

# The influence of intermittent fasting on the circadian pattern of melatonin while controlling for caloric intake, energy expenditure, light exposure, and sleep schedules: A preliminary report

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## Abstract:

**AIMS:** We hypothesized that if we control for food composition, caloric intake, light exposure, sleep schedule, and exercise, intermittent fasting would not influence the circadian pattern of melatonin. Therefore, we designed this study to assess the effect of intermittent fasting on the circadian pattern of melatonin.

**METHODS:** Eight healthy volunteers with a mean age of  $26.6 \pm 4.9$  years and body mass index of  $23.7 \pm 3.5$  kg/m<sup>2</sup> reported to the Sleep Disorders Center (the laboratory) on four occasions: (1) adaptation, (2) 4 weeks before *Ramadan* while performing Islamic intermittent fasting for 1 week (fasting outside *Ramadan* [FOR]), (3) 1 week before *Ramadan* (nonfasting baseline [BL]), and (4) during the 2<sup>nd</sup> week of *Ramadan* while fasting (*Ramadan*). The plasma levels of melatonin were measured using enzyme-linked immunoassays at 22:00, 02:00, 04:00, 06:00, and 11:00 h. The light exposure, meal composition, energy expenditure, and sleep schedules remained the same while the participants stayed at the laboratory.

**RESULTS:** The melatonin levels followed the same circadian pattern during the three monitoring periods (BL, FOR, and *Ramadan*). The peak melatonin level was at 02:00 h and the trough level was at 11:00 h in all studied periods. Lower melatonin levels at 22:00 h were found during fasting compared to BL. Cosinor analysis revealed no significant changes in the acrophase of melatonin levels.

**CONCLUSIONS:** In this preliminary report, under controlled conditions of light exposure, meal composition, energy expenditure, and sleep-wake schedules, intermittent fasting has no significant influence on the circadian pattern of melatonin.

## Keywords:

Circadian rhythm, fasting, light exposure, meals, Ramadan, sleep

The month of *Ramadan*, the 9<sup>th</sup> month of the Muslim lunar calendar, is a unique model of intermittent fasting, where people abstain from food and drink between dawn and sunset. Moreover, *Ramadan* is accompanied by several personal lifestyles

and societal changes, including a delay in starting work, nocturnal prayers, and the opening of stores and shopping malls until predawn time.<sup>[1]</sup> Furthermore, habits and

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meal frequency and timing change during *Ramadan*, for example, some cultures prefer fried foods and consume an excessive amount of sweets.<sup>[1]</sup> Studies have shown that high fat and sugar intake can lead to changes in clock gene expression in the suprachiasmatic nucleus (SCN) and other peripheral brain nuclei (non-SCN brain nuclei).<sup>[2]</sup> During *Ramadan*, the usual circadian pattern of eating shifts suddenly, which results in an increase in caloric intake at night. The increased caloric intake at night has been proposed as a mechanism that possibly affects the circadian rhythm and results in a loss of the temporal control (circadian disruption) of metabolic processes during fasting,<sup>[3]</sup> given that the timing of meals and glucose levels have been shown to affect the circadian rhythm.<sup>[4-6]</sup>

The melatonin rhythm is one biological marker of circadian rhythmicity, which conveys messages to the body about the daily light/dark cycle.<sup>[7]</sup> Although several tissues in the body produce melatonin, the circulating neurohormone is derived almost exclusively from the pineal gland.<sup>[8]</sup> Melatonin is believed to be one of the best markers for circadian rhythm disruption because individual melatonin profiles are highly reproducible and are less affected by masking factors than are other markers of circadian rhythms, such as core body temperature or cortisol level.<sup>[9]</sup>

Two previous studies have suggested a shift delay in the peak of melatonin during *Ramadan*. However, neither study controlled for food intake, sleep/wake schedule, energy expenditure (exercise) nor the social habits that accompany *Ramadan*. In addition, there was no proper control for light exposure. Moreover, in both studies, the melatonin concentrations were not measured in the early morning, thus neglecting possible early morning peaks in melatonin concentration. Thus, whether Islamic intermittent fasting influences the circadian pattern of circulating melatonin remains undefined. We hypothesized that if we control for the above confounders, *Ramadan* daytime fasting will not influence the circadian pattern of circulating melatonin. Therefore, we designed the present study to assess the circadian pattern of plasma melatonin during and outside the *Ramadan* month while controlling for the sleep/wake schedule, sleep duration and quality, meal composition, energy expenditure, and light exposure.

## Methods

### Subjects

This study is a part of a research project to assess the effects of intermittent fasting on sleep architecture, circadian rhythm, hormonal changes, and different cellular biomarkers.<sup>[10,11]</sup> However, the current melatonin data have not been reported before. The study protocol

has been described in details before.<sup>[10]</sup> Eight nonsmoking, healthy Muslim male volunteers between the ages of 20 and 35 years (weight =  $69.1 \pm 8.4$  kg) provided written informed consent to participate in the study. Participants were informed of the project through advertising on bulletin boards in the university. The advertisement clearly stated that the volunteers were being recruited for research purposes. The selected volunteers were not on any medications, did not drink alcohol, did not perform shift work, and were not on vacation during the study period. None of the participants had sleep complaints or was on any dietary program. In addition, the selected volunteers were not practicing fasting as routine voluntary rituals before. Moreover, all participants have regular sleep/wake schedule and have morning chronotype confirmed by a validated Arabic version of the reduced Horne and Ostberg morningness-eveningness questionnaire.<sup>[12,13]</sup> The regularity of the sleep/wake schedule was assessed by wrist actigraphy monitoring (Philips/Respironics, Inc., Murrysville, Pennsylvania, USA) in all participants for 1 week (it was worn on the nondominant wrist). A regular sleep/wake schedule was defined as <1 h of daily variability in the bedtime and wake-up time.<sup>[10]</sup> None of the participants traveled 2 weeks prior to or during the study. Their work hours were from 07:30 to 16:30 h before *Ramadan* and from 10:00 to 15:00 h during *Ramadan*. The participants were instructed not to wear sunglasses during the study period.

The study protocol was approved by the College of Medicine Institutional Review Board at King Saud University, and written informed consent was obtained from the participants.

### Study protocol

This study was performed during the last week of *Rajab* (month 7, *Hijri*), the first and last weeks of *Shaban* (month 8, *Hijri*), and the 2<sup>nd</sup> week of *Ramadan* (month 9, *Hijri*). The study period corresponded to the dates between June 25 and August 15, 2011, on the Gregorian calendar. Participants reported to the Sleep Disorders Center (the laboratory) on 4 occasions. During each visit, the participant spent 24 h at the laboratory and underwent overnight polysomnography (PSG) [Figure 1]:

#### Initial visit

The participants came to their initial visit during the last week of *Rajab* (month 7, *Hijri*) to receive a medical checkup, to adapt to the sleeping environment and equipment, and to undergo an overnight sleep study (not included in the analysis) to reduce the “first night effect”, which is known to affect the sleep architecture of the first night of PSG recording<sup>[14]</sup> and to rule out sleep disordered breathing. All the participants were given an actigraph

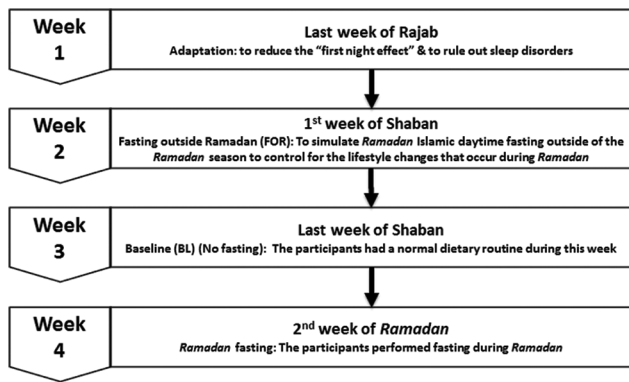


Figure 1: Study protocol

to wear on their nondominant wrist, to wear at home to monitor their sleep/wake pattern.<sup>[15]</sup> The body weight was monitored during the study period.

### Fasting outside Ramadan

This fasting period is not an element of the normal routine of religious *Ramadan* fasting. We introduced this protocol to simulate *Ramadan* Islamic daytime fasting outside of the *Ramadan* season to control for the lifestyle changes that occur during *Ramadan*, such as changes in the sleep/wake pattern, sleep duration, physical activity, and meal composition, which may influence melatonin secretion and circadian rhythmicity. This fasting period was completed 3 weeks before *Ramadan* to avoid any overlap with the baseline (BL) (nonfasting) or the *Ramadan* fasting and any carryover effects. The participants performed fasting for 7 days during the 1<sup>st</sup> week of the *Shaban* (month 8, *Hijri*). The participants reported to the laboratory for the last 24 h of the fasting week.

### Baseline (nonfasting)

This visit occurred during the last week of the *Shaban* (month 8, *Hijri*) (BL without fasting). The participants had a normal dietary routine during this week and reported to the laboratory for the last 24 h of the week.

### Ramadan (R)

This visit to the laboratory occurred during the last day of the 2<sup>nd</sup> week of *Ramadan* (month 9, *Hijri*) after fasting for 2 weeks.

The details of the protocol are described in previous publications.<sup>[10,11]</sup> Participants reported to the laboratory at approximately 18:00 h. During the laboratory stay, the participants received meals with fixed caloric intake (35 kcal/kg/24 h) based on their ideal body weight, and fixed proportions of carbohydrates, fats, and proteins. This was organized by a dietitian. Food items containing large amounts of 5-hydroxytryptamine or melatonin were avoided on the study nights and days.<sup>[16]</sup>

### Meal timing

During BL, the participants had three meals: dinner at 20:00 h, breakfast at 07:15 h, and lunch at 12:00 h mid-day. During fasting outside *Ramadan* (FOR) and *Ramadan*, the participants had the following meals: breakfast (at sunset, between 18:30 and 18:55 h), dinner at 21:00 h, and suhur before dawn (between 03:00 and 03:15 h).

### Blood specimen collection

On the day of admission to the laboratory, at 18:00 h, an intravenous cannula was placed in the antecubital vein for blood withdrawal. Blood samples were collected at 22:00, 02:00, 04:00, 06:00, and 11:00 h. The samples were immediately centrifuged at 4°C and stored at -70°C.

### Fasting protocol

The fasting protocol has been described elsewhere.<sup>[10,11]</sup> During the FOR period, the participants were asked to fast for the 1<sup>st</sup> week of *Shaban* (7 days) to assess the effects of Islamic intermittent fasting in the absence of the lifestyle changes and eating habits that occur during *Ramadan*. During the following 3 weeks of *Shaban*, the participants maintained their routine activities and eating habits. The participants performed fasting during the month of *Ramadan*. During the FOR period, dawn (the beginning of fasting) occurred at approximately 03:37 h and sunset (the end of fasting) occurred at approximately 18:47 h, and during the 2<sup>nd</sup> week of *Ramadan*, dawn occurred at approximately 04:00 h and sunset occurred at approximately 18:34 h.

### Polysomnography

A standard PSG recording was performed.<sup>[11]</sup> During PSG, the following parameters were monitored: four leads of electroencephalography (EEG) (EEG: C1-A4, C2-A3, O1-A4, and O2-A3), electrooculography, chin electromyography, electrocardiography, oxygen saturation, chest and abdominal wall movements, air flow (thermistor), and sleep position. Scoring was performed manually according to established scoring criteria.<sup>[17]</sup> The PSG recordings are described in a previous publication.<sup>[11]</sup>

The participants were asked to avoid any naps during the study day in the laboratory. During BL, the PSG recording began at 23:00 and was concluded at 07:00 h. During FOR and *Ramadan*, the PSG recording began at 23:00 h. During *Ramadan*, the participants had the suhur meal at 03:00 h, and the study was resumed from 03:45 until 07:45 h. During FOR, the participants had suhur at 03:15 h (to account for the shift in the dawn prayer time), and the study was resumed from 04:00 until 07:45 h. Alice 5 diagnostic equipment was used for data acquisition (Philips/Respironics, Inc., Murrysville, Pennsylvania, USA). The arousal index,

which is a measure of sleep fragmentation, was defined as the number of arousals per 1 h of sleep. "Stage shifts" was defined as the total number of changes in sleep stage from lights out to lights on.

### Light exposure in the laboratory

While in the laboratory, light exposure was controlled during the three study periods (BL, FOR, and *Ramadan*). From 18:00 h until bedtime, the light level was maintained at 50 lux. During the PSG recording, all lights were turned off and the light level was <1 lux. During suhur meal, light intensity was maintained <30 lux.<sup>[18]</sup> The light intensity was measured using SpectralStar Light Meter LX-1 (Japan).

### Energy expenditure measurement

To control for the effect of exercise on melatonin secretion,<sup>[19]</sup> the energy expenditure was measured while the participants were at the laboratory using a SenseWear Pro Armband™ (Body Media, Pittsburgh, Pennsylvania, USA). The technical characteristics of the armband have been previously described.<sup>[20,21]</sup> Briefly, the armband is a small portable sensing device worn on the triceps of the right arm. The device provides information regarding the total energy expenditure and total sleep time. The sensors in the armband measure movements, skin temperature, galvanic skin response, and heat flux from the body. The measured physiological data were processed by advanced algorithms to calculate the total energy expenditure.<sup>[22]</sup> The accuracy of the armband has been validated in many studies and comparisons with double-labeled water and metabolic carts.<sup>[22-24]</sup>

### Determination of melatonin levels

Plasma melatonin levels were determined by an enzyme-linked immunoassay (GenWay Biotech Inc., San Diego, California, USA). The limit of detection was 1.6 pg/mL, and the mean recovery was 102.4% (83%–125%). The intra-assay and inter-assay precision were 8.8–151.7 pg/mL and 5.6–134.3 pg/mL, respectively (the intra-assay and inter-assay coefficients of variation were 3.0%–11.4% and 6.4%–19.3%, respectively). The measurement procedure was carried out according to the manufacturer's instructions. During the blood sample collection, the illumination level was always <30 lux. The samples were constantly kept on ice and protected from light radiation to avoid melatonin degradation. All samples for melatonin were run in duplicate.

### Statistical analysis

The data are expressed as the mean ± standard deviation. Energy expenditure was expressed as metabolic equivalents (METs). The average METs were calculated to obtain an overall hourly average for each day.

To obtain the best estimates of the overall acrophase for melatonin, we used a 24 h cosinor rhythmometry model ( $f(t) = M + A \times \text{Cos}\left(\frac{2\pi t}{T} + \emptyset\right)$ , where  $M$  = mesor;  $A$  = amplitude;  $\emptyset$  = acrophase, and  $T = 24$  h.<sup>[25,26]</sup> The model was implemented for each participant's data during the three periods. Comparisons of the BL, FOR, and *Ramadan* fasting groups were performed using a repeated measures (RMs) ANOVA. When the normality test failed, Friedman's RM ANOVA on ranks test was used. *Post hoc* analyses were performed to determine the significant differences between individual groups. The results were considered statistically significant for  $P < 0.05$ . The data were analyzed using Statistical Package for Social Sciences (SPSS), IBM version 22 (SPSS Inc., Chicago, IL, USA) software.

## Results

The study group had a mean age of  $26.6 \pm 4.9$  years and a body mass index of  $23.7 \pm 3.5$  kg/m<sup>2</sup>. No differences were detected in body weight during the three study periods:  $69.1 \pm 8.4$  kg,  $67.5 \pm 7.8$  kg, and  $66.3 \pm 12.3$  kg for BL, FOR, and *Ramadan*, respectively. Moreover, no differences were found in serum glucose measurements at 15:30 between BL, FOR, and *Ramadan* ( $6.1 \pm 0.5$ ,  $5.7 \pm 0.4$ , and  $5.8 \pm 0.5$  mmol/L, respectively).

Table 1 shows the sleep pattern at home. There was a significant delay in bedtime and wake-up time during *Ramadan* due to the accompanying delay in the start time of work. No significant differences in bedtime and wake-up time were found between BL and FOR. No differences were detected between the nocturnal sleep duration and total sleep duration (nocturnal sleep and naps) among the three study periods.

Table 2 shows the sleep parameters and the energy expenditure during monitoring in the laboratory. The sleep efficiency was relatively lower than normal (BL =  $79.3\% \pm 9.6\%$ , FOR =  $70.7\% \pm 11.1\%$ , and *Ramadan* =  $74.2\% \pm 7.3\%$ ), which could be related to the disruptive effect of blood collection. Otherwise, no changes were detected in sleep parameters. Energy expenditure was reported for 24 h (day and night), during the daytime (from dawn to sunset), and at night (sunset to dawn). No significant changes were

**Table 1: Sleep pattern while at home**

	BL	FOR	<i>Ramadan</i>	P
Mean bedtime±SD (24 h)	00:36	01:30	02:42*	0.004*
Mean wake-up time±SD (24 h)	05:30	06:12	08:48*	0.034*
Nocturnal sleep duration (h)	5.3±1.2	5.3±1.2	5.1±1.3	0.134
Nocturnal sleep duration + naps (h)	5.9±1	6.2±2.9	7±2.9	0.539

\*Indicates p-value is significant compared to BL. SD = Standard deviation, BL = Baseline, FOR = Fasting outside *Ramadan*



**Table 2: Sleep parameters and energy expenditure during monitoring at the Sleep Disorders Center (the laboratory)**

	BL	FOR	Ramadan	P
Sleep parameters during monitoring at the laboratory using polysomnography				
Total sleep time (min)	375.8±46.4	333.0±59.8	353.9±42.7	0.165
Sleep efficiency	79.3±9.6	70.7±11.1	74.2±7.3	0.336
Sleep latency	29.6±24.2	33.7±33.6	32.8±29.3	0.224
Arousal index	9.1±3.6	8.9±4.8	7.9±3.3	0.369
Stage shifts	66.3±13.9	74.9±20.5	64.1±16.7	0.327
Energy expenditure based on the SenseWear Pro Armband				
Energy expenditure (24 h) (kcal/min)	1.6±0.2	1.6±0.2	1.6±0.2	0.846
Energy expenditure (daytime) (kcal/min)	1.7±0.3	1.6±0.2	1.5±0.2	0.069
Energy expenditure (night) (kcal/min)	1.5±0.1	1.6±0.2	1.6±0.2	0.260
METs	1.4±0.2	1.4±0.1	1.3±0.2	0.738
METs (day)	1.4±0.2	1.3±0.1	1.3±0.1	0.069
METs (night)	1.3±0.2	1.4±0.1	1.4±0.2	0.311

BL = Baseline, FOR = Fasting outside Ramadan, METs = Metabolic equivalents

found in energy expenditure during the three monitoring periods (BL, FOR, and Ramadan).

Figure 2 shows the pattern of melatonin concentrations during BL, FOR, and Ramadan fasting. Clearly, the melatonin levels followed the same circadian pattern during BL, FOR, and Ramadan. During the three study periods (BL, FOR, and Ramadan), the melatonin levels showed the same peak level at 02:00 h. The documented trough level was at 11:00 h in all studied periods (BL, FOR, and Ramadan). However, the plasma melatonin level at 22:00 h was lower during fasting (FOR and Ramadan) than at BL, with a significantly lower level for Ramadan versus BL ( $28 \pm 18.7$  pg/mL versus  $25.7 \pm 19.5$  pg/mL, respectively,  $P < 0.05$ ). Moreover, at 11:00 h, the melatonin levels were significantly higher during FOR than at BL ( $28 \pm 18.7$  pg/mL versus  $25.7 \pm 19.5$  pg/mL, respectively,  $P < 0.05$ ). Cosinor analysis of the melatonin plasma levels [Table 3] revealed no significant changes in the acrophase during the three periods.

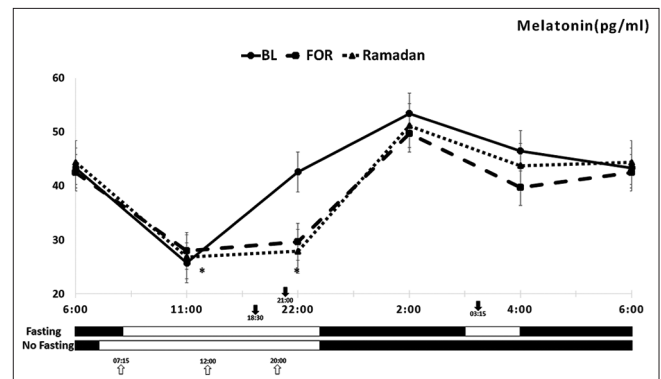
### Discussion

Several lifestyle changes that accompany Ramadan fasting, such as changes in the sleep/wake pattern, sleep duration, physical activity, and meal composition, may influence melatonin secretion and circadian rhythmicity.<sup>[1,21,27,28]</sup> Indeed, current evidence shows that changes such as light exposure,<sup>[29]</sup> sleep/wake schedule and sleep duration,<sup>[29]</sup> meal composition,<sup>[16]</sup> and exercise<sup>[19]</sup> influence plasma melatonin levels. The present study was designed to assess the changes in the plasma levels of melatonin during intermittent fasting, while controlling for potential confounders that might affect the levels of the hormone, such as the sleep/wake schedule, sleep duration and quality, physical activity, light exposure, and meal composition. Moreover, to control for potential confounders that accompany the

**Table 3: Cosinor summary of plasma melatonin circadian rhythm during baseline, baseline fasting, and Ramadan**

Melatonin circadian rhythm	BL	FOR	Ramadan	P
Mesor	35.9±11.7	31.1±15.8	30.1±16	0.197
Amplitude	15.5±16.6	14.5±14.6	18.1±26	0.325
Acrophase	5.5±7.5	4.0±2.1	4.0±1.7	0.417

BL = Baseline, FOR = Fasting outside Ramadan



**Figure 2:** Circadian pattern of plasma melatonin concentrations in eight healthy male volunteers before and during Ramadan daytime fasting. Each point represents the mean and standard deviation of eight participants. Values at baseline (...♦...), baseline fasting before Ramadan (---■---), and Ramadan (-▲-) are presented. The dark-headed arrows indicate the meal time during fasting (Ramadan and baseline fasting), and the white-headed arrows indicate the meal time during baseline. (□) indicates awake, and (■) indicates sleep

lifestyle changes during Ramadan, we assessed the effects of intermittent Islamic FOR. To our knowledge, this is the first study to assess the effects of intermittent Islamic fasting on melatonin plasma concentrations outside of the Ramadan season.

The main finding of this study is that melatonin levels followed the same circadian pattern during BL, FOR, and Ramadan, although there was a decrease at 22:00 h during the 2<sup>nd</sup> week of Ramadan compared to BL [Figure 1].

However, in our study, when a cosinor model was used; no shift delay was detected in the acrophase during the three periods.

During *Ramadan*, food and fluid intake are shifted to the hours of darkness, thus partially reversing the normal circadian pattern of eating and drinking. In theory, these changes could affect circadian rhythmicity in fasting individuals, with effects including changes in several hormone systems and the core body temperature.<sup>[1]</sup> A limited number of studies has objectively assessed the circadian rhythm changes during *Ramadan*, and the results suggested a shift delay in the circadian rhythmicity of body temperature.<sup>[3,21,30]</sup> In a well-designed study, Roky *et al.* continuously recorded body temperature for 24 h in six healthy participants during *Ramadan* using a rectal thermistor probe and reported delays in both the occurrence of the acrophase and bathyphase of body temperature (i.e., the times at which the calculated maximum and minimum values, respectively, occur in the cycle).<sup>[30]</sup> The shift delay in core body temperature was attributed to the shift to night time meals during *Ramadan*.<sup>[30]</sup> Whether a shift delay also occurs in the circadian rhythm of melatonin secretion remained unknown.

Theoretically, changes in the circadian pattern of body temperature during *Ramadan* should also be associated with alterations in the circadian pattern of melatonin secretion, although some studies suggested that shifting the meal time influences the core body temperature rhythm but not the melatonin rhythm.<sup>[31,32]</sup> To date, two studies have assessed melatonin secretion during *Ramadan*. In the first study, the plasma melatonin levels were measured in blood samples obtained every 4 h (omitting the 02:00 time point to avoid sleep disturbances) before and on the 23<sup>rd</sup> day of *Ramadan*.<sup>[33]</sup> The investigators reported a smaller and delayed night peak and a flatter slope in serum melatonin concentrations during *Ramadan*.<sup>[33]</sup> In the second study, saliva samples for melatonin were collected from eight healthy volunteers at three-time points over a 24 h period (08:00, 16:00, and 00:00) 1 week before *Ramadan* and on the 7<sup>th</sup> and 21<sup>st</sup> days of *Ramadan*.<sup>[28]</sup> A significant decrease in melatonin concentrations at 00:00 and 16:00 h was reported during *Ramadan* compared to BL levels; however, similar trends in melatonin profiles were observed during *Ramadan* but with a flatter slope.<sup>[28]</sup> A major limitation in those previous two studies was that melatonin was not measured in the early morning hours, which overlooks the possibility of a late peak in melatonin concentrations. Moreover, neither study properly controlled for differences in light exposure, sleep schedule, or the social habits that accompany *Ramadan*.

To overcome the shortcomings of these studies, we assessed circadian patterns in melatonin during intermittent fasting outside the month of *Ramadan* and during the 2<sup>nd</sup> week of *Ramadan* after controlling for light exposure, sleep schedule, sleep duration, and meal composition. A trough in melatonin levels was observed at 11:00 h and a peak at 02:00 h during all studied periods, which indicates that intermittent fasting has no significant influence on the circadian pattern of melatonin.

The current study concurs with the previous studies<sup>[28,33]</sup> in reporting lower nocturnal melatonin levels. In general, most studies consistently found a decrease in nocturnal melatonin concentrations during fasting, even during short-term experimental fasting.<sup>[34-36]</sup> Although the exact mechanisms involved in the reduction of melatonin levels during fasting are unknown, this finding could be related to the shift in meal time. In a study that assessed the effects of a single morning and evening carbohydrate-rich meal for 3 consecutive days on the circadian phase of salivary melatonin rhythms under controlled constant routine conditions in ten healthy participants, the nocturnal melatonin secretion after the last morning meal was elevated compared to that for the evening meal.<sup>[31]</sup> Another plausible mechanism is the rise in nocturnal cortisol levels during *Ramadan*, which could be associated with a drop in melatonin levels.<sup>[37,38]</sup> In addition, the decrease in melatonin synthesis could be due to a decrease in glucose supply.<sup>[39]</sup> The latter hypothesis is supported by findings showing that melatonin levels are decreased secondary to mild hypoglycemia and that glucose supplementation during fasting ameliorates the decrease in melatonin levels.<sup>[35]</sup> Nevertheless, hypoglycemia is not believed to occur in healthy individuals during *Ramadan* fasting.<sup>[28,40,41]</sup> Moreover, glucose level did not change significantly in the current study.

Although the present study was conducted under well-controlled conditions, the meal time during fasting periods could not be matched to the BL because the shift in meal time is an essential part of the religious practice during fasting. The effect of meal time on melatonin levels in humans remains undefined. Indeed, there are very few uncontrolled studies investigating the effects of meal timing on circadian rhythms in humans.<sup>[31]</sup> In shift workers, meal timing does not appear to be a strong synchronizer of circadian rhythms.<sup>[42]</sup> In fact, daily meal timing does not usually entrain circadian rhythms controlled by the SCN clock in the presence of the photic synchronizer.<sup>[43,44]</sup> In contrast to the peripheral organs, the SCN clock seems to be invulnerable to feeding time and remains in phase with the light-dark cycle.<sup>[45,46]</sup>

A number of limitations of the present study must be considered. An important one is the relatively small number of participants examined due to the time constraints of the study protocol. Nevertheless, small participant numbers are typical in studies that use objective continuous assessment methods under controlled conditions and must be conducted within a limited time (the month of *Ramadan*) as both of these factors limit the number of recruited volunteers.<sup>[10,21,28,30,31,33]</sup> Second, the fact that the participants were recruited within a university setting, which may limit how well the sample represents the general population since individuals in this group are more likely to be more educated than the general public. Third, females were not included as females have to break their fast while having menstruation.

## Conclusion

Our investigation of plasma melatonin concentration in eight fasting participants during and before Ramadan revealed that under controlled conditions of light exposure, meals, and sleep-wake schedules, intermittent fasting does not significantly influence the circadian pattern of circulating melatonin. Larger studies are needed to assess the circadian pattern of melatonin during intermittent diurnal fasting while controlling for potential confounders.

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## Conflicts of interest

There are no conflicts of interest.

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