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Effects of photoperiod variations and alpha-lipoic acid treatment on melatonin, cortisol, and oxidative stress levels in the blood of rats

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Abstract: This study aimed to investigate the effects of frequently changing photoperiods and alpha-lipoic acid treatment on melatonin, cortisol, oxidative markers, and some blood parameters. Eighty-four rats were randomly and equally divided into 7 groups: a control group, 3 prolonged light groups, and 3 prolonged dark groups. The study was completed in 2 stages, with 1- and 4-week periods, respectively. Blood samples were obtained at the end of the first and fourth weeks, and at the end of the dark period for the first and second stages. Serum aspartate aminotransferase and creatinine levels were significantly elevated in the prolonged dark olive oil group in the second stage. These values were similar to those of the control group in the prolonged dark water and alpha-lipoic acid groups. There was no statistically significant difference in total oxidant status between the prolonged dark olive oil group and the control group; however, the numerical total oxidant status value was increased. Higher urea and blood urea nitrogen levels were noted in the prolonged dark groups as compared with those in the prolonged light groups in the second stage. Melatonin and cortisol levels did not significantly change. The applied photoperiod changes in this study did not constitute a direct metabolic stressor for rats.

Key words: Photoperiod, melatonin, oxidative stress, alpha-lipoic acid

1. Introduction

The biological rhythm of many organisms is regulated by a 24-h cycle of biochemical, physiological, and behavioral processes, and/or inner circadian molecular mechanisms that depend on abiotic external factors such as light (Zhang et al., 2010). The biological functions of sleep, heart rate, blood pressure, body temperature, hormone concentrations, hepatic metabolism and detoxification, and renal elimination take place at specific times of the day (Schulz and Steimer, 2009). Many organisms manifest seasonal morphological and biological responses to environmental factors, including the photoperiod. These changes are associated with seasonal variations in the secretion of pineal melatonin, which is directly related to the photoperiod. Changes in emotional state and behavior may also contribute to adaptation (Wen et al., 2004; Benabid et al., 2008). Moreover, the photoperiod has been reported to affect the immune, endocrine, and reproductive systems, and may be a powerful modulator of immune system functions (Pawlak et al., 2009). Decreased light exposure due to shorter days causes changes in the energy balance, reproductive physiology, and immune functions. Prolonged decreased light exposure and a secondary prolonged melatonin secretion period can affect immune functions (Prendergast et al., 2003, 2008; Çetin, 2005). The development of decreased natural killer cytolytic capacity and spontaneous blastogenesis has been reported in response to shorter days (Yellon et al., 1999), and stimulatory areas in the hypothalamus are the source of regular hormone-releasing cycles of the endocrine system. A self-stimulatory endogenous circadian clock that is regularly activated at the same time of the day under laboratory conditions is claimed to be located in the suprachiasmatic nucleus (SCN); some SCN neurons synthesize neurotransmitters (Berne et al., 2008). The photoperiod is also known to affect the reproductive systems of sheep, mares, hamsters, and deer. The reproductive cycle time and reproductive system in these mammalian species are controlled by daily rhythms of melatonin secretion, which regulate seasonal reproduction in response to photoperiodic changes. Melatonin has a stimulatory effect on the gonads of animals that have cyclic activity on shorter days (sheep, goats, and deer) and a suppressive effect on animals that have cyclic activity on longer days (horses, hamsters, and camels) (Reiter, 1968; Uyar and Alan, 2008). Melatonin is pivotal in exerting

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photoperiod effects on various systems. Apart from regulating the circadian cycle, melatonin also affects blood pressure, body temperature, and seasonal reproductive functions. Melatonin is both water and lipid soluble with the ability to reach any cellular organelle, including the nucleus, and is reportedly an antioxidant that can prevent lipid peroxidation, promote immune response, delay aging, and exhibit antitumoral activity. It has also been reported to have a protective effect on the cardiovascular system, prevent atherosclerosis, and regulate blood cholesterol and low-density lipoprotein metabolism (Yerer and Aydoğan, 2006). It also induces sleep, shortens the time needed to fall asleep, and improves sleep quality (Cajochen et al., 2003).

Cortisol influences carbohydrate, protein, and lipid metabolism, protecting the organism from changes in its physiological balance. Cortisol levels increase in times of stress, affecting various systems for the adaptation of the organism. Reactive oxygen species are chemical compounds with 1 or more uncoupled electrons in their orbital; they are also produced in response to stress (Dündar and Aslan, 2000). Series of chain reactions caused by free radicals are suppressed by antioxidants. Alpha-lipoic acid (α-LA), a lipid- and water-soluble antioxidant, activates vitamin E and glutathione, and prevents free radical damage. α-LA is also thought to induce regeneration of vitamins E and C and can be obtained through the diet or synthesized by the body. Both animal and plant tissues abundant in mitochondria contain α-LA. However, the effects of α-LA on biochemical and physiological responses to photoperiod variations are not well known.

Considering the various photoperiod effects on organisms, changes in oxidant–antioxidant capacity, melatonin and cortisol levels, biochemical values in response to more variable and irregular light–dark periods, and the contribution of an antioxidant such as α-LA to these changes are important aspects. In this study, we aimed to investigate the levels of oxidative markers, melatonin, cortisol, and blood parameters to study the effects of a biorhythm-type photoperiod over a 2-day period. Moreover, we monitored the effect of α-LA on this process.

2. Materials and methods

2.1. Animals and study groups

Our study was approved by the Afyon Kocatepe University Animal Experiments Local Ethics Committee (17 February, 2010; number 37). Eighty-four male Sprague–Dawley rats, weighing 250–300 g, were included in the study. The animals were fed ad libitum with standard rat food containing 2600 kcal/kg metabolic energy and 23% raw protein. The animals were then separated into 7 groups, with each group comprising 6 animals in each phase of this study. They were kept in groups of 3 in a cage at a room temperature of 22–23 °C. The 2-phase study was started after a 1-week washout period to allow the rats to acclimatize to their new environment. The first and second phases consisted of 1- and 4-week periods, respectively, and the second phase started with new rats immediately after the completion of the first one. The photoperiods were 16-h light and 8-h dark or 8-h light and 16-h dark (Wen et al., 2004; Benabid et al., 2008). The lights were automatically switched on at 0700 (Ashkenazy-Frolinger et al., 2010); a 600-lux light source was used (Benabid et al., 2008). α-LA gel capsules (GNC, Pittsburgh, PA, USA) were administered via oral gavage at 100 mg/kg. The 7 groups were as follows: Group 1: control group (CG) that was exposed to a 12/12-h light/dark (L/D) cycle; Group 2: prolonged light olive oil group (LOG), where the animals were fed 2 mL/kg per day olive oil via oral gavage in daily turns of 16/8 L/D and 12/12 L/D; Group 3: prolonged dark olive oil group (DOG), where the animals were fed 2 mL/kg per day olive oil via oral gavage in daily turns of 8/16 L/D and 12/12 L/D; Group 4: prolonged light water group (LWG), where the animals was fed 2 mL/kg per day water via oral gavage in daily turns of 16/8 L/D and 12/12 L/D; Group 5: prolonged dark water group (DWG), where the animals were fed 2 mL/kg per day water via oral gavage in daily turns of 8/16 L/D and 12/12 L/D; Group 6: prolonged light α-LA group (LLG), where the animals were fed 100 mg/kg per day α-LA dissolved in 2 mL of olive oil via oral gavage in daily turns of 16/8 L/D and 12/12 L/D; Group 7: prolonged dark α-LA group (DLG), where the animals were fed 100 mg/kg per day α-LA dissolved in 2 mL of olive oil via oral gavage in daily turns of 8/16 L/D and 12/12 L/D.

2.2. Blood collection

Blood samples of 8–10 mL were obtained at the end of the dark period (at 0700) and transferred to heparin and gel tubes for whole blood and serum analysis, respectively, at the end of the first week of the first phase, and at the end of the fourth week of the second phase after performing a cardiac puncture under anesthesia following injection of 50 mg/kg ketamine HCl + 10 mg/kg xylazine HCl. The samples in the gel tubes were centrifuged for 10 min at 4 °C for the determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, blood urea nitrogen (BUN), creatinine, cortisol and melatonin levels, and the total antioxidant status (TAS) and total oxidant status (TOS). AST, ALT, urea, BUN, creatinine, and cortisol tests were measured after 24 h, and the remainder of the serum samples was kept at –20 °C until further analysis for measuring melatonin levels, TAS, and TOS. AST, ALT, urea, BUN, creatinine, cortisol, and melatonin levels, and TAS and TOS were determined in the Afyon Kocatepe University Medical School biochemistry laboratory.

2.3. Measurement of AST, ALT, urea, BUN, and creatinine levels

Serum AST, ALT, urea, BUN, and creatinine were measured by the spectrophotometric enzymatic method using Roche Diagnostics kits (cat. # 0764949, 0764957, 4460715, and 4810716, respectively) (Basel, Switzerland) in a Roche Diagnostics autoanalyzer (Cobas c501 model).

2.4. Measurement of cortisol levels

Serum cortisol was measured by the chemiluminescent enzyme immunoassay method with a Roche Diagnostics kit (cat. # 1875116) (Mannheim, Germany) in a Roche Diagnostics autoanalyzer (Cobas e601 model).

2.5. Measurement of melatonin levels

Serum melatonin levels were measured with a competitive ELISA assay kit provided by CUSABIO Biotech (cat. # CSB-E13433R) (Wuhan, China), in a Trinity Biotech Captia ELISA reader (Bray, Ireland), according to the manufacturer's instructions.

2.6. Measurement of TAS and TOS

Serum TAS and TOS were determined with Mega Tıp Rel assay diagnostics kits (cat. # RL0017, RL0024) (Gaziantep, Turkey) developed by Erel.

Serum TAS levels were determined using a novel automated measurement method, developed by Erel (2004). In this method, the antioxidative effect of the sample against the potent free radical reactions, initiated by the produced hydroxyl radical, is measured. The results are expressed as mmol Trolox equiv./L.

Serum TOS levels were determined using a novel automated measurement method developed by Erel (2005). The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide (H_2O_2) and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv./L).

2.7. Statistical analysis

SPSS was used for all statistical analyses (Chicago, IL, USA). Intergroup analysis was performed by one-way analysis of variance. Duncan's multiple range test was used for the post hoc evaluation to determine differences between the groups, and $P < 0.05$ was considered significant.

3. Results

The urea levels were significantly decreased in the DWG in comparison with the LWG, LOG, DLG, and CG ($P < 0.05$) in the 1-week period. Intervariance was evident between the LOG and DOG and between the LWG and DWG in the 4-week period (Table 1). Dark exposure increased urea levels in the 4-week period, unlike the 1-week period.

Table 1. Influence of photoperiod changes implemented over 1- and 4-week period on urea, BUN, and creatinine levels in serum of rats.

GROUP	ONE-WEEK PERIOD			FOUR-WEEK PERIOD		
	Urea (mg/dL) MEANS ± SE (n = 42)	BUN (mg/dL) MEANS ± SE (n = 42)	Creatinine (mg/dL) MEANS ± SE (n = 42)	Urea (mg/dL) MEANS ± SE (n = 42)	BUN (mg/dL) MEANS ± SE (n = 42)	Creatinine (mg/dL) MEANS ± SE (n = 42)
CG	$63.63 \pm 1.68^{a*}$	$29.73 \pm 0.78^{a*}$	0.29 ± 0.01	$69.06 \pm 1.02^{abc*}$	$32.27 \pm 0.47^{abc*}$	$0.35 \pm 0.01^{b***}$
LOG	$59.76 \pm 1.90^{a*}$	$27.92 \pm 0.89^{a*}$	0.30 ± 0.02	$65.01 \pm 2.20^{bc*}$	$30.38 \pm 1.03^{bc*}$	$0.35 \pm 0.01^{b***}$
DOG	$55.56 \pm 1.29^{ab*}$	$25.96 \pm 0.60^{ab*}$	0.32 ± 0.00	$76.21 \pm 6.98^{a*}$	$35.61 \pm 3.26^{a*}$	$0.44 \pm 0.02^{a***}$
LWG	$61.53 \pm 3.25^{a*}$	$28.75 \pm 1.51^{a*}$	0.29 ± 0.01	$62.90 \pm 2.98^{c*}$	$29.39 \pm 1.39^{c*}$	$0.31 \pm 0.01^{b***}$
DWG	$50.00 \pm 3.39^{b*}$	$23.36 \pm 1.58^{b*}$	0.29 ± 0.00	$75.51 \pm 3.25^{ab*}$	$35.29 \pm 1.51^{ab*}$	$0.34 \pm 0.01^{b***}$
LLG	$56.45 \pm 3.58^{ab*}$	$26.37 \pm 1.67^{ab*}$	0.34 ± 0.01	$60.93 \pm 2.35^{c*}$	$28.47 \pm 1.10^{c*}$	$0.32 \pm 0.01^{b***}$
DLG	$58.96 \pm 2.12^{a*}$	$27.55 \pm 0.99^{a*}$	0.27 ± 0.01	$69.53 \pm 1.81^{abc*}$	$32.49 \pm 0.84^{abc*}$	$0.35 \pm 0.01^{b***}$
P value	0.019	0.019	0.091	0.019	0.019	0.000

[a,b,c]Vertical columns with different letters have significant differences between them.
*: $P < 0.05$ **: $P < 0.01$ ***: $P < 0.001$.
CG: Control group
LOG: Prolonged light olive oil group
DOG: Prolonged dark olive oil group
LWG: Prolonged light water group
DWG: Prolonged dark water group
LLG: Prolonged light lipoic acid group
DLG: Prolonged dark lipoic acid group

BUN levels significantly decreased in only the DWG in comparison with the LWG, LOG, DLG, and CG ($P < 0.05$) in the 1-week period. Intervariance was evident between the LOG and DOG, and between the LWG and DWG in the 4-week period (Table 1). Dark exposure increased BUN levels in the 4-week period, unlike the 1-week period.

There was no significant change in creatinine levels after the 1-week period. There was a significant increase in creatinine levels in the DOG in the 4-week period ($P < 0.001$) (Table 1), and the creatinine levels in all other groups were similar to those in the CG.

In the DOG, there was a significant increase in AST levels in the 4-week period ($P < 0.01$); however, there was no change in AST levels after the 1-week period and in ALT levels in the 1- and 4-week periods (Table 2).

No change was noted in TOS in the 1-week period and in TAS in the 1- and 4-week periods. The LLG and DLG showed decreased TOS in the 4-week period as compared with the CG. The other groups were similar to the CG; however, TOS was significantly higher in the DOG than in the LOG ($P < 0.05$) (Table 3).

There were no changes in cortisol and melatonin levels in the 1- and 4-week periods, as shown in Table 4.

4. Discussion

Nitrogen compounds from amino acids and other sources are excreted as ammonium, urea, or uric acid. One nitrogen atom in urea comes from free ammonium and the other from aspartate (Tokullugil et al., 1997; Sözbilir and Bayşu, 2008). Urea is synthesized in the liver and is transported to the kidneys via the blood, undergoing glomerular filtration and reabsorption during renal passage. Glucagon, glucocorticoids, prolonged fasting, protein-rich diets, and N-acetyl glutamate all increase urea synthesis (Konukoğlu, 2006). BUN is a measure of the nitrogen in urea and is an indicator of decreased effective circulation volume. Several nonrenal causes may affect BUN levels (Set and Şahin, 2003). Dehydration, renal failure, gastrointestinal bleeding, decreased urine output, high-protein diets, steroids, tetracyclines, and catabolic conditions can increase BUN levels. Conversely, liver disease, fasting, anabolic conditions, polyuria, pregnancy, low-protein diets, and the syndrome of inappropriate secretion of antidiuretic hormone can lead to decreased BUN levels (Konukoğlu, 2006). Higher urea and BUN levels were noted in the prolonged dark groups in the 4-week second phase as compared with the prolonged light groups. Considering that laboratory animals are active at night, higher urea and BUN levels can be attributed to the increased amino acid catabolism due to insufficient amounts of carbohydrates to meet increased energy expenditure, leading to gluconeogenesis. A study conducted on goats showed no specific seasonal rhythm of urea (Piccione et al., 2007). However, to our knowledge, there are no studies indicating the effects of experimental photoperiodic changes on urea and BUN level. Our study is novel considering the prolonged photoperiodic intervals.

Table 2. Influence of photoperiod changes implemented over 1- and 4-week period on AST and ALT levels in serum of rats.

GROUP	ONE-WEEK PERIOD		FOUR-WEEK PERIOD	
	ALT (U/L) MEANS ± SE (n = 42)	AST (U/L) MEANS ± SE (n = 42)	ALT (U/L) MEANS ± SE (n = 42)	AST (U/L) MEANS ± SE (n = 42)
CG	57.51 ± 4.78	154.70 ± 10.74	57.06 ± 2.91	134.81 ± 7.39^{bc**}
LOG	51.15 ± 3.16	165.25 ± 18.03	62.31 ± 8.23	138.98 ± 12.32^{bc**}
DOG	52.03 ± 2.90	166.15 ± 22.02	56.51 ± 6.26	178.30 ± 9.02^{a**}
LWG	50.90 ± 2.92	157.8 ± 12.40	61.35 ± 4.16	134.40 ± 11.87^{bc**}
DWG	53.55 ± 3.65	151.45 ± 10.48	57.28 ± 3.35	149.13 ± 10.32^{b**}
LLG	54.46 ± 4.11	154.36 ± 7.05	62.60 ± 2.40	116.71 ± 6.55^{c**}
DLG	52.86 ± 3.86	159.11 ± 17.87	64.06 ± 5.53	140.16 ± 10.81^{bc**}
P value	0.884	0.990	0.888	0.007

^{a,b,c}Vertical columns with different letters have significant differences between them.
*: $P < 0.05$ **: $P < 0.01$ ***: $P < 0.001$.
CG: Control group
LOG: Prolonged light olive oil group
DOG: Prolonged dark olive oil group
LWG: Prolonged light water group
DWG: Prolonged dark water group
LLG: Prolonged light lipoic acid group
DLG: Prolonged dark lipoic acid group

Table 3. Influence of photoperiod changes implemented over 1- and 4-week period on TAS and TOS levels in serum of rats.

GROUP	ONE-WEEK PERIOD		FOUR-WEEK PERIOD	
	TAS (mmol/troloxequiv/L) MEANS ± SE (n = 42)	TOS (μmol H_2O_2 equiv./L) MEANS ± SE (n = 42)	TAS (mmol/troloxequiv/L) MEANS ± SE (n = 42)	TOS (μmol H_2O_2 equiv./L) MEANS ± SE (n = 42)
CG	1.17 ± 0.01	7.91 ± 2.40	1.23 ± 0.02	$5.97 \pm 0.61^{ab*}$
LOG	1.16 ± 0.04	6.45 ± 1.08	1.23 ± 0.05	$4.81 \pm 0.16^{bc*}$
DOG	1.19 ± 0.02	5.87 ± 1.46	1.29 ± 0.02	$6.55 \pm 0.28^{a*}$
LWG	1.14 ± 0.04	5.10 ± 0.46	1.26 ± 0.01	$4.88 \pm 0.63^{bc*}$
DWG	1.09 ± 0.04	5.97 ± 0.66	1.25 ± 0.03	$5.16 \pm 0.15^{bc*}$
LLG	1.12 ± 0.03	5.69 ± 0.96	1.22 ± 0.02	$4.61 \pm 0.33^{c*}$
DLG	1.13 ± 0.06	8.95 ± 1.64	1.26 ± 0.01	$4.54 \pm 0.43^{c*}$
P value	0.746	0.453	0.699	0.018

[a,b,c]Vertical columns with different letters have significant differences between them.
*: P < 0.05 **: P < 0.01 ***: P < 0.001
CG: Control group
LOG: Prolonged light olive oil group
DOG: Prolonged dark olive oil group
LWG: Prolonged light water group
DWG: Prolonged dark water group
LLG: Prolonged light lipoic acid group
DLG: Prolonged dark lipoic acid group

Table 4. Influence of photoperiod changes implemented over 1- and 4-week period on melatonin and cortisol levels in serum of rats.

GROUP	ONE-WEEK PERIOD		FOUR-WEEK PERIOD	
	Cortisol (μg/dL) MEANS ± SE (n = 42)	Melatonin (ng/mL) MEANS ± SE (n = 42)	Cortisol (μg/dL) MEANS ± SE (n = 42)	Melatonin (ng/mL) MEANS ± SE (n = 42)
CG	0.72 ± 0.27	20.91 ± 0.55	0.90 ± 0.20	19.92 ± 0.60
LOG	1.17 ± 0.17	19.93 ± 0.87	1.03 ± 0.35	18.94 ± 0.60
DOG	1.10 ± 0.18	19.81 ± 0.70	1.06 ± 0.29	19.53 ± 0.92
LWG	1.05 ± 0.21	20.28 ± 0.50	1.08 ± 0.26	20.00 ± 0.62
DWG	1.52 ± 0.33	19.79 ± 0.25	0.74 ± 0.24	19.50 ± 0.65
LLG	1.17 ± 0.41	19.75 ± 0.72	0.85 ± 0.24	20.27 ± 0.59
DLG	1.29 ± 0.30	18.68 ± 0.59	0.83 ± 0.21	18.93 ± 0.73
P value	0.624	0.371	0.956	0.751

[a,b,c]Vertical columns with different letters have significant differences between them.
*: P < 0.05 **: P < 0.01 ***: P < 0.001.
CG: Control group
LOG: Prolonged light olive oil group
DOG: Prolonged dark olive oil group
LWG: Prolonged light water group
DWG: Prolonged dark water group
LLG: Prolonged light lipoic acid group
DLG: Prolonged dark lipoic acid group

Creatine synthesis starts in the kidneys; creatine then enters the muscle tissue via a transport system. It undergoes glomerular filtration and is abundantly reabsorbed from the proximal tubules. Approximately 2% of creatine is converted to creatinine; however, the amount of creatinine produced is related to muscle mass and can be increased by meat consumption, acute infection, injuries, emotional stress, heavy exercise, acute muscle damage, and increased muscle mass (Konukoğlu, 2006). Serum creatinine levels are also used as an indicator of kidney function because they increase in renal diseases. In our study, creatinine levels increased in the 4-week period in the DOG, which may have been due to oxidative stress. However, it could also be associated with increased muscle mass in rats induced by increased physical activity.

Transaminases are enzymes that form α-keto acids from α-amino acids. ALT and AST are the 2 main enzymes of this group and are often measured for diagnostic purposes. Both are intracellular enzymes and are abundant in hepatocytes. ALT and AST are released from damaged hepatocytes into the blood following hepatocellular injury (Ersoy, 2012). These enzymes increase glucose synthesis from noncarbohydrates taking part in gluconeogenesis (Friedman et al., 1996). AST produces aspartate during amino acid catabolism with the amino groups being transferred to oxaloacetate; aspartate, the product, then enters the urea cycle. During amino acid catabolism, ALT synthesizes glutamate, which acts as a nitrogen collector (Tokullugil et al., 1997; Ersoy, 2012). Serum ALT and AST levels increase in almost all liver diseases, and are also elevated in diabetic and obese patients. Fatty acids mobilized from the adipose tissue accumulate in the liver after excessive dietary fat intake. Further, fatty acid catabolism forms acetyl-CoA, which inhibits pyruvate dehydrogenase and activates pyruvate carboxylase, which converts pyruvate into oxaloacetate. Oxaloacetate is then used in gluconeogenesis instead of entering the tricarboxylic acid cycle. Aspartate is obtained from the transamination of oxaloacetate by AST (Champe et al., 2007). In the present study, there was a significant increase in AST levels in the DOG in the 4-week period. AST elevation in the DOG increases aspartate formation from oxaloacetate following increased amino acid catabolism due to gluconeogenesis. The increased formation of oxidative stress products in hepatocytes as a result of fatty acid oxidation may cause hepatocyte damage; increased hepatocyte permeability may lead to the migration of AST outside the cell, thus elevating serum levels. TOS, an indicator of oxidative stress, was also increased in the DOG. AST levels in the DLG were similar to those in the CG, probably due to the preventive effects of α-LA on hepatocyte damage (Zacharias, 2003; Kumar et al., 2006). A study by Karaca and Sözbilir (2007) indicated that 100 mg/kg per day of α-LA prevented the elevation of AST levels.

Glucocorticoid secretion has a circadian rhythm. Cortisol secretion is managed by the hypothalamic–pituitary–adrenal axis, and some factors in this axis can affect cortisol synthesis (Johansson et al., 2003), including physiological and psychological stress factors (Johansson et al., 2003; Kılıçoğlu and Gülcan, 2007). Another factor is light; cortisol secretion is diurnal and is regulated by the corticotropin-releasing hormone. The minimum and maximum levels are found in the morning and at night, respectively (Kılıçoğlu and Gülcan, 2007). Lemos et al. (2009) determined cortisol levels hourly in monkeys in groups exposed to 8/16 L/D, 12/12 L/D, and 16/8 L/D photoperiods and reported that maximum cortisol levels were prolonged in the 8/16 L/D group. Moreover, Johansson et al. (2003) drew blood samples from goats every 2 h over a 24-h period to study seasonal cortisol levels and found that the winter-like seasonal photoperiod led to a higher consistency of elevated cortisol levels as compared with that in early spring-, late spring-, summer-, and late autumn-like photoperiods. The elevation in the winter-like photoperiod was more prominent after midnight. Seasonal changes in cortisol levels were reported; however, the cortisol levels did not significantly change after turning the lights on or off at any time of the year. Pawlak et al. (2009) studied photoperiod-related changes in hamsters by measuring cortisol levels in response to short and long days at the beginning of the light period. The cortisol levels were higher for the shorter days than for the longer days, with the hamsters perceiving shorter days as chronic stress. Gutzler et al. (2009) applied 14/10 L/D and 8/16 L/D photoperiods in hamsters and obtained hourly blood samples 3 h before entering the nocturnal period with continued sampling until the fourth hour of the nocturnal period. Cortisol concentrations were significantly lower 2–3 h after turning the lights off for hamsters exposed to a shorter day as compared with those exposed to a longer day. Shorter days led to the suppression of the cortisol rhythm. The authors claimed that shorter days may affect the hypothalamic–pituitary–adrenal axis. However, studies emphasize higher cortisol levels in shorter days. We found that photoperiodic changes did not affect cortisol rhythm and that hormone levels were similar across all groups, suggesting that the cortisol rhythm and photoperiodic change durations did not cause metabolic stress in our study. However, unlike the above-mentioned studies, we obtained a single blood sample at the end of the dark period. Frequent blood sampling to analyze cortisol levels in the above studies during both daytime and nighttime periods may explain the differences in the results between previous studies and our study, in which hormone determination at the end of the dark period was performed only once.

Melatonin is synthesized and secreted at night in all organisms, and secretion is dependent on the length of the nocturnal period. In other words, the longer the nighttime period, the longer melatonin is secreted for. The period of melatonin secretion is longer for shorter days and shorter for longer days (Çam and Erdoğan, 2003). Light exposure at night reportedly decreases melatonin secretion (Srinivasan et al., 2008). Pawlak et al. (2009) studied photoperiod-associated changes in hamsters for both short and long days and determined melatonin levels in the middle of the light period, 2 h after turning the lights off, in the middle of the dark period, and 2 h before turning the lights on. The melatonin elevation was delayed through the end of the dark period for short days, whereas this elevation occurred in the middle of the dark period in hamsters exposed to long days. The plasma melatonin levels in the middle of the light period were significantly higher in the short-day group as compared with those in the long-day group, unlike the levels in the middle of the dark period, which showed no significant differences between the 2 groups. Yerer and Aydoğan (2006) exposed rats to 0/24 L/D, 24/0 L/D, 8/16 L/D, 16/8 L/D, and 12/12 L/D photoperiods, and the highest melatonin levels were reported in the 0/24 L/D group. The plasma melatonin levels decreased with increasing exposure to light in other groups, and the melatonin levels of rats with constant dark exposure were similar to those in the CG. Another study found prolonged elevation of melatonin levels in the short-day group as compared with those in the long-day group (Gündüz, 2002), and the melatonin levels remained elevated for the whole dark period in both the short- and long-day groups. There were no changes in the melatonin levels of the hamsters exposed to light for 24 h. Edmonds et al. (1995) determined melatonin levels in rats exposed to 10/14 L/D, 12/12 L/D, 14/10 L/D, and 16/8 L/D photoperiods; the melatonin levels remained at basal levels during the day, started increasing after the lights were turned off, and remained elevated during nighttime. The melatonin levels remained elevated until the beginning of the day period, particularly in the 10/14 L/D group, and significantly decreased 1 h after turning the lights on. The melatonin levels were determined every 20 min during the first hour in the 16/8 L/D group and were found to gradually increase during the first hour with a significant decrease 20 min after turning the lights on. The photoperiod rhythm did not affect the melatonin secretion cycle. Unlike the above study, we only determined melatonin levels once at the end of the dark period. These findings suggest that neither metabolic nor endocrinological cycles are affected by these applications or that changes in melatonin cannot be recognized with a single measurement.

Oxygen is vital for cell functions due to its key role in oxidation. However, high oxygen concentrations have toxic potential for cells (Gökpınar et al., 2006), and the most important free radicals are those formed by oxygen (Mercan, 2004). Oxygen turns into water after it is reduced, forming reactive metabolites during the intermediary reaction steps (Gökpınar et al., 2006). While the measurements taken at the end of the nocturnal period before the initiation of the light cycle showed higher TOS levels in the DOG than in the CG, no statistically significant difference was found. There was a prominent increase in the DOG in the interanalysis of the prolonged dark period groups. The elevation in the prolonged dark period and olive oil groups may be associated with the use of night-active rats in the present experiment, which then resulted in the increased expenditure of energy and use of olive oil for energy. This consequently led to increased oxygen consumption and an increase in the midproducts during the conversion of oxygen to water. The DOG also exhibited higher neutrophil counts than the other groups, although this was not statistically significant. TOS may also increase secondary to neutrophil activation. The antioxidant properties of α-LA did not result in higher neutrophil counts in the prolonged dark group administered α-LA-treated olive oil; in fact, the neutrophil counts in this group were lower than those of the CG rats. Baran et al. (2000) studied the effects of photoperiodic changes on free radical levels in rats and reported that superoxide dismutase, catalase, and glutathione levels decreased, glutathione peroxidase levels increased after 48 h, catalase levels increased after 72 h, and malondialdehyde levels were highly elevated.

In conclusion, 2-day periods of 16/8 L/D and 8/16 L/D photoperiods did not cause measurable acute or chronic stress in rats. Prolonged dark periods and secondary increased metabolism enhance the oxidation of nutritional fatty acids, leading to the increased formation of oxidative stress products. It is considered that oxidation triggers the elevation of AST levels, and α-LA is effective in suppressing the oxidative status at the CG level. As the period of activity in the animals is prolonged, amino acid catabolism may increase, contributing to gluconeogenesis, the process of glucose synthesis from noncarbohydrate resources, and therefore increasing urea and BUN levels in rats exposed to prolonged dark periods as compared with those exposed to prolonged light periods. Interval photoperiodic changes did not cause statistically significant changes in stress markers either acutely or in the long term, suggesting that the source of stress is related to different factors. New studies on rats and in humans are needed to confirm the findings of the present study.

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