Effects of Controlled Photoperiod on Body Development in Growing Juvenile Rats

Seunghoon Lee[1,#], Han-Ki Lee[2,#], Jinhee Shin[3], Yunkyung Hong[1], Sang-Kil Lee[4], Sangun Lee[5], Takao Suzuki[5], Taeyoung Kang[6] and Yonggeun Hong[1,3,4,†]

[1]Department of Rehabilitation Science in Interdisciplinary PhD Program, Graduate School of Inje University, Gimhae 621-749, Korea
[2]Deaprtment of Physical Therapy, Masan College, Masan 630-729, Korea, [3]Department of Physical Therapy,
[4]Cardiovascular & Metabolic Disease Center, College of Biomedical Science & Engineering, Inje University, Gimhae 621-749, Korea
[5]Department of Physical Therapy, Aomori University of Health and Welfare, Aomori 030-8505, Japan
[6]College of Veterinary Medicine, Jeju National University, Jeju 690-756, Korea

ABSTRACT

Melatonin is induced by light information through the retina and leads to growth factor activation. Thus, we investigated the effects of melatonin by controlling the photoperiod of growing young rats. Male Sprague-Dawley rats (n=6; 4 weeks old) were divided into two experimental groups: the L/D group (normal photoperiod; light/dark: 12/12 h; lights on at 9:00 a.m.) and the L/L group (light/light: 24 h). Rat body weight and food consumption were measured daily for 8 weeks. After 8 weeks, the rats were anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) and sacrificed. Tissue was then collected for RNA isolation (from brain, heart, liver, kidney, adrenal gland, testis, tibia, hind limb muscles). Also, serum was isolated from blood using a centrifugal separation. The L/L group had significantly lower body weight than the L/D group from 4 to 6 weeks ($p<0.05$). The L/D group had increased tissue mass, compared with the L/L group, but the difference was not statistically significant. The L/D group had a significantly higher melatonin concentration than the L/L group between the hours of midnight and 2:00 a.m ($p<0.01$). These results indicate that photoperiod length may affect the secretion of melatonin from the pineal gland. Also, the reduction of nocturnal melatonin secretion may retard the development of growing young rats. In future studies, we plan to compare exogenous melatonin administration with endogenous melatonin concentration induced by photoperiod control. Moreover, we will confirm whether the effects seen in pathological animal models can be reversed by controlling the photoperiod.

(Key words : Controlled photoperiod, Melatonin, Development, Juvenile rat)

INTRODUCTION

N-acetyl-5-methoxytryptamine (melatonin) is produced by pinealocytes in the pineal gland, located in the brain (Paulose et al., 2009). Production of melatonin by the pineal gland is under the influence of the suprachiasmatic nuclei (SCN) of the hypothalamus, which receives information from the retina about the daily pattern of light and dark. Both SCN rhythmicity and melatonin production are affected by non-image- forming light information traveling through the recently identified retinohypothalamic tract (RHT) (Ackermann & Stehle, 2006; Hardeland, 2005). Melatonin is an anti-oxidant, anti-aging, and anti-inflammatory molecule that affects development as a master controller of circadian rhythm. It is used for the treatment of jet lag and in patients suffering from insomnia (Hardeland, 2005; Altun, 2007). A previous study reported that the neurotransmitter serotonin plays an important role in the photic and non-photic regulation of circadian rhythms and is a precursor of melatonin (Jagota & Kalyani, 2008). Age-induced changes in daily serotonin rhythmicity in the brain could affect age-induced circadian function disorders (Richardson, 2005). Moreover, downregulation of melatonin secretion leads to reduced physiological activity, drinking, and core temperature (Wonden-Hanson et al., 2000). Additionally, melatonin influences the release of growth hormones and cortisol (Makay et al., 2009).

Developmental stage is affected by sex hormones. The male hormone testosterone plays a key role in

health and has anabolic effects that include increasing muscle mass and strength, increasing bone density and strength, and stimulation of linear growth and bone maturation (Oner *et al.*, 2008). The female hormone estrogen promotes the development of female secondary sex characteristics and reduces muscle mass, bone resorption, and low density lipoprotein (LDL) levels. Conversely, estrogen can also increase bone formation, high density lipoprotein (HDL), and triglyceride levels (Uslu *et al.*, 2007). Several studies reported that melatonin treatment induced hypertrophy of skeletal muscles in castrated animal models, similar to the observed effects of estrogen in ovariectomized rats (Oner *et al.*, 2008; Uslu *et al.*, 2007). Edmons *et al.* (2005) reported that reproductive organs affected photoperiod-dependent hypertrophy. Also, body weight was increased after long photoperiods in male marsh rice rats. Wolden- Hanson *et al.* (2000) suggested that melatonin administration affected physiological features, including reducing body weight, visceral fat mass and hormone (e.g. insulin and leptin) levels in mid-age male rats. Moreover, Sanchez-Hidalgo *et al.* (2007) reported that rodents showed body weight retardation and accumulation of visceral fat following low-dose melatonin administration. Pathological animal models (e.g. fracture, osteoporosis, stroke) are positively affected by melatonin treatment. Another interesting finding is that pinealectomized rats do not have a greater body weight than intact rats.

Quiroz *et al.* (2008) suggested that melatonin administration might improve the course of chronic renal failure in rats with renal mass reduction, and ameliorate oxidative stress and inflammation. Glial cells play an important role in brain development and recovery after damage. An *in vitro* study showed that pinealocytes affected the differentiation and proliferation of astrocytes in co-culture conditions (Paulose *et al.*, 2009). Also, fibroblast growth factor-2 (FGF-2), an activator of osteoblasts, was up-regulated after melatonin treatment in a material implantation animal model (Takechi *et al.*, 2008). Numerous studies suggest that melatonin has multipotent effects on aspects of biology and physiology (Ostrowska *et al.*, 2003; Lu & Cassone, 1993).

Melatonin secretion is affected by the season. Environmental day length affects nocturnal pineal melatonin secretion, which, in turn, generates or entrains seasonal cycles of physiology, reproduction, and behavior in mammals (Mishima *et al.*, 2001). Numerous studies have shown that controlled photoperiods induce melatonin synthesis and secretion by the pineal gland (Hance *et al.*, 2009). These *in vivo* findings demonstrate that rhythmicity of melatonin secretion affects physiological homeostasis. Bartness and Wade (1985) reported that a long photoperiod induced weight loss without changes in food intake in Siberian hamsters.

Exogenous melatonin administration reverses some pathological conditions, retards aging, and facilitates growth (Koh, 2008; Oner *et al.*, 2008; Dominguez-Rodriguez *et al.*, 2007). However, it is unclear whether a controlled photoperiod affects growth through changes in endogenous melatonin secretion. Thus, in the present study, we investigated whether alteration of melatonin secretion induced by a controlled photoperiod affected growth in young, developing juvenile rats.

MATERIALS AND METHODS

Animals

Fifteen 4-week-old male Sprague-Dawley rats (Hyochang Science, Daegu, Korea) weighing 90~100 g were used in all studies. Rats were randomly divided into a control group (L/D; light/dark condition: 12/12 h) and an experimental group (L/L; light/light condition: 24 h). They were provided with 70 g of rodent standard chow diet (Hyochang Science, Daegu, Korea) daily and tap water *ad libitum* for 8 weeks. All animal procedures were approved by the Ethics Committee for Animal Care and Use at Inje University, which is certified by the Korean Association of Accreditation of Laboratory Animal Care. All rats were housed one per cage under controlled environmental conditions (22±1°C; lights on at 9:00 am).

Experimental Design

Rats were randomly divided into two groups, control (L/D) and experimental (L/L). The L/D group (n=7) had a normal photoperiod (light/dark: 12/12 h, lights on at 9:00 am). The L/L group (n=8) had a controlled photoperiod (long photoperiod; light/light: 24 h).

Tissue Collection

Body weight and food intake were measured daily for 8 weeks. After 8 weeks, rats were anesthetized with a mixture of ketamine (50 mg/kg: Yunhan Co., Seoul, Korea) and xylazine (10 mg/kg; Rompun, Bayer Healthcare, Seoul, Korea). Serum was isolated from blood using a centrifugal separator (HSL-05A; Hanil Industrial Co., Korea). Tissue (from brain, heart, liver, kidney, spleen, adrenal gland, testis, tibia, hindlimb skeletal muscle) was collected and stored frozen at −70°C until analysis. Then, all tissues were weighed to the nearest 0.01 g on an electronic balance (CB-200; A&D Electronic Balance, Toyko, Japan) and an image was captured immediately (Canon-450D; Tokyo, Japan). Tibia length was measured using calipers (531 series; Mitutoyo Korea Co., Seoul, Korea).

Analysis of Plasma Melatonin Concentration

Serum was isolated from blood using a centrifugal

separator. Measurement of plasma melatonin was preceded by an extraction procedure with chloroform. Briefly, a 500 µl aliquot of plasma was mixed (1:1, v/v) with 0.1 M acetic acid buffer (pH 4.6) and 2 ml of chloroform was added. The mixture was vortexed for 1 min, centrifuged (3,800×g, 10 min), and the aqueous phase was removed. The organic layer was separated and cleaned once with 2 ml 0.1 N NaOH. After cleaning and centrifugation, the aqueous phase was aspirated and the organic layer was dried to vacuum under at 40℃. The residue was dissolved in 200 µl of mobile phase and filtered through a 0.5 µm filter. A 20 µl aliquot of the filtrate was injected into the high-performance liquid chromatography (HPLC) system. HPLC analysis was performed on a HPLC system (Agilent 1100; Agilent Technologies Inc., Santa Clara, CA, USA) with fluorescence detector (λex=280 nm, λem=360 nm). Melatonin was separated on a Phenomenex C18 (3 um particles, 75×4.6 mm i.d.) (Luna C18; Phenomenex Inc., Torrance, CA, USA). The mobile phase consisted of solution of methanol 40% and water 60%. All analyses were performed at 24℃ at a flow rate of 1 ml/min. Acquisition and integration of chromatograms was performed using the Agilent Chemstation software. Quantification of sample peaks was done by comparing peak areas with those of standards.

Statistical Analyses

Data were collected from repeated experiments and are presented as means±SD. A one-way ANOVA was used for statistical analysis. Differences were deemed to be statistically significant when $p<0.05$. All analyses were performed using the SPSS software (ver. 17.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Variation in Food Intake

We compared the food intake in the light/light (L/L) group with the light/dark (L/D) group. Food consumption was measured daily at 10:00 a.m. for 8 weeks. Food consumption did not significantly differ between the L/D and L/L groups (Fig. 1). Thus, food intake was apparently not affected by the controlled photoperiod.

Change in Body Weight

We compared the body weight of the light/light (L/L) group with the light/dark (L/D) group. Body weight was measured weekly at 10:00 a.m. for 8 weeks. Percent body weight change from the initial body weight was also compared. The L/L group body weight was significantly retarded at weeks 5 and 6 ($p<0.05$). Body weights did not significantly differ from week 7 to 12
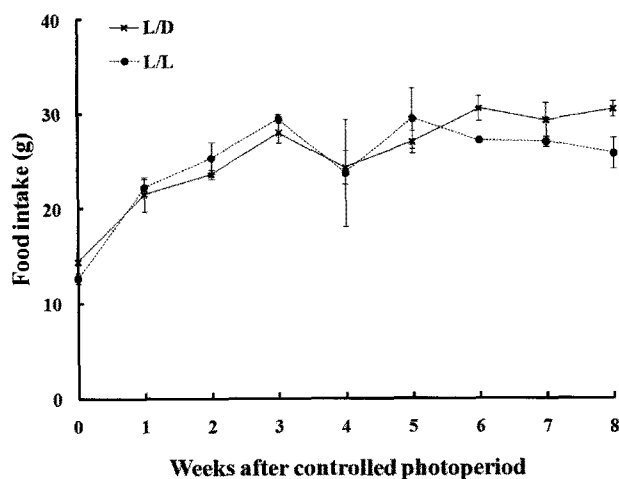

Fig. 1. Daily food consumption over 8 weeks. L/D; light/dark condition, L/L; light/light condition.
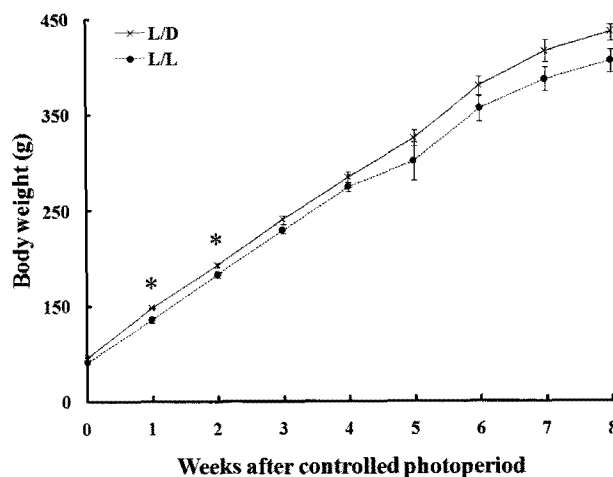

Fig. 2. Variation of body weight by controlled photoperiod. L/D; light/dark condition, L/L; light/light condition. * $p<0.05$: L/D vs. L /L.

(Fig. 2). These data suggest that a controlled photoperiod may affect the development of juvenile rats.

Change in Individual Tissue Weight

After 8 weeks, rats were anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) and tissue was isolated. After isolation, the tissue was weighed to the nearest 0.01 g on an electronic balance. There was a non-significant trend towards greater tissue mass in the L/D group versus the L/L group in brain, heart, spleen and adrenal gland. L/D kidney was significantly heavier than in the L/L group ($p<0.01$). Hind limb muscles were not significantly different between the L/D and L/L groups. However, gastrocnemius and plantaris muscle mass was greater in the L/D than the L/L group. In contrast, the soleus of the L/L group

Lee *et al.*

was heavier than that of the L/D group (Table 1).

Change in Ratio of Heart/Tibia Length Due to Controlled Photoperiod

We analyzed the relationship between cardiac hypertrophy due to overdevelopment of heart and controlled photoperiod using measurement of heart/tibia ratio. This ratio was not significantly different between the L/D and L/L groups (Fig. 3).

Table 1. Change in individual tissue weight

	L/D (g)	L/L (g)
Brain	2.09±0.08	1.97±0.06
Heart	1.61±0.05	1.50±0.14
Liver	13.11±0.41	13.15±0.46
Kidney	3.68±0.01	3.46±0.02**
Spleen	0.94±0.14	0.93±0.09
Testis	3.11±0.06	3.24±0.18
Adrenal grand	0.07±0.01	0.06±0.00
Rt. gastrocnemius	2.16±0.07	2.05±0.08
Lt. gastrocnemius	2.18±0.08	2.12±0.05
Rt. plantaris	0.38±0.01	0.36±0.01
Lt. plantaris	0.39±0.02	0.37±0.00
Rt. soleus	0.16±0.01	0.16±0.00
Lt. soleus	0.15±0.00	0.16±0.02

Comparison of tissues mass in L/D vs. L/L. ** $p<0.01$: L/D vs. L/L.
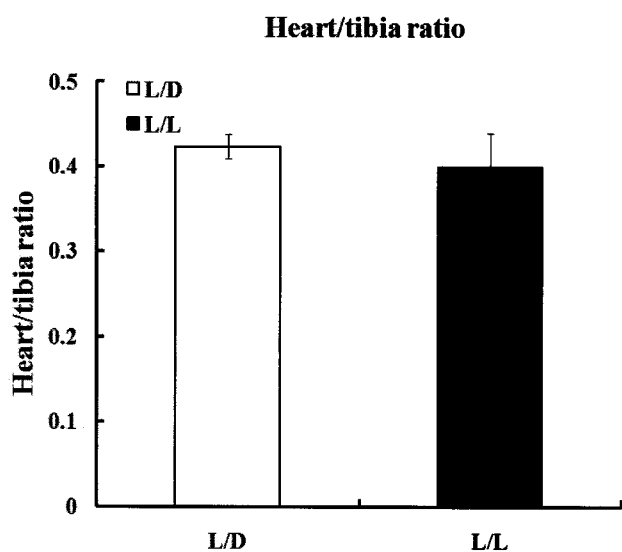
Heart/tibia ratio

Fig. 3. Heart/tibia ratio following 8 weeks of controlled photoperiod. L/D; light/dark condition, L/L; light/light condition.

Table 2. Change in biochemical components

Test	Normal range	L/D	L/L
Alkaline phosphatase(IU)	62~209	257±165.85	150.67±203.81
Alpha amylase(IU)	1,691~3,615	312.33±7.09	286.33±41.00
Albumin(g/dL)	2.5~4.8	3.40±0.00	3.23±0.12
Calcium(mg/dL)	5.9~9.4	3.73±3.18	3.90±3.99
Cholesterol FL(mg/dL)	36~96	37.33±7.64	57.67±8.50*
Creatinine(mg/dL)	0.2~0.8	0.03±0.06	0.10±0.10
LDH (IU)	1,105~3,993	289.33±241.38	471.00±90.22
T-BIL(mg/dL)	0.1~0.9	0.20±0.17	0.30±0.17
ALT(IU)	28~132	12.33±1.53	11.00±1.00
AST(IU)	59~247	2.00±3.46	21.00±13.89
Phosphorus(mg/dL)	6.1~10.1	9.41±0.38	10.45±0.00*
Total protein(g/dL)	3.6~6.6	5.87±0.06	5.97±0.32
BUN(mg/dL)	18~29	17.67±4.16	18.00±4.00

Comparison of biochemical components in L/D vs. L/L. * $p<0.05$: L/D vs. L/L.

Change in Biochemical Components

After 8 weeks, rats were anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) and serum was isolated from blood. The L/L group had a significant higher concentration of cholesterol and phosphorus than L/D group ($p<0.05$). However, the other parameters were not statistically significant difference as showing at Table 2.

Plasma Melatonin Concentration

After 8 weeks, rats were anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg). Serum was isolated from blood using a centrifugal separator after collection between midnight and 2:00 a.m. We confirmed the plasma melatonin concentration using HPLC. The L/D group had a significantly increased melatonin concentration compared to the L/L group from midnight to 2 a.m. ($p<0.01$) (Fig. 4). These data indicated that melatonin was controlled by light at night.

DISCUSSION

The photoperiodic mammal undergoes quite remarkable changes in physiology as part of its natural adaptation to seasonal fluctuations in the environment. Indeed, seasonal alternations induce endogenous hormone
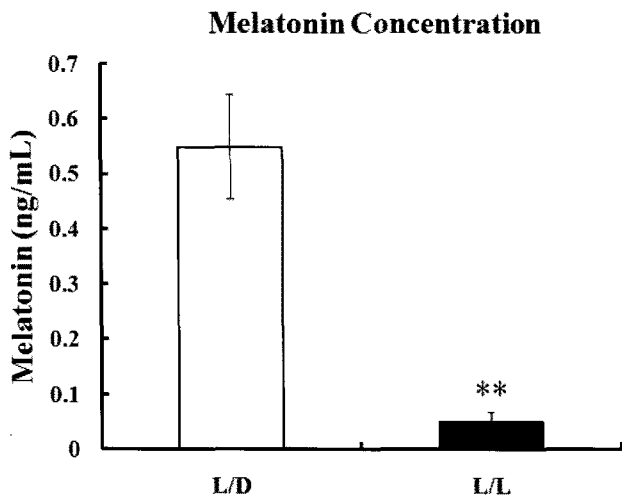
Melatonin Concentration

Fig. 4. Plasma melatonin concentration following 8 weeks of controlled photoperiod. L/L; light/light condition. ** $p<0.01$: L/D vs. L/L.

secretion. Specifically, melatonin secretion is characterized by photoperiod duration. Melatonin also controls the secretion of growth hormones. Ekman (2008) suggested that nocturnal melatonin secretion might affect the secretion of growth hormone and also reported that brown-eyed children experience more nighttime growth pain than light-eyed children. Edmons et al. (2005) reported that body weight was increased after long photoperiods in male marsh rice rats. Strikingly, opposite results were found compared previous reports that developing rats in the L/L group had significantly retarded body weight at 5 and 6 week following the controlled photoperiod, respectively (Fig. 2). Also, The L/L group tended to retardation of increasing body weight from 6 to 12 week. However, there no change was observed in food consumption between the L/D and L/L groups (Fig. 1). Thus, we suggest that a longer photoperiod may affect the secretion of melatonin and retard the development of body composition. L/D group kidneys were significantly heavier than the kidneys of the L/L group ($p<0.01$) (Table 1). The L/D group had a non-significant increase in tissue mass versus the L/L group after the controlled photoperiod. The gastrocnemius and plantaris hind limb muscles were heavier in the L/D group than the L/L group; however, the soleus was lighter than that in the L/L group (Table 1). We also confirmed differences in melatonin concentration by photoperiod. The dark phase induced melatonin secretion by the pineal gland. Melatonin concentration was highest from midnight to 2:00 a.m; thus, we obtained blood samples from midnight to 2:00 a.m. As shown at Fig. 4, the L/D group had a significantly higher melatonin concentration than the L/L group ($p<0.05$). Also, Makay et al. (2009) reported that melatonin promoted growth factor secretion. Thus,

our data suggest that nocturnal melatonin secretion could affect the development of body composition by activation of growth factors.

Melatonin secretion was affected by light information; growth hormones were also controlled by melatonin. Thus, we examined whether melatonin secretion was affected by a controlled photoperiod and whether melatonin affected the development of the growth stage. Prendergast (2010) reported that environmental photoperiod drives nocturnal pineal melatonin secretion, which in turn generates or entrains seasonal cycles of physiology, reproduction. Also, long day length downregulated the expression MT1/MT2 receptors. We confirmed that melatonin secretion was affected by the photoperiod. A long photoperiod inhibited the secretion of melatonin by the pineal gland. Also, decreased melatonin concentration may affect the development of body composition by controlling growth hormone secretion. In future studies, we plan to compare exogenous melatonin administration and endogenous melatonin, induced by photoperiod control. Moreover, we will determine whether the effects seen in pathological animal models can be reversed by a controlled photoperiod.

REFERENCES

1. Paulose JK, Peters JL, Karaganis SP, Casonne VM (2009): Pineal melatonin acts as a circadian zeitgeber and growth factor in chick astrocytes. J Pineal Res 46:286-294.
2. Ackermann K, Stehle JH (2006): Melatonin synthesis in the human pineal gland: advantages, implications, and difficulties. Chronobiology International 23 (1&2):369-379.
3. Hardeland R (2005): Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. Endocrine 27(2): 119-130.
4. Altun A, Ugur-Altun B (2007): Melatonin: therapeutic and clinical utilization. Int J Clin Pract 61(5): 835-845.
5. Jagota A, Alyani D (2008): Daily serotonin rhythms in rat brain during postnatal development and aging. Biogenrontology 9:229-234.
6. Pazo D, Cardinali DP, Cano P, Reyes Toso CA, Esquifino AI (2002): Age-related changes in 24-hour rhythms of nor-epinephrine content and serotonin turnover in rat pineal gland: effect of melatonin treatment. Neurosignals 11:81-87.
7. Richardson G (2005): The human circadian system in normal and disordered sleep. J Clin Psychiatry Suppl 9:3-9.
8. Wolden-Hanson T, Mitton DR, McCants RL, Yellon

SM, Wilkinson CW, Matsumoto AM, Rasmussen DD (2000): Daily melatonin administration to middle-aged male rats suppresses body weight, intra-abdominal adiposity, and plasma leptin and insulin independent of food intake. Endocrinology 141(2): 487-497.

9. Makay B (2009): Is there a role of melatonin in the development of growing pains? Med Hypotheses 72 (2):225.

10. Oner J, Oner H, Sahin Z, Demir R, Ustünel I (2008): Melatonin is as effective as testosterone in the prevention of soleus muscle atrophy induced by castration in rats. Anat Rec (Hoboken) 291(4): 448-455.

11. Uslu S, Uysal A, Oktem G, Yurtseven M, Tanyalçin T, Başdemir G (2007): Constructive effect of exogenous melatonin against osteoporosis after ovariectomy in rats. Anal Quant Cytol Histol 29(5):317-325.

12. Edmonds K, Riggs L, Masden T (2005): Effects of photoperiod, melatonin, and the pineal gland on compensatory gonadal hypertrophy during postnatal development in the Marsh Rice rat (*Oryzomys palustris*). Zoological Science 22:763-774.

13. Sanchez-Hidalgo M, Lu Z, Tan DX, Maldonado MD, Reiter RJ, Gregerman RI (2007): Melatonin inhibits fatty acid-induced triglyceride accumulation in ROS17/2.8 cells: implications for osteoblast differentiation and osteoporosis. Am J Physiol Regulatory Integrative Comp Physiol 292:2208-2215.

14. Quiroz Y, Ferrebuz A, Romero F, Vaziri ND, Rodriguez-Iturbe B (2007): Melatonin ameliorates oxidative stress, inflammation, proteinuria, and progression of renal damage in rats with renal mass reduction. Am J Physiol Renal Physiol 294:336-344.

15. Takechi M, Tatehara S, Satomura K, Fujisawa K, Nagayama M (2008): Effect of FGF-2 and melatonin on implant bone healing: a histomorphometric study. J Mater Sci Mater Med 19(8):2949-2952.

16. Ostrowska Z, Kos-Kudla B, Nowak M, Swietochowska E, Marek B, Gorski J, Kajdaniuk D, Wolkowska K (2003): The relationship between bone metabolism, melatonin and other hormones in sham-operated and pinealectomized rats. Endocr Regul 37(4): 211-224.

17. Lu J, Cassone VM (1993): Daily melatonin administration synchronizes circadian patterns of brain metabolism and behavior pinealectomized house sparrows, *Passer domesticus*. J Comp Physiol A 173: 775-782.

18. Mishima K, Okawa M, Shimizu T, Hishikawa Y (2001): Diminished melatonin secretion in the elderly caused by insufficient environmental illumination. J Clin Endocrinol Metab 86:129-134.

19. Hance MW, Mason JI, Mendis-Handagama SM (2009): Effect of photo stimulation and nonstimulation of golden hamsters (*Mesocricetus auratus*) from birth to early puberty on testes structure and function. Histo Histopathol 24(11):1417-1424.

20. Bartness TJ, Wade GN (1985): Photoperiodic control of seasonal body weight cycles in hamsters. Neurosci Biobehav Rev 9(4):599-612.

21. Koh PO (2008): Melatonin attenuates the cerebral ischemic injury via the MEK/ERK/p90RSK/Bad signaling cascade. J Vet Med Sci 70(11):1219-1223.

22. Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez MJ, Kaski JC, Reiter RJ, Jimenez-Sosa A (2007): A unicenter, randomized, double-blind, parallel-group, placebo-controlled study of melatonin as an adjunct in patients with acute myocardial infarction undergoing primary angioplasty the melatonin adjunct in the acute myocardial infarction treated with angioplasty (MARIA) trial: study design and rationale. Contemp Clin Trials 28(4):532-539.

23. Muñoz JL, Ceinos RM, Soengas JL, Míguez JM (2009): A simple and sensitive method for determination of melatonin in plasma, bile and intestinal tissues by high performance liquid chromatography with fluorescence detection. J Chromatogr B Analyt Technol Biomed Life Sci 877(22):2173-2177.

24. Prendergast BJ (2010): MT1 melatonin receptors mediate somatic, behavioral, and reproductive neuroendocrine responses to photoperiod and melatonin in Siberian hamsters (*Phodopus sungorus*). Endocrinology 151(2):714-721.