery, mothers and their offspring were immediately returned to LD photoperiod (i.e., 12 h light/12 h dark), because present study is focused on the role of maternal chronodisruption in pubertal development. In order to obtain uniformity in the development of the pups, on the day of birth each litter was adjusted to 12 pups per dam by cross-fostering some pups from larger litters within treatment groups. Pups remained with the mother until weaning on day 21 (birth=day 0). We study female offspring according to Ojeda´s classification concerning postnatal maturation [54]. The vaginal opening (VO) is usually used as an external marker to determine the sexual development of female rats. We daily examined the female rat's vulva as described in other studies[55]. The day of VO was recorded as the day on which the vaginal orifice transitioned from tightly closed to patent [52].

The day of VO pups were decapitated and immediately blood was collected from the trunk for the evaluation of kisspeptin and irisin plasma levels. Samples were centrifuged for 10 minutes at 8.000 rpm and serum was stored at -80ºC for later determination of irisin and kisspeptin.

Serum determinations

The irisin concentration was measured with a highly specific commercially available ELISA kit (catalog no. EK-067-029: Phoenix Pharmaceuticals). Serum levels of kisspeptin were determined by using ELISA

kits (catalog no. MBS7255578 MyBiosource) according to the manufacturer instructions. Assessments were carried out in Servicios Científico Técnicos from Oviedo University. Sensitivity of the assays were 1.29 ng/ml (Irisin) and 1 pg/ml (Kisspeptin). Intra-assay variation:<10% for both; Inter-assay variation: <15% for irisin and < 10% for kisspeptin.

Statistical analysis

Data were evaluated using SPSS package (15.0) Results are mean±SEM. Kruskal–Wallis and Wilcoxon' s signed rank test were performed to compare the inter-group and intra-group differences, respectively. Differences were considered statistically significant at $p < 0.05$.

Results

Vaginal Opening. - The time of vaginal opening in rats is closely associated with the start of estrous cyclicity. Our study showed that maternal photoperiod promotes changes in offspring's VO (Figure 1). In this sense, rats from LL group showed a delay in VO compared to control and LL-Mel offspring (p 0.05).

Irisin levels. - Figure 1 shows irisin serum levels in our experimental groups. The intragroup comparisons showed a similar performance in Control, LL and LL-Mel animals, irisin levels increased significantly throughout the experiment, reaching the highest levels at AV. Intergroup comparisons does not reach statistical significance. At AV moment the offspring from LL mothers shower the lowest levels of irisin, although the differences are not significant.

Kisspeptin levels. - Figure 2 showed kisspeptin serum levels in our experimental groups. In rats from control mothers (C Group), kisspeptin was significantly higher at the end of experiment (AV time) than at 25 days. Rats from LL mothers showed the highest levels at AV time. Intragroup comparisons showed that at day 25 of postnatal development and at AV time, kisspeptin levels were highest in LL-Mel than in C and LL offspring. At day 30, these levels were lower un C than in LL-mel offspring.

Discussion

In present work, we investigated whether maternal chronodisruption is related to changes on kisspeptin and irisin plasma levels in female offspring and if there is relationship with the time of vaginal opening. Our findings support a role of gestational chronodisruption in kisspeptin levels at time of vaginal opening that it cannot be reversed by melatonin treatment. However, our experimental model showed that maternal exposure to continuous light during pregnancy, promotes a delay on vaginal opening that can be reversed by melatonin treatment.

It is clearly demonstrated that the absence of the maternal melatonin rhythm by chronodisruption affects the postnatal reproductive axis development both in males and females. In this way, previously we have demonstrated that maternal pinealectomy alters LH secretion mainly at juvenile period in female rats [19], also that maternal pineal gland participates in cellular and nuclear volumes of prepubertal oocytes

development [20], even that exogenous melatonin treatment to mothers during pregnancy altered the maturation of the gonadotropin and prolactin feedback system to estradiol in female offspring [21].

Alterations in several neuropeptides levels in hypothalamus, pituitary or striatum, through development, in rat offspring from pinealectomized or melatonin treated mother were also observed by our group [22,23].

In addition, other authors had been suggested a role for maternal photoperiod on offspring development. The absence of maternal melatonin rhythm during pregnancy had a marked effect on the newborn temperature rhythm, one of the main circadian rhythms, and melatonin treatment to mothers synchronized this newborn rhythm [24]. On the other hand, It has been proposed that maternal melatonin acts on the foetus hypothalamus-pituitary unit to sets the trajectory of reproductive and metabolic development in pups and has a persistent effect on their subsequent sensitivity to the photoperiod through thyroid metabolism [25].

Vaginal opening is an external marked of puberty onset. This event depends on the adequate functional status of the reproductive neuroendocrine system. In this sense, the discovery that the neuropeptide kisspeptin play a critical role in regulating the hypothalamus reproductive axis and shed new perspectives on the understanding of puberty. The essential role of kisspeptin in the onset of puberty can be attributed to the critical role of hypothalamic arcuate nucleus kisspeptin neurons to generate the pulsatile GnRH release required for pubertal activation of the reproductive axis [26].

Our results show increased plasma kisspeptin levels at puberty, in female offspring from control mothers, according to results of previous authors from female animals [27,28,29,30].

Despite the scientific evidence about the role of the neurohormone melatonin on Kisspeptin system, there is not studies about the possible role of the maternal melatonin on developmental offspring kisspeptin levels. In this way, we found that both the absence of melatonin caused by continuous light exposure and the excess, affects plasma kisspeptin levels at puberty. It is known that melatonin is the pivotal cronobiotic to the circadian system and light/dark cycle is the most important synchronizer of the system [31]. It seems clear that the cronodisruption to which the mothers were exposed during gestation exerts its influence on developmental female offspring reproductive axis. Melatonin can cross placental barrier [32], then during intrauterine life foetuses were exposed to maternal circadian signals. In addition, newborns were also exposed to maternal melatonin rhythm [33]. Alteration of the maternal melatonin levels has been associated with disruption of the brain programming and developmental long effects [34]. Based on our results, we deduce that maternal chronodisruption by constant light exposure exert an excitatory influence on kisspeptin hypothalamic neurons at puberty since significant high kisspeptin values were found in female offspring from continuous light exposed mothers, so affecting the onset of puberty in the offspring but it is striking that those animals showing increased kisspeptin values are correlated to delayed vaginal opening. In any case, one of the strength of our study is that shows in an animal model that continuous light exposure at night during fetal life affect the postnatal neuroendocrine-reproductive axis affecting the pivotal process of puberty. In today`s society pregnant mothers are exposed to bright light at night for various reasons: night work, shift work, or constant abuse of electronic devices at night,

as it was verified in several studies [35,36,37,38]. Then, we considered that the present results allow us to infer the danger of future mothers' exposure to night light during pregnancy for an event as capital as puberty for their offspring.

Further, exogenous melatonin treatment during pregnancy was not able to restore plasma kisspeptin values to those found in control group, even values are higher in female offspring from melatonin treated mother rats exposed to continuous light.

Most of the studies looking for a relation between melatonin and kisspeptin at puberty have been carried out in seasonal breeders in which it has been demonstrated that kisspeptin levels are influenced by photoperiod changes related to changes in melatonin secretion to reduce kisspeptin levels [39,40]. Some authors suggest that in those animals, melatonin may alter the negative feedback effects of sex steroids on kisspeptin expression. These data are not agreeing with our results but It should be noted that our study was carried out in a non-seasonal animal, also that exposure to the hormone melatonin in our model occurred not during postnatal life but during intrauterine life. However, we propose that the ineffectiveness of exogenous melatonin to act as a chronobiotic and repair the effects of chronodisruption caused by continuous exposure to light may be due that longer exposure to melatonin may cause that the neuroendocrine-reproductive axis escape to its influence. In this way, it was demonstrated that acute exogenous melatonin induces a reduction in kisspeptin gene expression, but longer effects lead to an increase in kiss gene expression [41].

Despite the clear influence of the maternal cronodisruption on kisspeptin levels at puberty, we did not find a direct relationship between these values and vaginal opening since as we described above, we found delayed vaginal opening in female offspring from mother exposed to continuous light with increased plasma kisspeptin values compared to control but the female offspring from melatonin treated mother rats exposed to continuous light even with the highest kisspeptin values show vaginal opening at the same time that control offspring. All this leads us to think that other factors in addition to kisspeptin are necessary for the beginning of puberty. In relation to this approach other authors have proposed that the time of puberty is dictated by kisspeptin independent mechanism controlling the ontogeny of GnRH pulse generation. These authors propose that the impact of loss of function by mutation of genes encoding kisspeptin or its receptor on the onset of puberty can be attributes to the critical role of the arcuate kisspeptin neurons in the generation of GnRH pulses, necessary for pubertal activation of the neuroendocrine reproductive axis but kisspeptin neurons do not determine the timing of puberty. Rather this event is achieved by upstream neuronal mechanism [26].

The onset of puberty is a very complex phenomenon in which a lot of different signalling systems both inhibitory or excitatory participates. Among them, metabolic signals as leptin, ghrelin or insulin have been already investigated [42,43,44].

In recent years it has been reported irisin changes in different stages of puberty [15,45]. Also, it has been hypothesized [13] that irisin serves as metabolic trigger for the onset of puberty based on the increase in

the expression of irisin gene (FNDCS) mRNA levels in mice during postnatal development and the systemic irisin levels increase close to puberty in humans as it was previously described by others [15,46].

We study the irisin levels throughout development during the juvenile period in female offspring rats from control mothers and we found a progressive increase from 25 to 30 days of age and to the day of vaginal opening being values at vaginal opening day 3-fold-times higher than those found at the beginning of the pre-pubertal period. Furthermore, we found a similar developmental pattern, to one found for control group, both in offspring of continuous light exposed mother rats and in offspring of continuous light exposed mother rats plus melatonin treatment during pregnancy, at 25 and 30 days of age. But different response was found at vaginal opening day since irisin concentration was lower in offspring from continuous light mother rats compared to controls, which allows us to suggest that the lack of maternal melatonin caused by chronodisruption during pregnancy affects to the secretion of isirin at the critical time of the pubertal onset. On the other hand, unlike what we found for kisspeptin, it seems that muscle or adipose tissue are not as sensitive to the refractory effect of melatonin. Since, exogenous melatonin received throughout pregnancy seems to be able to reverse the effects of continuous light on muscle and/or adipose tissue on irisin synthesis.

To our knowledge this is the first study to evaluate the effect of maternal chronodisruption on developmental irisin in the female offspring. Given the importance of this molecule for issues related to reproduction as the onset of puberty, as well as its importance on other fields such as health of the musculoskeletal system, metabolic diseases, inflammatory processes, bone formation and functioning the nervous system [46,47,48], we posit that this result contribute one more with a novel reason to consider exposure to light at night as a health risk factor especially during pregnancy in view of the consequences for the offspring.

In relation to melatonin influence on irisin levels, scientific evidence is very scarce although recently it was reported that pharmacological concentration of melatonin may modulate irisin signalling pathways on remote ischemic preconditioning after myocardial ischemia-reperfusion injury in rats [49, 50]. On the other hand, chronic continuous melatonin administration reduces weight gain and the serum total cholesterol levels and additionally, it enhances the circulating irisin [51]. But in any case there is no scientific evidence about the direct influence of melatonin on irisin at the onset of puberty and much less in relation to the influence of maternal melatonin on the onset of puberty. Our results allow us to affirm not only that the absence of melatonin during intrauterine life may alter the secretion of irisin at the onset of puberty but treatment with melatonin can restore levels to those observed in offspring from control mother rats.

Further, looking at our results, we infer that it is possible that irisin plays an important role in the timing of the onset of puberty along with kisspeptin because as we previously exposed the altered kisspeptin levels at the onset of puberty in the two experimental groups does not seem to explain the different behaviour in terms of timing of vaginal opening, but it is possible that the decrease in plasma levels of the irisin hormone is related to the delay in vaginal opening observed in the group of offspring of mothers exposed to continuous light, which is reinforced by the fact that the treatment of mothers with melatonin allows a

recovery in the plasma concentration of irisin in their offspring, while they do not show a delay in vaginal opening.

In conclusion, our results demonstrated that maternal chronodisruption alters kisspeptin and irisin plasmatic levels at the critical phase of the onset of puberty, however pharmacological melatonin influence it is not the same on the kisspeptin hypothalamic neurons as on muscle and/or adipose cells that synthesize irisine.

Declarations

Author contributions:

E.D. designed the study, B.D. performed the experiments and A.A., C.G. and E.D. analysed the data and wrote the manuscript and.

The authors declare no competing interests

Present study was carried out in compliance with the ARRIVE guidelines

References

1. Tanner, J. M. Growth at adolescence: with a general consideration of the effects of hereditary and environmental factors upon growth and maturation from birth to maturaty. 2nd ed. 1962. P. 325. Springfield, III.
2. Kuiri-Hänninen, T., Sankilampi, U. & Dunkel, L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. *Horm. Res. Paediatr.* **82**, 73-80 (2014).
3. Bianco, S. D. & Kaiser, U. B. The genetic and molecular basis of idiopatic hypogonadotropic hypogonadism. *Nat. Rev. Endocrinol.* **5**, 569-576 (2009).
4. Pescovitz, O. H. *et al.* True precocious puberty complicating congenital adrenal hyperplasia: treatment with a luteinizing hormone-releasing hormone analog. J. Clin. Endocrinol. Metab. **58**, 857-861 (1984).
5. Schwanzel-Fukuda, M. Origin and migration of luteinizing hormone-releasing hormone neurons in mammals. *Micros. Res. Tech.* **44**, 2-10 (1999).
6. Kaprio, J. *et al.* Common genetic influences on BMI and age at menarche. Hum. Biol. **67** , 739-753 (1995).
7. Pinilla, L., Aguilar, E., Dieguez, C., Milar, R. P. & Tena-Sempere, M. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. *Physiol. Rev.* **92**, 1235-1316 (2012).
8. Manfredi-Lozano, M., Roa, J. & Tena-Sempere, M. Connecting metabolism and gonadal function: Novel central neuropeptide pathways involved in the metabolic control of puberty and fertility. *Front. Neurobiol.* **48**, 37-49 (2018).

9. Amoss, M., *et al*. Purification, amino acid composition and N-termius of the hypothalamic luteinizing hormone releasing factor (LRF) of ovine origin. *Biochem. Biophys. Res. Commun.* **44**, 205-210 (1971).
10. Bohlen, T.M. *et al.* A Short-Day Photoperiod Delays the Timing of Puberty in Female Mice via Changes in the Kisspeptin System. *Front. Endocrinol.* **9**, 44 (2018).
11. Tena-Sempere, M. Interaction between energy homeostasis and reproduction: central effects of leptin and ghrelin on the reproductive axis. *Horm. Metab. Res.* **45**, 919-927 (2013).
12. Castellano, J.M. & Tena-Sempere, M. Metabolic control of female puberty: potential therapeutic targets*. Expert. Opin. Ther. Targets.* **20**, 1181-1193 (2016).
13. Wahab, F., Shahab, M. & Behr, R. Hypothesis: Irisin is a metabolic trigger for the activation of the neurohormonal axis governing puberty onset. *Med. Hypotheses.* **95**, 1-4 (2016).
14. Ulker, N. *et al.* Irisin may have a role in pubertal development and regulation of reproductive function in rats. *Reproduction.* **160**, 281-292 (2020).
15. Reinehr, T., Elfers, C., Lass, N. & Roth, C.L. Irisin and its relation to insulin resistance and puberty in obese children: a longitudinal analysis. *J. Clin. Endocrinol. Metab.* **100**, 2123-2130 (2015).
16. Wahab, F. *et al.* Irisin in the primate hypothalamus and its effects on GnRH in vitro. *J. Endocrinol.* **241**, 175-187 (2019).
17. Horton, T. H. Fetal origins of developmental plasticity: Animal models of induced life history variation. *Am. J. Hum. Biol.* **17**, 34-43 (2005).
18. Prata Lima, M. F., Bacarat, E. C. & Simoes, M. J. Effects of melatonin on the ovarian response to pinealectomy or continuous light in female rats: similarity with polycystic ovary syndrome. *Braz. J. Med. Biol. Res.* **37**, 987-995 (2004).
19. Díaz, B*. et al.* Effect of pinealectomy amd melatonin treatmnet during pregnancy on the sexual development of the female and male offspring. *Europ. J. Endocrinol.* **132**, 765-770 (1995).
20. Fernández, B., Díaz, E., Colmenero, M. D. & Díaz, B. Maternal pineal gland participates in prepubertal rats' ovarian oocyte development. *Anat. Rec.* **243**, 461-465 (1995).
21. Díaz, B*. et al.* Maternal melatonin influences rates of somatic and reproductive organs postnatal development of male rat offspring. *Neuro. Endocrinol. Lett.* **20**, 69-76 (1999).
22. Vázquez, N., Debeljuk, L., Díaz, E., Fernández, C. & Díaz, B. Influence of maternal pineal gland on the developmental pattern of neurokinin A (NKA) and substance P (SP) in male rat offspring relationship to the season of the year. *Neurosc Lett.* **368**, 243-248 (2004).
23. Díaz, E., Debeljuk, L., Arce, A., Esquifino, A. & Díaz, B. Prenatal melatonin exposure affects luteinizing hormone and hypothalamic and striatal neuropeptide Y in the male rat offspring. *Neurosc. Lett.* **292**, 143-146 (2000).
24. Serón-Ferré, M. *et al.* Impact of chronodisruption during primate pregnancy on the maternal and newborn temperature rhythms. *Plos One.* **8**, e57710 (2013).

25. Sáenz de Miera, C., Bothorel, B., Jaeger, C., Simonneaux, V. & Hazlerigg, D. Maternal photoperiod programs hypothalamic thyroid status via the fetal pituitary gland. *Proc. Natl. Acad. Sci. U S A.* **114**, 8408-8413 (2017).
26. Terasawa, E., Guerriero, K.A. & Plant, T, M. Kisspeptin and puberty in mammals. *Adv. Exp. Med. Biol.* **784**, 253-273 (2013).
27. Shahab, M. *et al.* Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. *Proc. Natl. Acad. Sci. U S A.* **102**, 2129-2134 (2005).
28. Guerriero, K. A., Keen, K. L., Millar, R. P. & Terasawa, E. Developmental changes in GnRH release in response to kisspeptin agonist and antagonist in female rhesus monkeys (Macaca mulatta): implication for the mechanism of puberty. *Endocrinology.* **153**, 825-836 (2012).
29. Oakley, A. E., Clifton, D. K., Steiner, R. A. Kisspeptin signaling in the brain. *Endocr. Rev.* **30**, 713-743 (2009).
30. Garcia, J. P., Keen, K. L., Seminara, S.B. & Terasawa, E. I. Role of Kisspeptin and NKB in Puberty in Nonhuman Primates: Sex Differences. *Semin. Reprod. Med.* **37**, 47-55 (2019).
31. Reiter, R. J. Neuroendocrine effects of light. *Int. J. Biometeorol.* **35**, 169-175 (1991).
32. Klein, D. C. Evidence for the placental transfer of 3 H-acetyl-melatonin. *Nat. New. Biol.* **237**, 117-118 (1972).
33. Reppert, S. M., Chez, R. A., Anderson, A. & Klein, D. C. Maternal-fetal transfer of melatonin in the non-human primate. *Pediatr. Res.* **13**, 788-791 (1979).
34. Serón-Ferré, M. *et al.* Circadian rhythms in the fetus. *Mol. Cell. Endocrinol.* **349**, 68-75 (2012).
35. Pea, R. *et al.* Media use, face-to-face communication, media multitasking, and social well-being among 8- to 12-year-old girls. *Dev. Psychol.* **48**, 327-336 (2012).
36. Pecor, K. *et al.* Sleep health, messaging, headaches, and academic performance in high school students. *Brain. Dev.* **38**, 548-553 (2016).
37. Touitou, Y., Touitou, D. & Reinberg, A. Disruption of adolescents' circadian clock: The vicious circle of media use, exposure to light at night, sleep loss and risk behaviors. *J. Physiol. Paris.* **110**, 467-479 (2016).
38. Young, K. S. The evolution of Internet addiction. *Addict. Behav.* **64**, 229-230 (2017).
39. Simonneaux, V. *et al.* Kisspeptin and the seasonal control of reproduction in hamsters. *Peptides.* **30**, 146-153 (2009).
40. Wagner, G. C., Johnston, J. D., Clarke, L. J., Lincoln, G. A. & Hazlerigg, D. G. Redefining the limits of day length responsiveness in a seasonal mammal. *Endocrinology.* **149**, 32-39 (2008).
41. Gingerich, S. *et al.* The generation of an array of clonal, immortalized cell models from the rat hypothalamus: analysis of melatonin effects on kisspeptin and gonadotropin-inhibitory hormone neurons. *Neuroscience.* **162**, 1134-1140 (2009).
42. Cameron, J. L. Metabolic cues for the onset of puberty. Horm. Res. **36**, 97-103 (1991).

43. Qiu, X. *et al.* Delayed puberty but normal fertility in mice with selective deletion of insulin receptors from Kiss1 cells. *Endocrinology.* **154**, 1337-1348 (2013).
44. Sanchez-Garrido, M. A. & Tena-Sempere, M. Metabolic control of puberty: roles of leptin and kisspeptins. *Horm Behav.* **64**, 187-194 (2013).
45. Hofmann, T., Elbelt, U. & Stengel, A. Irisin as a muscle-derived hormone stimulating thermogenesis–a critical update. *Peptides.* **54**, 89-100 (2014).
46. Colaianni, G., Cinti, S., Colucci, S. & Grano, M. Irisin and musculoskeletal health. *Ann. N. Y- Acad. Sci.* **1402**, 5-9 (2017).
47. Korta, P., Pocheć, E. & Mazur-Biały, A. Irisin as a Multifunctional Protein: Implications for Health and Certain Diseases. Medicina (Kaunas). 55, 485 (2019).
48. Polyzos, S. A. et al. Irisin in metabolic diseases. Endocrine. 59, 260-274 (2018).
49. Aslan, G., Gül, H. F., Tektemur, A. & Sahna, E. Ischemic postconditioning reduced myocardial ischemia-reperfusion injury: The roles of melatonin and uncoupling protein 3. Anatol. J. Cardiol. 23,19-27 (2020).
50. Gul-Kahraman, K., Yilmaz-Bozoglan, M. & Sahna, E. Physiological and pharmacological effects of melatonin on remote ischemic perconditioning after myocardial ischemia-reperfusion injury in rats: Role of Cybb, Fas, NfκB, Irisin signaling pathway. Pineal Res. 67, e12589 (2019).
51. Tung, Y. T., Chiang, P. C., Chen, Y. L. & Chien, Y. W. Effects of Melatonin on Lipid Metabolism and Circulating Irisin in Sprague-Dawley Rats with Diet-Induced Obesity. Molecules. 25, 3329 (2020).
52. Ojeda, S.R., Wheaton, J.E., Jameson, H.E. & McCann, S.M. The onset of puberty in the female rat: changes in plasma prolactin, gonadotropins, luteinizing hormone-releasing hormone (LHRH), and hypothalamic LHRH content. Endocrinology. 98, 630-638 (1976).

Figures

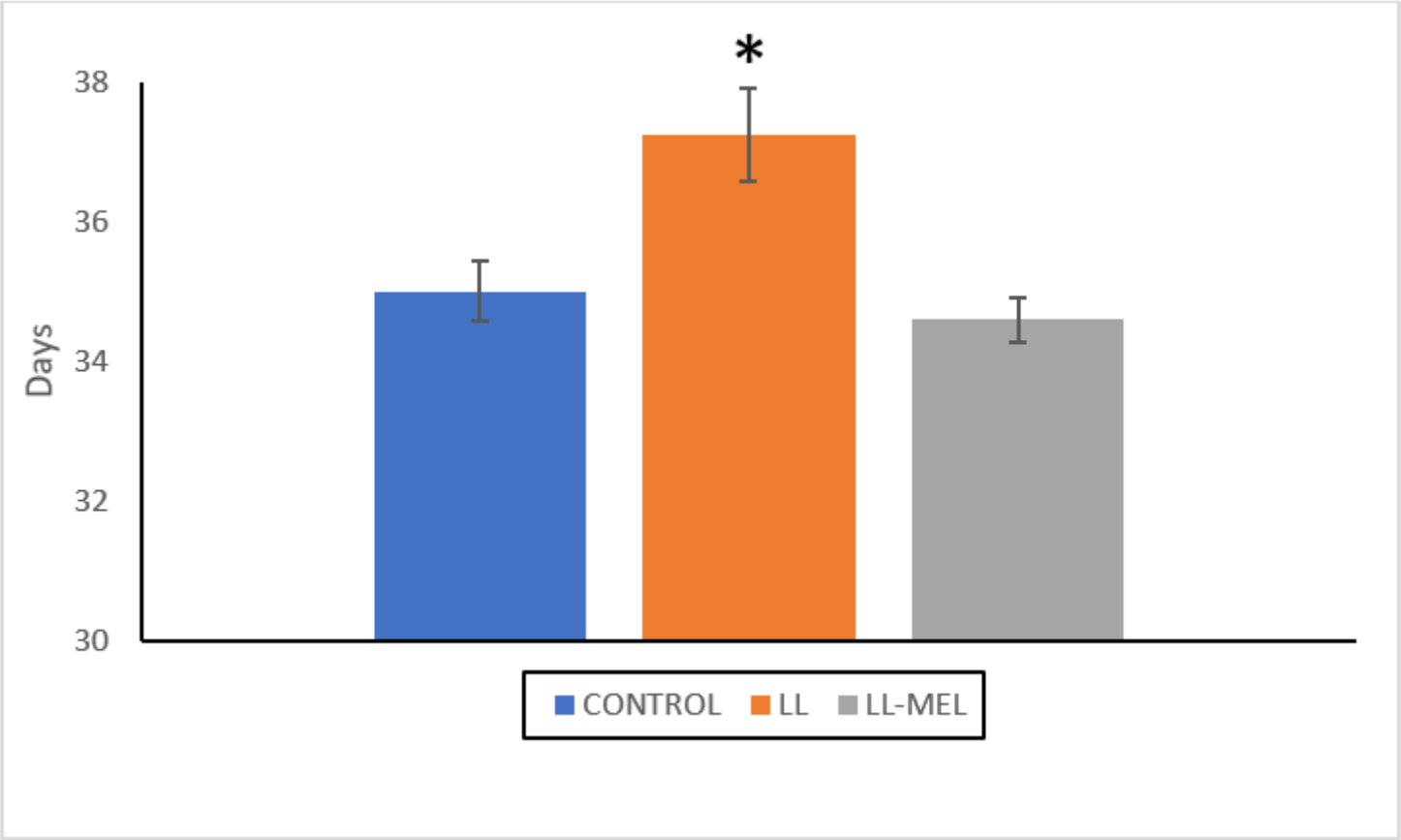
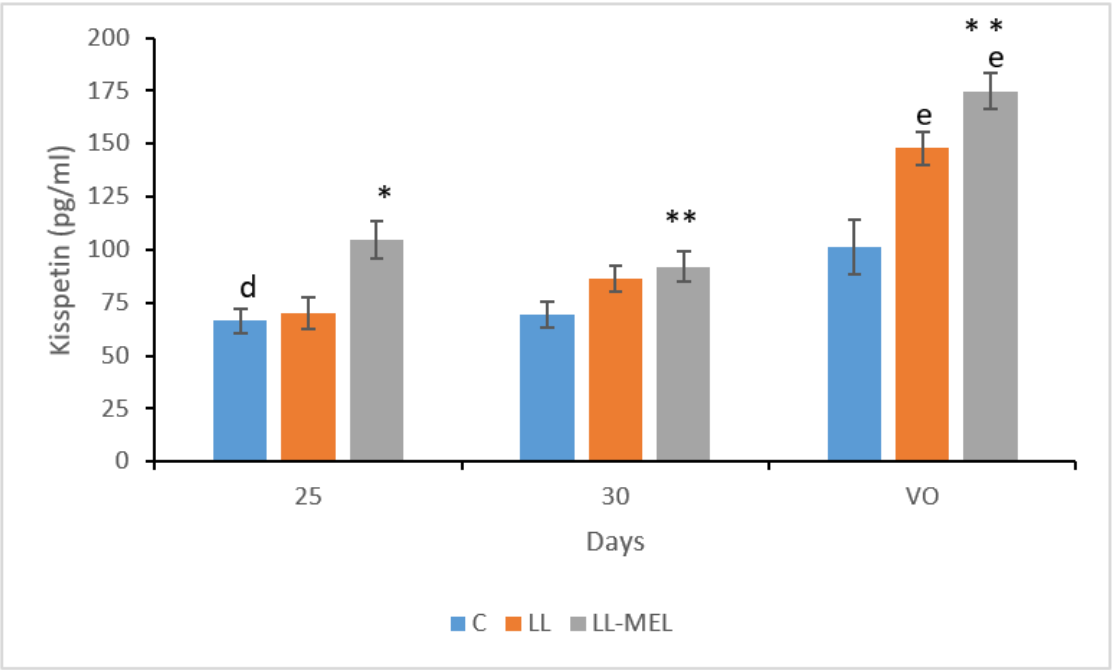


Figure 1

The effect of light exposure during pregnancy on the time of vaginal opening (VO) is showed in the offspring of normal photoperiod group (C), continuous light exposure group (LL) and continuous light exposure group treated with melatonin (LL-MEL). Rats from LL group showed a delay in the time of AV compared to C and LL-Mel groups ($p < 0.05$) All values are expressed as mean ± SEM, n=8 for C, LL and LL-Mel groups.

A



B

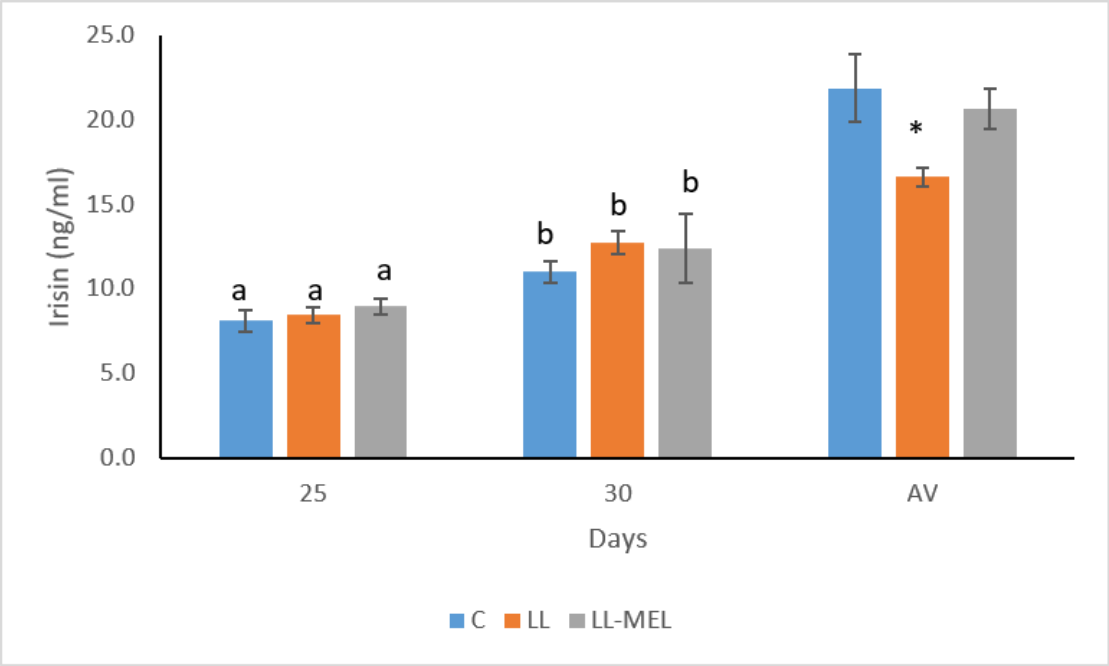


Figure 2

Kisspeptin (2A) and Irisin (2B) plasma levels in the offspring of normal photoperiod group (C), continuous light exposure group (LL) and continuous light exposure groups treated with melatonin (LL-MEL) at 25, 30 vaginal open (VO) days. All values are expressed as mean ± SEM. Different symbols about the bars indicate statistical differences, a value of $P < 0.05$ was considered statistically significant. n=8 for C, LL

and LL-Mel groups. Intragroup comparisons were a = 25 vs 30, VO; b= 30 vs VO; d = 25 vs VO; e= VO vs 25, 30 and Intergroup comparisons, *= LL-MEL vs C, LL; ** = LL-MEL vs C.