Original Article

The Possible Association Between Circadian Rhythm Chronotypes and Unhealthy Metabolic Phenotypes in Overweight/ Obese Women

Moein Askarpour, Habib Yarizadeh, Niloofar Rasaei, Negin Badrooj, Kurosh Djafarian and Khadijeh Mirzaei*

Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran

*** Corresponding author**

Khadijeh Mirzaei, PhD
Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences (TUMS), Tehran, Iran
Tel: +98 21 88955569
Fax: +98 21 88984861
Email: mirzaei_kh@tums.ac.ir

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Abstract

Background: Circadian rhythm affects individual body function and health. Although it can regulate many biological processes in the body such as hormone release and eating habits, little is known about the relationship between circadian rhythm and metabolic disorders. Therefore, the purpose of the present research was to examine the association between Circadian Rhythm Chronotypes (CRC) and metabolic health status components, in patients with overweight and obesity.

Methods: A cross-sectional study was conducted at the healthcare centres of Tehran. Patients with Overweight and Obesity (OW/OB) (*n* 374) aged 18 to 56 years were rectruited. All participants were assessed for metabolic health status components based on Karelis criteria (HDL, LDL, TG, hs-CRP and HOMA-IR). Morningness - Eveningness Questionnaire (MEQ) was used for chronotype categorizing. Also, bioelectrical impedance analysis (BIA) was applied to evaluate body composition. Regression model in crude and adjusted model was used to assess the relation between CRC and metabolic status.

Results: The differences in CRC were not significant in terms of Karelis criteria components except hs-CRP (p=0.02). Results had shown that the trend of hs-CRP tends to decrease from eveningness chronotype to morningness chronotype and it significantly increased in crude model (p=0.007) and adjusted model (p=0.03). In addition, hs-CRP had decreased significantly in morningness chronotype in comparison with eveningness chronotype in crude model [OR (95% CI): 0.28 (0.10 to 0.79), p=0.01].

Conclusion: This study indicated an association between hs-CRP and CRC in women patients with overweight and obesity, which indicates that inflammation may have a relation to the CRC and metabolic status.

Keywords: Circadian rhythm chronotypes, Obesity, Unhealthy metabolic status

Introduction

The prevalence of obesity has increased worldwide in recent years making it a major public health concern (1). Obesity is associated with a reduced life expectancy, largely because obese individuals are at an increased risk of type 2 diabetes, Cardiovascular Disease (CVD), and metabolic disorders (2). However, based on previous studies, not all obese subjects are at a high risk of mortality. According to this point, obesity can be discussed in terms of Metabolically Healthy Obesity (MHO) that is defined in patients with obesity (Body Mass Index-BMI≥30 kg/m^2) who show no sign of conditions associated with the metabolic syndrome (Mets) (impaired glucose metabolism, hypertension and dyslipidemia with a normal CVD risk profile). Second, Metabolically Unhealthy Obesity (MUO) is defined in obese individuals (BMI≥30 kg/m^2) with at least two mets risk factors (3,4).

Obesity, regardless of MUO and MHO status is associated with inflammation due to inflammatory response to pathogens that includes systemic increases in circulating inflammatory cytokines such as interleukins, tumor necrosis factor α, and High Sensitive C-Reactive Protein (hs-CRP) and other inflammatory factors (5). Previous studies demonstrate that MUO individuals have higher inflammation compared to their MHO counterparts and experience more adverse effect on body function (6-8).

It has been suggested that inflammatory response can be exacerbated by circadian rhythm misalignment (9). Circadian rhythms are physiologic and behavioral cycles with periodic recurrence of approximately 24 hours generated by the Suprachiasmatic Nucleus (SCN) located at the base of the brain in front of the pituitary gland for rhythm oscillation (10). The rhythms control a variety of biological processes, such as sleep-wake cycle, body temperature, feeding, hormone secretion, glucose homeostasis, lipid metabolism and immune system function (11-13). Different Circadian Rhythm Chronotypes (CRC) have shown the ability to change circadian oscillations of many body functions. Moreover, circadian rhythm misalignment can be induced by sleep deprivation in evening chronotypes. However, there is no study to examine the association between circadian rhythm and metabolic status in overweight and obese women. Therefore, the overall goal of this project was to define the association between circadian rhythm misalignment and metabolically unhealthy status in overweight and obese women.

Materials and Methods

Study population

This cross-sectional study included 374 overweight and obese adult women aged 18 to 56 years, who referred to healthcare centers in Tehran, Iran. Participants were in good general health with a BMI of 25 kg/m^2 or greater. The study protocol was approved by the Ethics Commission of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1395.1480). All participants signed a written informed consent. The exclusion criteria were as follows: history of hypertension, impaired renal and liver function, regular use of medicine other than birth control pills, cardiovascular disease, diabetes mellitus, and menopause, intake of alcohol, smoking, and pregnancy or lactation period. In addition, those women with chronic disease affecting their diet, those who followed special regimen, and those with any body weight fluctuations over the last year were excluded from the study.

Measurement of body composition and physical activity

The body composition analyzer (in body 720, Korea) was used to measure weight, BMI, Total Body Water (TBW), Fat Mass (FM), Fat-Free Mass (FFM), and body fat percentage (%) of the subjects following a standardized procedure according to guidelines. This electrical impedance analyzer measures the resistance of body tissues to the flow of an electrical signal sent through both feet and hands. The proportion and amount of body fat and fat-free mass can be calculated when the current flows more easily through certain parts of the body. International Physical Activity Questionnaire (IPAC) was used to assess Physical Activity (PA). Activity levels were classified into low, moderate, and high groups, as described by the IPAQ scoring protocol (14).

Determination of metabolic health status

MHO was defined based on Karelis cardiometabolic abnormalities, presenting individuals afflicted with obesity (BMI≥30 kg/m^2) with at least four of the followings: HOMA ≤2.7, triglycerides

(TG)≤1.7 *mmol/l*, High - Density Lipoprotein -Cholesterol (HDL-C)≥1.3 *mmol/l*, Low-Density Lipoprotein-Cholesterol≤2.6 *mmol/l*, high-sensitivity C-reactive protein (hs-CRP)≤3.0 *mg/l*. On the other hand, metabolically unhealthy status was defined as obesity (BMI≥30 kg/m^2) with at least two risk factors of metabolic components based on Karelis *et al* criteria (15). In order to compare the differences in circadian rhythm status, participants were assigned into two groups of MHO, and MUO.

Morningness-Eveningness Questionnaire (MEQ)

A validated Morningness - Eveningness Questionnaire (MEQ) was used to measure whether a participant's circadian rhythm produces peak alertness in the morning, in the evening, or in between. Reliability and validity of the MEQ has been already approved in Iran (16). Completed extensive MEQ comprised of components like sleep habits, sleep history, family history, sleep quality, morningness - eveningness traits, depression, mania, and seasonality of symptoms. MEQ scores ranged from 16 to 86; scores of 41 and below indicate "evening chronotype", scores between 42-58 indicate "intermediate chronotype", and scores of 59 and above indicate " morningness chronotype" (17).

Statistical analysis

Descriptive analysis was used to describe the mean (standard deviation) and study population characteristics. The relationship between qualitative variables and MEQ values was assessed with the chi-square test. The relationship between quantitative variables and MEQ values was determined by one-way analysis of variance. In order to analyze the potential association between MUO and circadian rhythm, binary general linear model was used in crude and adjusted models (Counfounding factors including age, BMI and physical activity). P-value lower than 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 22.0 (SPSS, Chicago, IL, US). All statistical analyses were performed using the IBM SPSS Version 22.0 (IBM Corp. Armonk, NY, USA) software.

Results

Study population characteristics

A sample of 374 overweight and obese adult women aged 18 to 56 years was recruited in the current study. The characteristics of study participants are shown in table 1. The mean age, height, and weight of women were 35.1 years, 160.9 *cm*, and 80.8 *kg*, respectively. Moreover, other characteristics including lipid profile, body composition, and biochemical parameters of participants have been shown.

In order to examine the association of circadian rhythm with metabolic status, participants were categorized based on circadian rhythm including morningness, intermediate and eveningness chronotypes. The differences between characteristics of participants among the MEQ chronotypes are shown in table 2. The results from this study revealed that weight (p=0.03) and Fat Mass Index (FMI) (p=0.006) showed significant differences among MEQ chronotypes. After adjustment for confounding factors, the effect of age (p=0.01) was more in morningness chronotype compared to other MEQ chronotypes.

Frequency of abnormal metabolic status based on Karelis criteria

The differences between MEQ chronotypes and Karelis criteria components of participants are shown in table 3. There were no statistically significant differences in LDL, HDL, TG, and HOMA index

Table 1. Characteristics of study population

	Min	Max	Mean	SD
MEQ-score	7.00	71.00	36.52	8.32
Age (year)	18.00	56.00	35.12	8.69
Weight (*kg*)	59.50	119.50	80.89	12.45
Height (*cm*)	147.50	179.00	160.92	10.14
BMI (kg/m^2)	25.00	40.70	30.33	3.65
FFM (*kg*)	35.30	67.70	46.62	5.43
FM (*kg*)	19.40	53.20	33.43	7.60
GLU (*mg/dl*)	67.00	202.00	88.57	11.38
TG (*mg/dl*)	37.00	512.00	118.39	64.54
HDL (*mg/dl*)	18.00	84.00	47.76	10.87
LDL (*mg/dl*)	34.00	282.00	96.98	26.86
hs-CRP (*mg/l*)	0.00	22.73	4.05	4.54
HOMA-IR	1.29	16.59	3.39	1.92
SGOT (*μKat/L*)	7.00	70.00	18.52	8.05
SGPT (*μKat/L*)	4.00	121.00	18.82	14.46

N=374, BMI body mass index, MEQ morningness- Eveningness Questionnaire, FFMI free fat mass index, FMI fat mass index, SGOT serum glutamic oxaloacetic transaminase, SGPT serum glutamic pyruvic transaminase, HDL high density lipoprotein، LDL low density lipoprotein, TG triglyceride, hs-CRP high–sensitivity c-reactive protein, GLU glucose, HOMA homeostatic model assessment

Table 2. Characteristics of study population sub grouped by MEQ

Circadian chronotypes [a]	Eveningness (n 127)	Intermediate (n 132)	Morningness (n 115)	p-value*	p-value[α]
Age (year)	36.19 ±8.18	35.89 ± 8.49	37.73 ±8.38	0.364	0.011
Weight (*kg*)	83.98±12.68[a]	79.49 ± 11.27	77.68 ± 14.62[a]	0.101	0.582
Height (*cm*)	161.15±6.60	160.81±5.63	159.37± 20.91	0.711	0.917
BMI (*kg/m²*)	32.30±4.14	30.65±3.63	30.44±3.83	0.086	0.411
FFMI (*kg*)	18.41±1.55	17.74 ±1.37	17.90 ±1.43	0.095	0.756
FMI (*kg*)	13.89± 3.10[a]	12.92 ±2.83[b]	12.53 ±3.08[a.b]	0.147	0.597
GLU (*mg/dl*)	90.80±9.82	87.55±9.73	85.74±7.40	0.150	0.952
SGOT (*units/L*)	18.60± 6.67	18.15± 7.20	17.56± 8.25	0.823	0.464
SGPT (*units/L*)	20.13 ±11.14	20.37 ±13.43	19.16± 14.10	0.964	0.524

BMI body mass index, FFMI free fat mass index, FMI fat mass index, GLU glucose, Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT)
[a] categorized based on Morningness -Eveningness Questionnaire, *p value resulted from ANOVA analysis
[α] P value resulted from general linear model after adjustment for age, BMI, physical activity

Table 3. Frequency of abnormal metabolic status based on Karelis criteria among different circadian chronotypes

Circadian chronotypes		Eveningness	Intermediate	Morningness	p-value[∞]
TG(*mg/dl*)	(>1.7, n= 67)	7(10.5%)	38(56.7%)	22(32.8%)	0.821
HDL(*mg/dl*)	(<1.3, n= 206)	26(12.6%)	119(57.7%)	61(29.7%)	0.972
LDL(*mg/dl*)	(>2.6, n= 129)	21(16.3%)	71(55.0%)	37(28.7%)	0.335
hs-CRP (*mg/l*)	(>3, n= 125)	21(16.8%)	79(63.2%)	25(20.0%)	0.044
HOMA-IR	(>2.7, n= 190)	28(14.7%)	108(56.9%)	54(28.4%)	0.547

HDL high density lipoprotein, hs-CRP high–sensitivity C- reactive protein, HOMA homeostatic model assessment, LDL low density lipoprotein, TG triglyceride
∞ p-value resulted from chi-squared analysis

among MEQ chronotypes. However, intermediate chronotype participants in comparison with MEQ chronotypes had higher level of hs-CRP.

Association of MUO components with CRC in crude and adjusted regression model

The association between circadian rhythm and karelis components among participants, in crude and adjusted models of binary regression model is presented in table 4. It has been shown that eveningness chronotype in comparison to other chronotypes had no abnormal metabolic status based on Karelis components. However, in crude model, participants in morningness chronotype had lower odds of high hs-CRP compared to eveningness chronotype (p=0.01). Moreover, hs-CRP trend in crude (p=0.007) and adjusted (p=0.03) models was ascending from morningness chronotype to eveningness chronotype.

Discussion

In the present study, the association between CRC and MUO characteristics in women was studied. Significant association between hs-CRP as a component of metabolic health status and CRC in participants was observed. However, no significant association was found in other karelis components (LDL, HDL, TG, and HOMA). Our findings showed hs-CRP caused significant difference among chronotype groups, so higher hs-CRP level was related to eveningness chronotype compared to morningness chronotype.

Based on previous studies, evening chronotype was associated with a higher prevalence of diabetes, metabolic syndrome, cardiovascular disease, and sleep disorders (18, 19). Similarly, Baron *et al* demonstrated that evening chronotype groups were 2.5 times more likely to have weak general health compared with morning chronotype groups (20). Eveningness chronotype groups such as shift workers have shorter sleep durations during working days compared with the other chronotype groups which induces circadian rhythm misalignment, a critical factor in disrupting energy metabolism, lipid and glucose metabolism leading to obesity with both physical and psychiatric consequences (21).

Our results showed no association between MUO lipid profiles and circadian rhythm chronotypes. Despite our

Table 4. Association of metabolic unhealthy status and its components with circadian rhythms chronotypes in crude and adjustment regression model among participants ∞

Total population						
	Crude model OR (95% CI)	p value	p trend	Adjustment model OR (95% CI)	p value	p trend
MUO						
Eveningness ¥						
Intermediate	1.37 (0.46 to 4.09)	0.562	0.507	0.94 (0.29 to 3.04)	0.912	0.881
Morningness	1.22 (0.60 to 2.48)	0.577		1.24 (0.57 to 2.65)	0.574	
TG						
Eveningness ¥						
Intermediate	1.36 (0.42 to 4.41)	0.601	0.588	1.89 (0.54 to 6.54)	0.312	0.572
Morningness	1.47 (0.42 to 5.10)	0.547		1.71 (0.46 to 6.37)	0.415	
HDL						
Eveningness ¥						
Intermediate	0.97(0.36 to2.59)	0.954	0.817	1.01(0.36 to 2.78)	0.981	0.770
Morningness	0.89(0.31 to0.57)	0.841		0.89(0.30 to 2.63)	0.844	
LDL						
Eveningness ¥						
Intermediate	0.55(0.22 to 1.38)	0.218	0.191	0.81(0.30 to 2.15)	0.677	0.260
Morningness	0.48(0.18 to 1.30)	0.150		0.58(0.20 to 1.67)	0.314	
hs-CRP						
Eveningness ¥						
Intermediate	0.67(0.27 to 1.66)	0.391	0.007	0.97(0.35 to 2.65)	0.962	0.031
Morningness	0.28(0.10 to 0.79)	0.017		0.38(0.12 to 0.96)	0.047	
HOMA-IR						
Eveningness ¥						
Intermediate	0.69(0.26 to 1.84)	0.461	0.460	0.84(0.30 to 2.38)	0.758	0.287
Morningness	0.56(0.20 to 1.52)	0.283		0.69(0.23 to 2.06)	0.518	

HDL high density lipoprotein, hs-CRP high–sensitivity C- reactive protein, HOMA homeostatic model assessment, LDL low density lipoprotein, MUO metabolically unhealthy obese, TG triglyceride

∞based on binary regression model in crude and adjustment models

¥Eveningness chronotype considered as reference group, OR=odds ratio, CI=confidence interval

findings, Wefers *et al* showed circadian misalignment can lead to high Free Fatty Acids (FFA) levels and low TG levels. It may happen due to circadian regulation or high clearance of very LDL (VLDL)-TG by the skeletal muscle (22). Esquirol *et al* observed that shift workers are affected by circadian misalignment, and they were associated with higher FFA and TG, and lower HDL compared with day workers (23). Similarly, De Bacquer *et al* in a prospective study observed that shift workers compared with day workers have higher triglycerides and lower HDL (24). But in contrast to HDL and TG fluctuations, Ghiasvand *et al's* as well as current study found no difference in the level of HDL and TG after adjustment for age and food type between different chronotype groups (25).

Some studies as well as our study have found no difference in TC and LDL levels between circadian chronotype groups (26-28). In spite of our results about LDL and TC, Merikanto *et al* examined the association between different chronotypes and cardiovascular disease factors in a large population in Finland and found that eveningness chronotype group had lower serum levels of cholesterol and LDL compared with other chronotype groups (21).

HOMA-IR index as an insulin resistance index was evaluated in our study, but showed no association with chronotypes. Despite our findings, Gonnissen *et al* showed circadian misalignment reduced Rapid Eye Movement (REM) sleep, specially sleep during the second part of the night. REM sleep was inversely associated with HOMA-IR index and can reduce insulin sensitivity (29).

In the current study, it was found that hs-CRP had a significant effect on chronotype groups, as higher hs-CRP level was related to eveningness chronotype compared to morning chronotypes. Several studies have shown high inflammatory markers in eveningness chronotype (30,31). Similar to our findings, Morris *et al* recently found that participants who experienced misalignment circadian rhythm had higher hs-CRP levels (32).

Mechanisms of increased hs-CRP level can be discussed in two ways; first, direct effect of circadian rhythm misalignment can be assessed on hs-CRP and, second, indirect effect of circadian rhythm misalignment on obesity and consequently on hs-CRP can be investigated. It was shown that circadian misalignment can have adverse effects by increasing the level of hs-CRP in participants who experienced sleep deprivation (33-35). This is in accordance with Leproult *et al* findings; moreover, Wright *et al* showed that this type of circadian misalignment not only increased the hs-CRP levels but also increased other inflammatory markers (36,37). The mechanism of increased hs-CRP is not well understood, but animal studies showed that inflammatory responses may be related to clock gene expression (38,39). Another potential factor is the role of melatonin. It has been suggested that melatonin has effects on the immune system via controlling cytokines action. Sleep deprivation may change the regulation of inflammatory responses in the absence of melatonin (40,41).

One more possible mechanism for the mentioned association can be explained by contribution of obesity in increasing inflammatory factors. Insufficient sleep may be a potential important contributing mechanism in increased accrual of adipocytes. Leptin, an adipocyte hormone signaling satiety to the brain, is reduced in acute and sustained partial sleep deprivation. Ghrelin, a hormone which signals hunger to the brain, increases during partial sleep deprivation. The combination of slow metabolism produced by sleep deprivation and increased appetite may theoretically lead to weight gain and increasing prevalence of obesity. It has been demonstrated that obesity is associated with low-grade inflammatory process characterized by the increase in circulating levels of pro-inflammatory cytokines such as IL-6, TNF-alpha and CRP in obese subjects (42,43).

Conclusion

To our knowledge, this is the first study that assessed the relationship between metabolic unhealthy obesity and circadian rhythm. The strength of the study was its adjustment for some major confounders. Since our study was a cross-sectional one, certainly further randomized clinical trials and observational prospective studies are needed to confirm these findings. In conclusion, our findings showed a significant association between hs-CRP and CRC in OW/OB women, which shows that inflammation may have a role in a CRC and metabolic status.

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Conflict of Interest

None declared.

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