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Variants in Circadian Rhythm Gene *Cry1* Interacts with Healthy Dietary Pattern for Serum Leptin Levels: a Cross-sectional Study

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ABSTRACT

Circadian disruption causes obesity and other metabolic disorders. There is no research considering the role of Cryptochromes (Cry) 1 body clock gene and major dietary patterns on serum leptin level and obesity. We aimed to investigate the interaction between *Cry1* circadian gene polymorphisms and major dietary patterns on leptin and obesity related measurements. This study was performed on 377 overweight and obese women. Mean age and body mass index (BMI) of study subjects were 36.64 ± 9.02 years and 30.81 ± 3.8 kg/m^2, respectively. Dietary assessment was done using a validated 147-item food frequency questionnaire. *Cry1* rs2287161 were genotyped using polymerase chain reaction-restriction fragment length polymorphism. Generalized linear models were used for interaction analysis. Healthy and unhealthy dietary pattern (HDP and UDP, respectively) were extracted using factor analysis (principal component analysis). Our study revealed a significant higher weight (p = 0.003) and BMI (p = 0.042) in women carrying CC homozygote compared with G allele carriers. Moreover, our findings showed a significant gene-diet interaction between HDP and *Cry1* rs2287161 on BMI (p = 0.034) and serum leptin level (p = 0.056) in which, BMI and serum leptin level were lower in subjects with CC genotype than in those with GG genotype while following HDP. This study suggests a significant interaction between *Cry1* rs2287161 polymorphisms and HDP on BMI and serum leptin and the lowering effects were apparent among C allele carriers compared to G allele ones. This data highlights the role of dietary pattern in relation of gene and obesity.

Keywords: Gene-environment interaction; Obesity; Body mass index; Leptin

Conflict of Interest
The authors declare that they have no competing interests.

INTRODUCTION

New evidences link circadian rhythm disruption to metabolic disorders such as obesity and other component of metabolic syndrome [1,2]. In mammals, the circadian rhythm affects many aspects of physiology and behavior, including sleep cycle, cardiovascular activity, endocrine system, and etc [3,4]. At the molecular level, the circadian clock is arranged by a regular network of transcription and translation factors that control the expression of genes related to the body clock. This network is regulated by positive and negative feedback in the suprachiasmatic nucleus (SCN) [5]. Cryptochromes (*Cry*) 1, the main components of the negative arm of core clock, plays several roles in metabolism [6]. In experimental studies, it has been demonstrated that circadian clock genes modolute the expresion of adipokines like leptin [7]. Moreover, SCN destruction has removed the leptin circadian rhythm in rodents which shows that circadian clock regulate the expresion of leptin [8]. Also, the studies have been shown the development of overweight, metabolic syndrom and hyperleptinemia in clock genes mutant mice [9].

As leptin regulates appetite and body weight, so impaired synthesis, signaling, or sensitivity to leptin can lead to impaired energy homeostasis and body composition [10]. Hyperleptinemia is a feature of obesity in humans and rodents in which it is accompanied by hyperphagia and leptin resistance [11,12].

Many studies on animal models have shown that leptin resistance is involved in the pathogenesis of diet-induced obesity [13]. Also, as shown in many rodent models, eating a high-fat diet increases resistance to central and peripheral leptin. Therefore, it seems that blood lipids play an important role in the development of leptin resistance by regulating cellular responses to the hormone. For example, some studies have shown that eating a high-fat diet causes leptin resistance in the arcuate nucleus and the ventral tegmental area of the hypothalamus and results in diet-induced obesity [14,15], so, nutrients from diet and dietary pattern can be another factor affecting the leptin resistance development. A cohort study have shown the positive association between adherance to western dietary pattern and leptin concentration in pregnant women [16]. In contrast, some other studies did not support these findings [17,18]. Therefore, genetic and environmental factors must be considered together for a comprehensive study of leptin and obesity. The present study have been addressed the issue of obesity and leptin resistance by considering genetic (*Cry1* polymorphysims) and environmental factors (dietary pattern) with a comprehensive approach.

MATERIALS AND METHODS

Study design and participants

A total of 377 participants were investigated for the current cross-sectional study conducted between February 2018 and May 2019. Study participants were recruited from overweight and obese women referred to 21 health centers of Tehran, Iran by a multistage cluster random sampling method and provided written informed consent. The inclusion criteria defined as: 1) body mass index (BMI) between 25 to 40 kg/m^2, and 2) an age over 18 years. Exclusion criteria comes as follow: 1) menopause, 2) pregnancy, 3) history of any chronic disease, 4) using nutritional supplements over the last 3 months, and 5) following any kind of weight loss regimen over the last 1 year. The assessment were conducted in the laboratory of the Faculty of Nutritional Science and Dietetics, Tehran University of Medical Science. The study

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was approved by the ethics committee of Tehran University of Medical Science (IR.TUMS.VCR.REC.1398.051).

Outcome measurements

Anthropometric measurements

All participants were evaluated for the main anthropometric measurements. Weight was assessed without shoes with the least clothing using a calibrated scale (Seca 808; Seca, Hamburg, Germany) with a sensitivity of 0.1 kg. Height was measured without shoes in standing position with a sensitivity of 0.5 cm by a stadiometer (Seca). BMI was calculated by the formula weight (kg)/height^2 (m). Waist circumference was measured by an unstreached tape and in expiratory state. The hip circumference was also measured from the largest part with the same tool.

Dietary assessment

A 147-item semi-quantitative food frequency questionnaire (FFQ) was adminstered to assess dietary intake during the 12 months before the study. A trained questioner interviewed with participants and obtained their amount and frequency of food intake on a daily, weekly, monthly, or yearly basis. The servings and portion sizes reported by study subjects were converted to grams per day. Then, food analysis was done using the Nutritionist IV software (version 7; N-Squared Computing, Salem, OR, USA). The 147 food item were categorized into 16 food groups (**Table 1**). The validity and reliability of the questionnaire were previousely evaluated in Tehran Lipid and Glucose Cohort and have shown good results [19].

Laboratory measurements

Between 7 and 10:30 a.m., 10 mL of venous blood samples were taken on fasting overnight for 12 hours and immediately divided, and 7 mL of them was kept at room temperature for 30 minutes until the blood clots formed. Then, blood samples were centrifuged at 1,500 g for 15 minutes, poured into several separate clean micro-tubes and stored in −80°C freezer until the analysis. The rest were not centrifuged, poured into acid-washed tubes without anticoagulants and stored at −20°C for genotyping analysis. Serum leptin concentrations (ng/mL) were determined using enzyme-linked immunosorbent assay commercial kits (Mediagnost, Reutlingen, Germany).

Table 1. Food grouping used in dietary pattern analysis

Food groups	Food items
Vegetables	Cucumbers, tomatoes, leafy greens, stewed vegetables, celery, green peas, green beans, green peppers, bell peppers, turnips, squash, pumpkin, mushrooms, raw onion, garlic, any kind of cabbage, carrots, spinach and lettuce
Fruits and fruit juices	Apples, cherries, apricots, plums, figs (dried or fresh), kiwi, strawberries, grapes or raisins, dates, bananas, pomegranates, melons, oranges, tangerines, grapefruits, pears, persimmons, cantaloupe, melons, watermelons, nectarines, peaches, greengage, lemons, berries (dried or fresh), and other dried fruits, orange juice, apple juice, cantaloupe juice and fruit compote
Dairies	Milk, yogurt, cheeses, buttermilk, curd, cream, strained yogurt, ice creams, cocoa milk
Grains	All kind of bread, rice, pasta, noodles, vermicelli, wheat flour, barley, oatmeal and corn
Legumes	Beans, peas, lentils, mung beans, chickpeas, soybeans, beans
Red meats and eggs	Beef, lamb and eggs
White meats	Chicken, fish and canned tuna fish
Organ and processed meats	Internal organs of lamb such as liver, heart, kidney and so on and processed meats like hamburgers, and hot dogs
Fast food and sauces	Pizza, french fries and sausages
Nuts and healthy oils	Olive oil, almonds, peanuts, walnuts, pistachios, hazelnuts, and seeds
Unhealthy oils	Hydrogenated oil, vegetable oil, animal oil, mayonnaise, fried onions, fried eggplant, butter and margarine
Snacks	Chips and corn puffs
Sugar, sweets and deserts	Biscuits, crackers, cakes, sugar, candy, chocolate, honey, soft drinks, commercially produced fruit juices, jam, and all kind of sweets
Salts	Salt, pickles, and olives
Tea and coffee	Tea and coffee
Spices	All kind of spices

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Physical activity assessment

Physical activity evaluation was performed using the short form of the International Physical Activity Questionnaire (IPAQ) which expressed the acceptable validity and reliability in previouse studies [20]. This form includes 7 questions that describe the level of physical activity at 3 levels: intense, moderate, and walking. Each question consists of 2 sections of frequency of each activity in each level and the time allocated (minute per week). Metabolic equivalents were calculated based on the guide of IPAQ and reported as metabolic equivalent (MET)-minutes/week.

Circadian rhythm evaluation

The circadian rhythm assessment was conducted by the Morning-Evening Questionnaire (MEQ), which first introduced by Horne and Östberg in 1976 [21]. This questionnaire provides a good estimate of the peak alertness time during the day (morning, evening or between). The range of scores varies from 16 to 86, and a higher score indicates more morning preference, and a lower score indicates more evening preference. The validity and reliability of this test in Iran has been tested by Rahafar and colleagues [22] and have been shown good results.

DND

Genomic DNA was isolated from about 3 mL prepheral whole blood leukocytes using GeneAll Mini Columns Type kit (GeneAll, Seoul, Korea). Then the purity and concentration of extracted DNA was measured by Nano Drop spectrophotometer (Thermo Scientific Company, Waltham, MA, USA). The *Cry1* rs2287161 located in between *Cry1* and *MTERF2* gene. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was done using forward: 5′-GGAACAGTGATTGGCTCTATCT -3′ and reverse primer: 5′-GGTCCTCGGTCTCAAGAAG-3′. The PCR proccess was conducted in 5 steps of 1) 4 minutes initial denaturation (94°C), 2) 30 seconds denaturation (94°C), 3) 30 seconds annealing (58°C), 4) 30 seconds extension (72°C), and 5) 5 minutes final extension (72°C); denaturation, annealing, and extension were done for 35 cycles and the rest of the steps were done once. PCR products were digested using BseYI (cataloge number: R0635S; New England Biolabs, Essex, MA, USA) which yielded 2 cuts and 3 fragments of 108, 50, and 226 base pairs (bps); and one cut and 2 fragments of 156 and 226 bps in the presence of G and C alleles, respectively.

Statistical analysis

Data were analyzed using the IBM SPSS version 20 (SPSS, Chicago, IL, USA). Data were reported as means and standard deviations. Kolmogrov–Smirnov was used to verify the normality of data distribution.

We used principal component analysis (PCA) to identify the major dietary patterns. Varimax rotation was used to clarify and interpret the relationship among factors. Factors with eigenvalues > 1 were retained. Loading factor greater than 0.3 were considered to determin the major dietary pattern. Based on positive and negetive loading, direct and inverse association between factors and foods were recognized. Finally, according to researchers' interpretation of the similarity of the factors loaded in each pattern, the major ditary patterns were named as healthy dietary pattern (HDP) and unhealthy dietary pattern (UDP). To examine the adherece of participants to each of HDP or UDP, we catogorized each dietary pattern into 3 groups (tertiles).

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The one-way analysis of variance was used to compare the study variables, based on dietary pattern tertiles and genotypes. We used the post hoc method (Tukey) to find the between groups differences in terms of study variables. In addition, study variables were compared through analysis of covariance across the dietary patterns tertiles and genotypes adjusted for age, BMI, calorie intake, and physical activity.

The interaction between *Cry1* polymorphisms and major dietary patterns on leptin and BMI were verified by Generalized Linear Models. BMI and serum leptin, were response variables, while *Cry1* genotypes and each dietary pattern were considered as factor variables, and age and calorie intake as covariates. The p value < 0.05 was considered as statistically significant for all the tests except for interaction models, in which p values < 0.1 were considered as significant.

RESULTS

General charachteristics

Our study included 377 overweight or obese apparently healthy women with mean age, BMI, serum leptin and circadian rhythm of 36.64 ± 9.02 years, 30.81 ± 3.80 kg/m^2, 27.70 ± 11.88 ng/mL, and 52.44 ± 9.86, respectively. The general charachteristics of study participants are given in more details in **Table 2.**

Dietary pattern

We used factor analysis (PCA) to find the major dietary patterns. Two major dietary patterns entitled HDP and UDP, were determined by factor analysis. The 147-item FFQ were summerized to 16 food groups based on the similarity between food items. Factors with eigenvalues greater than one were retained. The loading factor lower than 0.3 were discarded, to specify the items of each food pattern. Food groups and loading factors are presented in **Table 3**. HDP was charachterized as consuming higher amounts of vegetables, fruits (fresh, dried, and fruit juices), dairy, meats (fish, poultry, and red meat), eggs; and lower amounts of unhealthy fats, grains, high-energy drinks, sweets, desserts, and industrial juices. In UDP, participants were more likely to consume higher quantities of processed and organ meats, fast foods, sauces, high-energy drinks, sweets, desserts, industrial juices, and lower quantities of legumes.

Each dietary pattern was divided into tertiles and study variables were reported and compared across the dietary pattern tertiles. There was a significant difference in physical activity level according to the HDP tertiles. Physical activity levels were significantly lower in people with the least adherance to HDP after the adjustment for age and calorie intake (p =

Table 2. Characteristics of study participants

Variables	Minimum	Maximum	Mean ± SD
Age (yr)	18	53	36.64 ± 9.02
Height (cm)	142	179	161.05 ± 5.82
Weight (kg)	59.50	122.40	79.90 ± 10.73
BMI (kg/m^2)	25.2	40.6	30.81 ± 3.80
Waist circumference (cm)	74.0	121.5	96.82 ± 9.65
Hip circumference (cm)	100	140	113.03 ± 7.75
Serum leptin (ng/mL)	4.18	51.92	27.70 ± 11.88
Circadian rhythm score	17	74	52.44 ± 9.86
Physical activity score (MET-minutes/week)	40	1,944	1,202.05 ± 1,085.34

MET, metabolic equivalent; SD, standard deviation; BMI, body mass index.

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Table 3. Food groups and loading factors for HDP and UDP

Food groups	Dietary patterns	
	HDP	UDP
Vegetables	0.733	
Unhealthy oils	−0.519	
Dairies	0.496	
Fruits, natural juices	0.495	
White meats	0.429	
Meat and egg	0.433	
Grains	−0.326	
Nuts and healthy oils	0.312	
Fast food and sauces		0.640
Organ and processed meats		0.633
Snacks		0.573
Legumes	0.306	−0.511
Sugar, sweets and deserts	−0.300	0.442
Salts		
Tea and coffee		
Spices		
Total variance	13.180	11.200

HDP, healthy dietary pattern; UDP, unhealthy dietary pattern.

0.023). Moreover, significant differences across the tertiles of UDP were observed for age ($p < 0.0001$), in which younger participants had the most compliance with the UDP. Acrosss the tertiles of UDP, a significant marginal statistical difference in serum leptin levels ($p = 0.058$) and circadian rhythm score ($p = 0.052$) has been observed after adjustment for confounding variable (**Table 4**).

Cry1 genotypes

Allele frequency of *Cry1* rs2287161 polymorphism was 51.98% and 48.02% for C and G allele, respectively (**Table 5**). Significant differences across *Cry1* rs2287161 genotypes were observed for weight ($p = 0.003$) and BMI ($p = 0.042$). Our study has revealed that study subject who carries CC genotype had higher weight and BMI compared with the other genotypes (GG and GC). Moreover, marginally significant difference was seen for hip circumference ($p = 0.052$), after adjusting for age, physical activity, and calorie intake.

Table 4. Characteristics of study participants according to adherence to HDP and UDP

Variables	HDP					UDP				
	T1	T2	T3	p value*	p value†	T1	T2	T3	p value*	p value†
Age (yr)	35.86 ± 9.44	36.49 ± 8.79	37.75 ± 9.05	0.235	0.303	37.72 ± 8.26[a]	38.28 ± 9.21[a]	34.09 ± 9.31[b]	**< 0.001**	**0.004**
Height (cm)	161.21 ± 5.78	160.02 ± 5.93	161.71 ± 5.83	0.070	0.211	161.80 ± 5.60[a]	160.04 ± 6.14[b]	161.13 ± 5.78[a,b]	**0.058**	0.445
Weight (kg)	79.66 ± 11.28	79.68 ± 9.69	80.17 ± 10.96	0.914	0.213	81.54 ± 10.78	78.50 ± 10.86	79.46 ± 10.13	0.069	0.365
BMI (kg/m^2)	30.73 ± 4.22	31.13 ± 3.57	30.65 ± 3.60	0.573	0.410	31.12 ± 3.56	30.70 ± 3.98	30.67 ± 3.89	0.576	0.497
Waist circumference (cm)	97.86 ± 9.63	96.21 ± 9.23	95.69 ± 14.16	0.403	0.378	97.02 ± 14.11	96.00 ± 9.10	96.85 ± 10.13	0.812	0.495
Hip circumference (cm)	112.93 ± 8.45	112.77 ± 7.42	113.63 ± 7.71	0.824	0.734	112.85 ± 7.56	114.38 ± 6.67	111.88 ± 8.29	0.262	0.092
Serum leptin (ng/mL)	27.91 ± 12.78	28.48 ± 11.39	26.59 ± 11.54	0.839	0.966	26.13 ± 12.11[a]	24.20 ± 11.34[a]	31.67 ± 11.25[b]	**0.036**	**0.058**
Circadian rhythm score	51.99 ± 9.73	52.88 ± 8.96	53.24 ± 9.68	0.604	0.501	54.21 ± 9.72[a]	53.39 ± 9.59[a,b]	50.60 ± 8.71[b]	**0.011**	**0.052**
Physical activity score (MET-minutes/week)	766.73 ± 721.12[a]	981.12 ± 1,071.73[a,b]	1,181.37 ± 1,280.46[b]	**0.037**	**0.023**	1,013.23 ± 1,132.90	953.38 ± 1,184.78	993.10 ± 856.10	0.933	0.897

Bold-faced values presented as significant association.
HDP, healthy dietary pattern; UDP, unhealthy dietary pattern; BMI, body mass index; MET, metabolic equivalent.
*The p value for analysis of variance test; †The p value for analysis of covariance test after adjustment for age, physical activity, and calorie intake. [a,b]Dissimilar letters indicate a significant difference between the tertiles after the post-hoc test.

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Table 5. Characteristics of study participants according to *Cry1* rs2287161 polymorphism

Variables	Genotypes (number, percent)			p value*	p value†
	GG (107, 28.4)	GC (148, 39.3)	CC (122, 32.4)		
Age (yr)	35.03 ± 8.30	36.83 ± 9.29	37.77 ± 9.30	0.070	0.081
Height (cm)	161.77 ± 5.59	160.27 ± 5.22	161.05 ± 6.10	0.128	0.536
Weight (kg)	78.25 ± 9.06[a]	77.49 ± 9.38[a]	82.56 ± 11.07[b]	< **0.001**	**0.003**
BMI (kg/m^2)	29.88 ± 3.05[a]	30.30 ± 3.33[a]	31.74 ± 4.13[b]	< **0.001**	**0.042**
Waist circumference (cm)	94.98 ± 8.84[a]	95.99 ± 8.36[a]	99.12 ± 10.76[b]	**0.013**	0.180
Hip circumference (cm)	111.37 ± 6.39[a]	112.02 ± 6.41[a]	115.38 ± 8.45[b]	**0.013**	**0.052**
Serum leptin (ng/mL)	26.41 ± 13.85	27.59 ± 11.98	28.43 ± 11.06	0.855	0.276
Circadian rhythm score	52.53 ± 10.06	52.22 ± 9.48	53.28 ± 10.19	0.350	0.940
Physical activity score (MET-minutes/week)	911.52 ± 827.49	1,034.29 ± 1,221.98	1,052.61 ± 1,168.78	0.702	0.374

Bold-faced values presented as significant association.
BMI, body mass index; MET, metabolic equivalent.
*The p value for analysis of variance test; †The p value for analysis of covariance test after adjustment for age, physical activity, and calorie intake. [a,b]Dissimilar letters indicate a significant difference between the tertiles after the post-hoc test.

Gene-diet interaction

The interaction between the *Cry1* rs2287161 polymorphisms and the major dietary patterns on leptin was tested by generalized linear models. Our study showed a significant interactive effect between HDP and *Cry1* rs2287161 genotypes on serum leptin. Serum leptin level was marginally significantly lower in women with 2 risk allele (CC genotype) in comparison to those with no risk allele (GG genotype) among those showing highest adherence to the HDP than among those showing lowest adherence to HDP (p = 0.056).

BMI follows a similar pattern to leptin. In obese and overweight women who carry the CC genotype compared to GG genotype, those who had higher adherence to HDP show the lower the weight (p = 0.034). Our study failed to confirm any link between UDP and *Cry1* genotypes on serum leptin and BMI. **Table 6** shows the data regarding the gene-diet interaction on BMI and leptin.

Table 6. Interaction of HDP and UDP with Cry1 on BMI and BFM

Interactions	BMI			Leptin		
	β	95% CI	p value*	β	95% CI	p value*
HDP						
CC*T3	−3.48	−6.71, −0.20	**0.034**	−14.82	−30.00, 0.35	**0.056**
CC*T2	−0.46	−3.81, 2.87	0.784	−6.30	−23.85, 11.24	0.481
CC*T1		Ref.			Ref.	
GC*T3	−2.26	−5.36, 0.80	**0.150**	−11.29	−26.56, 3.96	0.147
GC*T2	−1.21	−4.42, 1.99	0.457	9.51	−7.78, 26.81	0.281
GC*T1		Ref.			Ref.	
GG*T3						
GG*T2		Ref.			Ref.	
GG*T1						
UDP						
CC*T3	0.48	−1.77, 2.74	0.674	−3.11	−17.96, 11.73	0.681
CC*T2	0.86	−1.45, 3.17	0.465	−9.4	−29.11, 10.3	0.350
CC*T1		Ref.			Ref.	
GC*T3	−1.07	−3.26, 1.10	0.330	2.32	−12.03, 16.68	0.751
GC*T2	−0.85	−3.14, −1.42	0.461	−9.96	−29.32, 9.39	0.313
GC*T1		Ref.			Ref.	
GG*T3						
GG*T2		Ref.			Ref.	
GG*T1						

Bold-faced values presented as significant association.
HDP, healthy dietary pattern; UDP, unhealthy dietary pattern; Cry, cryptochromes; T, tertile; BMI, body mass index; BMF, body fat mass; CI, confidence interval.
*The p value for generalized linear models after adjust for age and calorie intake.

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DISCUSSION

The present cross-sectional study investigated the interaction between *Cry1* genotypes and dietary patterns on leptin hormone levels and obesity. According to our results, CC genotype predisposed a person to higher weight and other obesity related measurements, by following a healthier dietary pattern, but to lower serum leptin levels and BMI than those with the GG genotype. This data once again reveals the significant role of environmental factors such as diet on a person's health status.

So far, various epidemiological studies have been conducted on the interaction of genetic predisposition to obesity and diet on body weight. However, no study has evaluated the interactive effect of the *Cry1* polymorphisms and dietary patterns on leptin and body weight. In line with our results, most previous studies have shown that dietary and lifestyle factors for example high intake of sugar-sweetened beverages [23], high intake of fried food [24], high saturated fatty acids intake [24] and sleep characteristics [25] have a greater association with BMI or weight in subjects who are more genetically predisposed to obesity. A large study including data from 18 cohorts investigated whether a healthy dietary score modified the associations between genetic predisposition and obesity using genetic risk score. They founded that this association was accentuated in subject with healthier dietary scores [26]. Similarly, our study shows that following a HDP which includes higher amounts of vegetables, fruits (fresh, dried, and fruit juices), dairy, meats (fish, poultry, and red meat), eggs, and lower amounts of unhealthy fats, grains, high-energy drinks, sweets, desserts, and industrial juices was more related to the decrease in leptin and BMI in subjects with CC genotype of *Cry1* as a genotype prone to obesity.

Leptin is a hormone whose secretion reduces food intake and body weight. However, obese people have high levels of circulating leptin and some degree of leptin resistance [27]. Two potential mechanisms have been defined that may mediate leptin resistance. The first is limitation of leptin entry through the blood-brain barrier to central nervous system (CNS) [28] and the second is inhibition of leptin receptor expression and second messenger signaling primarily signal transducer and activator of transcription-3 in CNS [29]. The evidences regarding to the effect of dietary components on leptin levels are somewhat controversial [17,30-32]. On the other hand, it has been shown that leptin has a circadian rhythm and therefore interacts with genes that regulate circadian rhythms [33]. An animal study indicated that circadian disruption lead to leptin resistance [34]. Evidence suggests that inactivation of circadian rhythm genes, including *Per1*/*Per2*, *Cry1*/*Cry2* and *Bmal1* in mice affects body weight [34]. The researchers proposed that the direct circadian control of leptin expression in white adipocyte cells regulated through *BMAL1*/*CLOCK*-modulated C/EBPα-mediated leptin transcription, independent of food intake [35]. So far, no studies have examined the effect of *Cry1* genotypes on leptin status in human subjects.

This study has limitations. The cross-sectional design and gender specificity of the study deprives the ability to infer cause and effect and generalize to other societies. Also, FFQ is prone to recall bias and under- or over-estimation of nutrients intake since participants are requested to report their intake retrospectively and usually refer to prolonged periods of time [36]. Moreover, circadian rhythm score did not show a significant difference between the *Cry1* genotypes. Lack of difference between *Cry1* genotypes for circadian rhythm score can be explained in 2 ways, first, inappropriate distribution of study participants in terms of circadian rhythm score, as only 3% of the participants in this study were at the 2 ends

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of the circadian rhythm score range (completely evening or morning preference), the rest were between. Second, the circadian rhythm assessed by the MEQ questionnaire may be influenced by various environmental factors such as job status, cultural and social factors that act independently of the individual's genetics. However, this is the first study discussing the interaction between major dietary patterns and *Cry1* rs2287161 variants on serum leptin levels which is provides good information for designing further studies on the interaction of circadian clock genes and dietary patterns on weight and related hormones.

CONCLUSION

The present study shows the role of diet in mediating the relationship between gene, leptin and obesity. According to our study, following a healthier dietary pattern not only reduced the effect of the obesity-prone genotype CC, but also reduced the severity of serum leptin levels and BMI in the CC compared to GG genotype.

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