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The Association between People's Chronotype and Hormones Related to Appetite

Samira Rabiei; PhD [1], Mazyar Haghgoo; MSc [2], Nasrin Borumandnia; PhD [3], Javad Nasrollahzadeh; PhD [2], Ehsan Hejazi; PhD [2] & Hakimeh Sadeghzadeh; MSc [*2]

[1] *Department of Nutrition Researches, National Nutrition & Food Technology Research Institute and School of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
[2] *Department of Clinical Nutrition, School of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
[3] *Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

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Corresponding author:
sadeghzaadeh87@gmail.com
Department of Clinical Nutrition, School of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Postal code: 1981619573
Tel: +98 2122357483

ABSTRACT

Background: Recently, it has been suggested that people's chronotype is involved in regulating food intake and obesity. This study aimed to investigate the association between chronotypes with hormones related to appetite in adults in Tehran. **Methods:** This cross-sectional study was conducted on 353 men and women aged 18-60 years using convenience sampling in Tehran municipality community centers. Information on morningness-eveningness choronotype and physical activity (PA) level were collected by validated questionnaires. The data for anthropometric measurements and 3-day food recalls were also collected. ELISA method was used to assess Neuropeptide Y (NPY) and leptin serum level. **Results:** The prevalence of eveningness, intermediate, and morningness chronotypes was 11%, 47.6%, and 41.4%, respectively. The participants with eveningness type were significantly younger (P = 0.001). The percentage of men with eveningness type was significantly higher than women (*P* = 0.001). The mean values of height, weight, body mass index (BMI), waist circumference, total calorie intake, protein, carbohydrate, fat, and calorie intake from each of the main meals and snacks, were not different among three chronotypes. Leptin and NPY did not show any significant association with chronotypes. **Conclusion**: Individual's chronotype was not associated with NPY and leptin serum level. Further studies on a population with more varied work shifts are suggested.

Keywords: *Circadian type; Chronotype; NPY; Leptin; Obesity; Iran*

Introduction

Considering the increasing trend of the prevalence of obesity and overweight, it seems necessary to focus on other probable responsible factors, in addition to current well-known causes of obesity. It has recently been observed that the circadian cycles are involved in the regulation of metabolism and appetite. There are different preferences in circadian lifestyle, individually. One of the most important of these differences is morningness/eveningness preferences. People are categorized to three chronotypes, including morningness, intermediate, and eveningness type

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according to their preferences for time of sleeping, waking, and doing mental and physical activity (PA) (Potter *et al.*, 2016). There are some evidences suggesting that the biological clock plays an important role in regulation of energy balance. So that intake of calories during the resting phase, regardless of its amount, may lead to obesity (Kim *et al.*, 2015, Reutrakul *et al.*, 2013, Scheer *et al.*, 2013). Recent studies have suggested that the metabolic efficiency of calorie in the morning is higher than in the afternoon (Oike *et al.*, 2014). It shows that the time of food intake may affect body weight (Garaulet *et al.*, 2013). Studies conducted on mice have shown that eating at conventional hours is associated with normal metabolism, while eating at unconventional hours leads to metabolic disorders and some of the chronic diseases, such as obesity and diabetes (Laermans and Depoortere, 2016, Potter *et al.*, 2016, Reutrakul *et al.*, 2013). It has been observed that the loss of synchrony between the body central clock and the peripheral clock may play an important role on the occurrence of some metabolic disorders, such as obesity, perhaps through changes in time of food intake and dietary patterns (Rahmani *et al.*, 2015). Therefore, it may be assumed that chronotype can affect body weight. Abnormal dark/light patterns likely dysregulate intracellular circadian clocks through molecular mechanisms. Dysregulation of these molecular mechanisms, especially within the adipocytes, may lead to metabolic disorders. It may lead to adipose accumulation. Disturbances in circadian rhythm may also lead to hormone imbalance (Vitaterna *et al.*, 2001). Neuropeptide Y (NPY) is one of the hormones releasing from hypothalamus. NPY increases appetite and fat storage. There is some evidence showing that NPY level in obese people is higher than non-obese people. Furthermore, NPY plays an important role on modulation of dark/light cycle and circadian rhythm (Mrosovsky, 1997). Leptin is one of the other hormones effecting on appetite, energy balance and obesity. Several studies have suggested that waking during dark phase, like what is seen in people with eveningness type, may decrease leptin level and consequently, increase appetite. Biological clock can affect NPY and leptin secretion, although, little is known about its molecular mechanisms (Froy, 2010). There is not enough information about secretion rhythm of NPY and leptin in the body of individuals with different chronotypes. It is not clear whether or not the biological clock is affected by the chronotype. Although 21.7% of Iranian adults are obese or overweight (Asghar *et al.*, 2015), there is not any population based study to investigate the association between chronotype with obesity and appetite in Iran. Given the high prevalence of obesity in Iran (Rahafar *et al.*, 2013), the current study was conducted on Iranian population to investigate the association between people's chronotype and hormones related to appetite to find an efficient and cost effective approach to prevent and control obesity.

Material and Methods

Study design and participants: It was a descriptive-analytical cross sectional study conducted on women and men aged 18-60 years who referred to the community centers of Tehran Municipality from September 2019 to January 2020. To consider economic status, community centers were randomly selected from 5 regions, including north, south, west, east, and center of Tehran. Through convenience sampling, 353 people from both genders took apart in this study. Questionnaires for general information, PA, circadian type, and 3-day food recalls were completed for them. Anthropometric measurements were also measured in the community centers. For biochemical measurements, a subsample, including 90 individuals were selected through convenience sampling. Blood sampling was conducted on diet therapy clinic of Shahid Beheshti University of Medical Sciences. Pregnant and lactating women, professional athletes, adherents of weight loss/gain diets or special diets, such as vegetarianism, traditional medicine, and homeopathy during the last two months, were not allowed to enter to the study. The participants whose energy intake was less than 800 or more than 4,200 kcal/day were excluded from the analysis.

General information assessment: The participants completed a general information form including the questions about age, job, smoking status, taking appetite and sleep effective drugs, nutritional or weight loss supplements, and anti-depression drugs during last month.

Circadian type assessment: To assess the circadian type, Morning-Evening Questionnaire-19 (MEQ) was used. It was developed by Horn-Ostburg (Albers and Ferris, 1984) and is the most common questionnaire to assess circadian type. The Persian version of this questionnaire was validated by Arash Rahafar *et al.* (Rahafar *et al.*, 2013). Their study showed an acceptable validity for this questionnaire (Cronbach's alpha coefficient 0.77). MEQ-19 included 19 questions. The score range is from 16 to 86. Getting higher score shows more probable for being morningness. The people are categorized to different chronotypes according to their score as follows: 16-30 definite eveningness, 31-4 moderate eveningness, 42-58 intermediate, 59-69 moderate morningness and 70-86 definite morningness.

Anthropometric measurements: A trained certified dietitian conducted all anthropometric measurements. Body weight was measured to the nearest 100 grams, without shoes, while wearing light clothes using Beurer digital scale. Height was measured to the nearest 0.5 mm, without shoes using a non-stretch tape meter fixed to a wall. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Waist circumference (WC) was measured at the end of normal exhalation to the nearest 0.5 cm in the minimum measurable environment. For some people whose minimal WC was not easily determined owing to obesity or wasting, the circumference at the last vertebra was measured, since the minimal WC is located in this area for most of the people.

Food intake assessment: To assess food intake, the three-nonconsecutive food recall questionnaires were used for two weekdays and one weekend. The first food recall was completed in community center and two other ones were completed through telephone interview during a week. The participants were asked about all the meals and snacks eating during previous day. The dietary intake data were converted to grams using household measures. Nutritionist IV software was used to calculate calorie and macronutrients intake.

Assessment of physical activity: To assess PA, International Physical Activity Questionnaire (IPAQ) was used. The Persian version of this questionnaire was validated by Vashghani-Farahani *et al.* (Vasheghani-Farahani *et al.*, 2011). According to their study, this questionnaire has acceptable validity and reliability (0.33, 0.7, respectively). The IPAQ used in the present study is the long interview-administered version (27 items), which covers 4 domains of PA, including occupational (7 items), transportation (6 items), household/gardening (6 items), and leisure-time activities (6 items). The questionnaire also includes 2 questions about the time spent on sitting as indicators of sedentary behavior (Ishida *et al.*, 1999). After multiplying the time dedicated to each activity class by the specific MET score for that activity, PA was calculated and reported as MET/min/week.

Biochemical assessments: The volunteers were asked to attend on diet therapy clinic of Shahid Beheshti University of Medical Sciences at the specific day. Two ml blood was collected after 8-12 hours overnight fasting from the antecubital vein in the arm. The serum samples were frozen immediately at −80 °C until assay at the end of the study. ELISA method was employed to determine NPY and leptin serum level through R & D kits produced by Germany.

Ethical considerations: The present study was approved by the Ethics Committee of the National Nutrition and Food Technology Research Institute, Iran. A signed hand-written informed consent was obtained from each subject before data collection.

Data analysis: SPSS version 21 software was used for statistical analysis of the data. The Kolmogorov-Smirnov test was used to determine normality distribution of variables. Distribution of NPY and leptin among three circadian types was analyzed by Kruskal-Wallis. To find the association between chronotypes and hormones related to appetite, multiple regression was used, after adjustment for covariates. In all tests, P-value < 0.05 was considered statistically significant.

Results

From 353 individuals who participated in this study, 29 were excluded from the analysis, since their calorie intake was lower than 800 kcal or more than 4200 kcal. Totally, 324 individuals were included for the analysis. The participants were categorized into three chronotypes (eveningness, intermediate, and morningness), based on their MEQ score. The prevalence of eveningness, intermediate, and morningness was 11%, 47.6%, and 41.4%, respectively. According to **Table 1**, the mean age of individuals with eveningness, intermediate, and morningness was 37, 40, and 46 years, respectively. The results of Bonferroni test as the post hoc comparison, showed that people with morningness type were significantly older than those with eveningness type and intermediate type (P < 0.001). Among the women, the prevalence of eveningness type was significantly lower than intermediate type and morningness type (P = 0.001), while distribution of three chronotypes was not different among the men. There was not any significant difference among the three chronotypes according to work shifts. It was found that 57.9% of morningness type, 36.8% of intermediate type, and 5.3% of eveningness type participants were taking medicine for reducing weight and appetite. The prevalence of smokers in intermediate type was higher than the other two types; however, it was not statistically significant. PA level in eveningness type was lower than intermediate and morningness types; however, it was not significant.

Table 2 shows the comparison of anthropometric measurements and dietary intakes among the three chronotypes. The median height and mean weight of eveningness type were higher than intermediate type and morningness type; however, these differences were not significant. The mean waist, calorie, carbohydrate, protein, and fat had a similar distribution among the three circadian types.

Table 3 shows the median and interquartile range for hormones related to appetite (NPY and Leptin). The results of Kruscal-wallis tests showed that the level of NPY and leptin did not have any significant difference among the three chronotypes. Although Leptin level in morningness type was higher than eveningness type, it was not statistically significant (P < 0.06).

Table 4 reveals the results of analysis of regression to investigate the association of NPY and Leptin serum level with chronotypes, after adjusting for gender and age. The association of eveningness and morningness type with NPY and Leptin was not significant compared to intermediate type, as the reference group.

Table 1. Distribution of general characteristics among the three circadian types.

Variables	Chronotypes			P-value
	Eveningness	Intermediate	Morningness	
Age (year)	39.32 ± 19.00[a]	39.73 ± 18.00	46.11 ± 18.00	<0.001[c]
Physical activity (Met/min/week)	1340 ± 1978	1929 ± 1830	1978 ± 2259	0.11 [c]
Gender				
Women	24 (8.3)[b]	144 (50.2)	119 (41.5)	0.001[d]
Men	4 (10.8)	13 (35.1)	20 (54.1)	
Employment Status				
Housekeeper/unemployed/Retired	22 (10.6)	104 (48.0)	89 (41.4)	0.18 [d]
Working on day shift	5 (5.6)	40 (50.0)	44 (49.4)	
Working night shift	1 (33.3)	2 (66.7)	0 (0.0)	
Working on both shifts	0 (0.0)	11 (64.0)	6 (36.0)	
Smoking				
Yes	2 (12.5)	8 (50.0)	6 (37.5)	0.8 [d]
No	26 (8.4)	149 48.4)	133 (43.2)	

[a]: Mean ± SD; *[b]: N (%)ant difference with intermediate; [c]:* Bonferroni *test; d: Chi-square*

Table 2. Distribution of anthropometric measurements and dietary intakes among the three chronotypes.

Variables	Chronotypes			P-value
	Eveningness	Intermediate	Morningness	
Height[a] (cm)	160.94 ± 12.66[c]	160.00 ± 10.00	160.00 ± 10.00	0.20
Weight[b] (kg)	71.841 ± 14.38	70.81 ± 13.69	71.40 ± 13.17	0.88
Body mass index[b] (kg/m^2)	27.24 ± 5.46	27.20 ± 5.51	27.45 ± 4.81	0.91
Waist[b] (cm)	94.75 ± 19.46	91.60 ± 12.68	93.76 ± 11.72	0.26
Calorie[b] (kcal)	1740.82 ± 623.11	1767.67 ± 636.93	1804.49 ± 607.56	0.89
Carbohydrate[b] (g)	236.28 ± 88.75	250.69 ± 110.60	261.29 ± 112.90	0.55
Protein[b] (g)	69.97 ± 28.63	71.64 ± 29.30	72.81 ± 23.15	0.59
Fat[b] (g)	58.79 ± 23.98	54.83 ± 21.11	54.59 ± 20.59	0.53

*[a]: Kruskal-Wallis test; **[b]:** ANOVA test; [c]: Mean ± SD.*

Table 3. Distribution of hormones related to appetite in the three circadian types.

Variables	Chronotypes						P-value[a]
	Eveningness		Intermediate		Morningness		
	Median	IQR	Median	IQR	Median	IQR	
NPY (pg/ml)	3097.5	8647.5	4147.5	11163.7	4685.0	10633.0	0.98
Leptin (ng/ml)	2643.2	1776.5	2367.0	1373.7	2970.1	1678.4	0.06

NPY: Neuropeptide Y; IQR: Interquartile range; [a]: Kruskal-Wallis test.

Table 4. Association of hormones related to appetite with chronotypes.[a]

Variables	Chronotypes	B[b]	CI (95%)	P-value
NPY (pg/ml)	Eveningness	-2075.3	-10293.5, 6142.9	0.6
	Morningness	3598.1	-3263.2, 10459.4	0.2
NPY (pg/ml)	Eveningness	-72.1	-685.4, 541.1	0.8
	Morningness	-75.2	-587.3, 436.7	0.7

NPY: Neuropeptide Y; [a]: Multiple regression adjusted for age and gender; [b]:: Intermediate type was considered as reference

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Discussion

Prevalence of different chronotypes among the participants: The current study investigated the prevalence of morningness, intermediate, and eveningness circadian types in adult men and women living in Tehran, Iran. The findings showed that the intermediate type had the highest prevalence (47.6%) and the eveningness type had the lowest prevalence (11%) in the population. The prevalence of circadian types in the present study was similar to another study conducted on Iranian nurses working in medical centers (Glass *et al.*, 2010). The prevalence of intermediate, morningness, and eveningness circadian types in that study was 57.9%, 36.1%, and 6%, respectively. The current study findings were partly similar to the other study conducted on Iranian women living in dormitories (Broussard and Van Cauter, 2016). In that study, the intermediate type had the highest prevalence, while the morningness type had the lowest prevalence. The prevalence of morningness type in the study by Isa Wiater (Wiater *et al.*, 2011) was similar to the present study (41%), while the prevalence of intermediate type was slightly lower (44%) and the prevalence of eveningness type was higher than the present study (15%). The results of different studies might not be similar to each other. For instance, Yasumoto *et al.* suggested that the intermediate type has the highest prevalence among circadian types, while the prevalence of morningness and eveningness types is almost equal (Yasumoto *et al.*, 2016). These differences, to some extent, may be due to the effects of gender, occupational status, and lifestyle on circadian type. In women participating in the current study, the intermediate type was significantly higher than the morningness, and eveningness types; however, in men, the morningness type was significantly higher than the intermediate and eveningness types. This finding may be due to the fact that men have to wake up early in the morning but women do not, since most of the women were housewives. Time of sleeping and waking may affect the coordination between the biological clock and the environmental clock. On the other hand, in the present study, the prevalence of morningness type was higher among older people. It may show a different lifestyle in young people from middle-aged and older people.

Circadian type and hormones related to appetite: In the current study, there was not any association between chronotypes and hormones related to appetite. Although NPY had a similar distribution among all circadian types in the population, it seems that NPY may have a role on synchronization of sleep time and meal time (Vettor *et al.*, 1994). However, Serova, *et al*. showed that nasal injection of NPY cannot lead to increase in appetite (Serova *et al.*, 2020), while Kalra (Kalra *et al.*, 1988) suggested that activation of NPY receptors lead to hyperphagia and weight gain in rats. Increase in activity of NPYergic neurons leads to continuing food intake in dark phase. It may be one of the effective factors on etiology of hyperphagia in individuals with eveningness type. Most of the studies have investigated circadian rhythm of releasing hormones, while few studies have assessed circadian rhythm of releasing hormones according to chronotype of individuals. Some studies have shown the effects of dark/light cycle on the amount of calorie intake. For instance, a study in the USA conducted on individuals with obesity suggested that calorie intake after 8 pm is associated with higher BMI (Reutrakul *et al.*, 2013). It showed that energy intake after 8 pm (like what is seen in an individual with eveningtype) is two times more than energy intake before this time (like what is seen in morningness type). It also suggested that individuals with eveningness type consume larger portion sizes than individuals with morningness type (Reutrakul *et al.*, 2013). It can be concluded that people with eveningness type have higher BMI because of higher intake of calorie in the last hours of the night. Although the exact mechanisms showing the association between food intake in unusual time and obesity have not been well understood, changes in leptin serum level may be one of the mechanisms. Leptin is a multifunctional hormone mostly produced by white adipose tissue in response to feeding (Damiola *et al.*, 2000). Leptin regulates energy homeostasis and food intake. Given biological clock controls leptin gene expression as an appetite suppressor, dysregulation of this clock leads to a decrease in leptin secretion (Friedman and Halaas, 1998). Food intake in dark phase (inactive phase), may lead to overeating that is partly due to the disruption of appetite regulation systems (Bray *et al.*, 2013), although there is not any consciousness, yet. Dibner *et al.* showed that food intake in unusual hours (dark or inactive phase) is not associated with leptin resistance. They suggested that food intake in dark phase disrupts regulation of environmental clock and then, may lead to obesity and metabolic disorders, but it is not due to leptin resistance. They suggested that disruption of synchronization between biological clock and environmental clock may lead to a low PA level and fat accumulation in liver (Dibner and Gachon, 2015).

There are some studies suggesting that leptin secretion has a similar rhythm in individuals with different chronotypes (Mariné-Casadó *et al.*, 2018, Nedeltcheva *et al.*, 2009, Rynders *et al.*, 2020, Serova *et al.*, 2020). According to findings of Morante, *et al.*, weight loss due to a low caloric diet changes the rhythm of leptin and ghrelin secretion, but it does not have any effect on feeling hunger, mentally. It also shows that weight loss may modify circadian rhythm of leptin secretion without any decrease in hunger/satiety feeling (Hernández Morante *et al.*, 2020). Some studies have shown that the amount of calorie intake is the most important factor to control circadian rhythm of leptin secretion (Marcheva *et al.*, 2009). Consumption of high amount of food in unusual time may lead to the disorder in leptin secretion rhythm. It can be seen in individuals whose circadian rhythm is dysregulated (Laposky *et al.*, 2008). It may be possible that leptin has no direct effect on circadian type of people. Considering the important role of meal timing as a significant stimulus of metabolic rhythms, it may be concluded that meal timing may play an important role in the association between obesity and circadian types, not entirely due to leptin. The association between chronotype and food intake and metabolic health requires further studies.

Circadian type, calorie and macronutrients intake, and anthropometric measurements: In the current study, the amount of calories and macronutrients intake, the mean BMI and waist circumference had no significant difference among different circadian types. Some evidence shows that food intake during the light phase (active phase) improves some metabolic pathways in the liver to protect against weight gain. These metabolic pathways are involved in the function of the biological clocks (Laermans and Depoortere, 2016, Reutrakul *et al.*, 2013). Changes in the light/dark cycle that act as a mutation in the regulatory genes, can dysregulate the body's circadian rhythm and lead to obesity (Shaw *et al.*, 2019). The findings of the study by Yasumoto *et al.* showed that feeding mice at night compared to feeding during day results in overeating (hyperphagia), weight gain, and obesity (Yasumoto *et al.*, 2016). This leads to the accumulation of fat in the liver and metabolic disorders (Shaw *et al.*, 2019). Furthermore, doing PA at the end of the active phase reduces obesity more than at the beginning of this phase. Therefore, exercise time is very important in alteringcircadian rhythm and metabolic disorders. In addition, exercise can be a useful and powerful factor in maintaining a regular circadian rhythm. On the other hand, intake of food at regular times can reduce the circadian fluctuations of metabolic clock genes and potentially reduce obesity due to high-fat diet (Duncan *et al.*, 2016, Sundaram and Yan, 2016). However, there are several studies, consistent with our study, showing no association between chronotype, calorie intake, and BMI (Askarpour *et al.*, 2019, Okauchi *et al.*, 2019, Seyedoshohadaee *et al.*, 2015, Shaw *et al.*, 2019). In the study conducted on 4,493 people aged 25-74 years, there were not any correlation between BMI, calorie intake, and the MEQ questionnaire score, which is in line with the present study. It was due the similarity in total calorie intake and the PA level in individuals with different chronotypes (Maukonen *et al.*, 2017). In a study conducted by Maukonen et al., there was no significant difference in the total amount of calories and macronutrients intake in different circadian types (Maukonen *et al.*, 2019). The findings of a cross-sectional study by Gontijo et al. (de Assis *et al.*, 2003) as well as a study by Kanerva et al. (Maukonen *et al.*, 2017) also showed that calorie and macronutrient intake had no significant association with the MEQ questionnaire score. In the study by Toktas *et al.*, there was also no association between circadian type and BMI (Toktas *et al.*, 2018). In their study, people with the eveningness type usually skipped one meal, mostly breakfast, but instead, the calorie of this meal was usually offset at dinner. Eventually, the total calorie intake was similar in people with morningness and eveningness types (Askarpour *et al.*, 2019). Despite the existence of mechanisms indicating the effect of light-dark phase on BMI, in the present study, no association was observed between NPY, Leptin, anthropometric measurements, calorie intake, and chronotype. It should be noted that the participants in the current study were only overweight, not obese (BMI 25-30 kg/m^2). Previous studies have shown an association between circadian types and obesity (Harb *et al.*, 2012, Kanerva *et al.*, 2012, Meule *et al.*, 2012, Tahara *et al.*, 2017), but there is not any evidence about the association between overweight and chronotypes. In addition, most of the studies investigating the association between circadian type and obesity have been conducted through intervention in light/dark cycle on animal samples, while the same intervention might not be possible in the studies conducting on human. The possibility of comparing the present with other studies may be difficult. Most of the participants were housewives, so it is probable that most of them have a similar circadian lifestyle. There might be another reason for not finding a significant association between chronotype and the anthropometric measurements in the present study.

Strengths, limitations, and suggestions: One of the strengths of the current study was the large sample size and using a validated questionnaire to determine chronotypes. Using certified ELISA kits was the other strengths of the study. This study has certain limitations as well. The cross-sectional design of this study cannot reveal causality. Moreover, dietary recall is based on memory, thus, possible memory and reporting biases may affect the data to some extent. The findings might be affected to some extent by the fact that majority of the participants were female. Women often tend to underreport their food intake (Kye *et al.*, 2014). The similarity in job status of most women in the study weakens the possibility of finding an association between work shift and appetite. It is recommended to conduct clinical trials on a population with more balanced gender distribution, more diverse work shifts, and more limited age range.

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Authors' contributions

Rabiei S designed the study, wrote the manuscript and supervised the study. Haghgoo M conducted data collection and wrote the manuscript. Borumandnia N conducted the statistical analysis. Nasrollahzadeh J and Hejazi E conducted biochemical analysis. Sadeghzadeh H conducted data collection and wrote the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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