CRY1 polymorphism may influence the association of low carbohydrate diet (LCD) score on glucose homeostasis in overweight and obese women

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Abstract

Background and aims: We sought to examine the interaction between *CRY1* genotypes and low carbohydrate diet (LCD) score and the effect on insulin resistance, insulin sensitivity, homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (ISQUKI).

Methods: This cross-sectional study was conducted with a total of 228 overweight and obese women. The data related to anthropometric and biochemical measures were collected and a food frequency questionnaire (FFQ), with 147 items, was used to assess dietary intake. Based on the FFQ, we calculated an LCD score for each study participant, ranging from 0 to 70. Biochemical assessments, including TC, HDL, LDL, TG, FBS, insulin and HOMA-IR, were performed. Deoxyribonucleic acid (DNA) samples were assessed to be genotyped for the rs2287161, which was genotyped by the restriction fragment length polymorphism (PCR-RFLP) method. A generalised linear model was performed for interaction analysis.

Results: The results of the study demonstrated that, after controlling for several confounders, increased adherence to an LCD (T3 vs. T1) in the interaction with one risk allele genotype (CG) increases ISQUKI level (β: 0.001, CI: 0.00, 0.002, p=0.041). Also, there was a marginally negative interaction between higher adherence to LCD and two risk alleles genotype (CC) on insulin level (β: -0.012, CI: 0-0.024, 0.001, p=0.054).

Conclusions: This study revealed a negative interaction of *CRY1* genotypes with two risk allele and higher LCD adherence on insulin level, and a positive interaction on ISQUKI. However, the mechanism of interaction between LCDs and *CRY1* genotypes remains unclear.

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Key words: obesity, *CRY1*, HOMA-IR, ISQUKI, low carbohydrate diet, interaction.

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Introduction

Obesity has become one of the biggest global health problems in the last century, and its incidence continues to rise.[1] Nearly one third of the world's population is now classified as overweight or obese,[2,3] and women are almost 50% more likely than males to be obese.[4,5] The etiology of obesity is multifactorial; however, in Iranian women, a lower level of physical activity, having had several pregnancies, lower socioeconomic status, being unemployed, a higher ratio of depression, and lower level of education predispose to or coincide with an increased obesity risk.[6-9] Increased body mass index (BMI) is the main risk factor for several non-communicable diseases, such as cardiovascular disease (CVD), cancers, metabolic syndrome (MetS) and insulin resistance.[1,10-12] In addition, it has been shown that dietary and biological factors, which include genetic background and cultural influences, can have a significant effect on BMI.[1,10,13]

Diet plays a vital role in obesity, and intake of a high-carbohydrate diet can lead to obesity and insulin resistance. A low-carbohydrate diet (LCD), which is defined as a restriction in carbohydrate intake, can elicit dramatic weight loss and decrease the risk of diabetes development.[14,15] This diet tends to have a low glycaemic index and glyceric load and leads to decreased serum glucose level, insulin responses and glycated haemoglobin, and to increased insulin sensitivity.[16,17] Based on a cohort study, LCD scores can reduce the risk of diabetes,[18] and might directly affect hepatic glucose output and glucose utilisation through the production of ketone bodies.[19]

At the molecular level, the central circadian clock is made up of *Clock* (circadian locomotor output cycles kaput), *Bmal1* (brain and muscle Arnt like protein-1), *Per* (period) 1,2,3, and

cryptochrome (*Cry*) 1,2 genes.[20] *Cry1* and *Cry2* are thought to be essential elements in the development of the circadian rhythm in mammals.[21]

The human circadian clock is responsible for modulating energy intake from diet and expenditure based on changes in external factors, including sunset/sunrise, physical activity and dietary intake.[22-24] Recently, researchers have looked into how the circadian clock affects insulin resistance.[25] The circadian system cycles through many hormones, including orexin, leptin and glucagon, that regulate insulin secretion, glucose balance and eating.[26] Due to this regulation, the loss of circadian rhythmicity brought on by the variations of genes that alter the coding regions can exert adverse influence over the homeostasis of glucose. Previous investigations have explicitly linked clock variations to diabetes and insulin resistance.[27,28] *Cry1* and *Cry2* make up the negative limb of the core body clock and seem to possess a crucial role in regulating gluconeogenesis and diminishing insulin resistance.[29] *Cry* suppresses gluconeogenesis by inhibiting protein kinase A–mediated phosphorylation of cAMP response element–binding protein (CREB) during fasting via blocking of glucagon-mediated increases in intracellular cAMP concentration.[29,30] Knockout of *Cry1* and *Cry2* causes glucose intolerance and elevated circulating glucose concentrations. As a result of this recognised role of cryptochromes in regulating glucose homeostasis, polymorphisms in *Cry1* and *Cry2* genes may be related to risk for insulin resistance or type 2 diabetes.

To our knowledge, no previous studies have investigated the interaction of adherence to LCD, insulin resistance and *Cry1* genotypes. Therefore, the purpose of the present study was to assess the interaction between *Cry1* genotypes and LCD score on insulin resistance and insulin sensitivity in overweight and obese women.

Materials and methods

Study population

A total of 404 obese and overweight women referred to health centres in Tehran, Iran, between 2017 and 2019 were entered into this cross-sectional study. Of the 404 subjects recruited, 377 were eligible for inclusion, and after assessing their medical history, 266 people met the final conditions for study entry. However, only 226 participants were available to be genotyped for rs228716. All women had self-certified good general health. In the school of Nutritional Sciences and Dietetics at Tehran University of Medical Sciences (TUMS), anthropometric measurements and blood samples were evaluated. The study protocol had ethical approval (ID: IR.TUMS.VCR.REC.1398.051) from the Ethics Commission of the Tehran University of Medical Sciences (Grant number: 99.3.212.50265). All subjects provided written informed consent before participation.

All participants were overweight or obese with body mass index (BMI) ranging between 25 and 45 kg/m^2 and they were aged between 18 and 48 years. No acute or chronic infection or inflammatory disease was present, and no excess intake of drugs/alcohol was reported. We excluded subjects who were lactating or pregnant at the time of the study and those with a history of hypertension, cardiovascular disease, diabetes mellitus, impaired renal and liver function, alcohol use, smoking and menopause and those with any significant body weight fluctuations over the previous year. According to the food frequency questionnaire (FFQ) responses, we excluded participants with reported total energy intake of <800 kcal/ day or >4200 kcal/ day as well as those with poor response to FFQ.

Dietary and glycaemic load (GL) assessment

Usual dietary intake over the preceding year and nutritional status were assessed using a 147-item semi-quantitative FFQ. This procedure was designed according to the Willett study, with a standard serving size for each nutrient, which was validated in 2010 in Iran.[31] Participants reported the frequency of intake and the amount of each food item during the previous year on a daily, weekly or monthly basis. Nutritionist-4 software was used to analyse the FFQ data . The nutritionist IV software (version 7.0; N-Squared Computing, Salem, OR) was used to convert grams of each item per day. Average glycaemic index (GI) values from the GI table which was reported by Foster-Powell was applied to calculate the GI for carbohydrate-containing food items (foods with ≥5 g carbohydrate per 100 g or 100 mL).[32] Average daily dietary GI was calculated by multiplying the GI of individual foods by the proportion of total energy contributed by carbohydrates ([GI of food item * (grams carbohydrate of consumed food/ total grams of carbohydrate consumed per day)]). Dietary glycaemic load (GL) was measured as [(daily GI * grams carbohydrate consumed per day) /100].

LCD score

Participants in this study were classified into seven groups according to their carbohydrate, refined grain, vegetable and protein intake, monounsaturated fatty acid (MUFA) and n3/n6 polyunsaturated fatty acid (PUFA), expressed as a percentage of energy intake, and also by their fibre consumption (g/1000Kcal) and glycaemic load (GL). Dietary GL was estimated based on total available carbohydrate per 100g and total glycaemic index expressed as g/d. Women with the lowest consumption of refined grains, carbohydrates and GL were given a score of 10, and those with the highest consumption were given a score of 0. For n3/n6 PUFA, MUFA, fibre and vegetable protein intake, the order of the strata was reversed. The points for the seven items were added together to create the overall score, named the "low carbohydrate diet score", which ranged from 0 to 70. Therefore, higher LCD scores demonstrated closer adherence to low-carbohydrate diets.[33]

Anthropometry measurements

Weight was measured with a calibrated digital Seca scale (803, Germany) with a sensitivity of 0.1 kg, and height was measured with a wall-mounted Seca 206 stadiometer (Germany). BMI was calculated by use of the equation weight (kg)/height^2 (m^2). Waist circumference (WC) was measured at the central point between the iliac crest and rib cage with tape with an accuracy of 0.1 cm, and waist to hip ratio (WHR) of subjects was calculated by dividing WC by hip circumference. A trained dietitian performed all of the measurements according to specific guidelines, to reduce interpersonal variation.

Clinical parameters assays

Fasting blood glucose (FBG) was measured after 10 to 12 hours of overnight fasting, using the glucose oxidase phenol 4-Aminoantipyrine Peroxidase (GOD/PAP) method, at the Nutrition and Biochemistry Laboratory of the School of Nutritional Sciences Dietetics, TUMS. Samples of serum were centrifuged for serum collection for 10 min at 300 rpm, diluted in 1 ml tubes, and stored at –80°C until analysis. Serum insulin concentrations were analysed through the enzyme-linked immunosorbent assay (ELISA) method (human insulin ELISA kit, DRG Pharmaceuticals GmbH, Germany). The concentration of serum triglycerides (TG) was assessed using the kits triacylglycerol (test Pars Inc, Tehran, Iran) glycerol-3-phosphate oxidase method using phenol 4-Mynvanty Pirin peroxidase (GPOPAP). Total cholesterol levels were measured using Endpoint Enzymatic. Also, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were measured by an enzymatic clearance test.

The homeostasis model assessment method was used to compute insulin resistance based on the following HOMA-IR formula: fasting serum insulin (mIU/L) × fasting blood glucose (mmol/l)/22.5.[34] The Insulin sensitivity quantitative insulin sensitivity check index (ISQUICKI) was assessed by: ISQUICKI = 1/[log (fasting insulin) + log (fasting glucose)].[35]

DNA extraction and gene sequencing

In all, 226 participants were assessed to be genotyped for the rs2287161. Genomic DNA extraction from whole blood samples was completed using Mini Columns (Type G Exgene; Genall, Korea) according to the manufacturer's protocol. The concentration and quality of the extracted DNA were calculated using a NanoDrop ND-2000 spectrometer. The rs2287161 (major allele: C; minor allele: G) was genotyped using a polymerase chain reaction-restricted length polymorphism (PCR–RFLP) technique.

PCR applied the following primers: forward 5′-GGAACAGTGATTGGCTCTATCT -3′; reverse 5′-GGTCCTCGGTCTCAAGAAG-3′. PCR reactions were carried out in a final volume of 20 µl made up of 2 µl primers, 1 µl extracted DNA, 7 µl distilled water and 10 µl Taq DNA Polymerase Master Mix (Amplicon; Denmark) with the next conditions in a DNA thermocycler: The DNA templates were denatured at 94°C for 4 minutes; amplification contained 35 cycles at 94°C, 58°C and 72°C (each stage for 30 seconds), with a final extension at 72°C for 7 minutes. Amplified DNA (10 microliters) was mixed with 2 microliters of DRI restriction enzyme (Thermo Fisher Scientific, USA) at 37°C. To ensure the PCR process and amplification of the desired parts, PCR product electrophoresis was performed on the agarose gel. Fragments including three possible genotypes were then determined: uncut homozygous GG (107 bp), cut heterozygous GC (107,48 and 226 bp), and cut homozygous CC (155 and 226 bp).

Assessment of other variables

Data pertaining to age, sex (male, female), education level (illiterate, below diploma, diploma, masters and above) and marital status (single or married) were collected by a trained interviewer.

To assess the physical activity of the participants, the short form of the International Physical Activity Questionnaire (IPAQ), designed by the World Health Organization, was used.[36] The validity and reliability of this tool has already been evaluated and shown to be acceptable in Iranian women. Scores were calculated based on Metabolic Equivalents minutes per week (MET-min/week).[37]

Statistical analyses

The normality of variable distribution was evaluated using the Kolmogorov-Smirnov test (<0.05). Participants were divided into tertiles of LCD scores. We analysed the study participants' characteristics according to the LCDS tertiles and *Cry1* genotype, using one-way analysis of variance (ANOVA) to compare continuous variables and $\chi 2$ tests for categorical variables. General variables across tertiles of LCDs and *Cry1* genotypes were reported as mean +/- standard errors (SE) for continuous variables; categorical variables were expressed as numbers and percentages. Analysis of covariance (ANCOVA) was used to assess a comparison of continuous and categorical variables between the tertiles of LCDs and genotypes, with adjustment for confounders including age, physical activity, BMI and total energy intake.

Genotypes were recoded according to numbers of risk alleles: code 0 for GG, 1 for CG, and 2 for CC genotypes. To examine the interactions, a generalised linear model (GLM) was used. Age, physical activity, BMI and total energy intake were adjusted in the GLM to assess the potential interactions of *Cry1* genotypes and LCDs on insulin, HOMA-IR and ISQUICKI. GG genotype, that lacked a risk allele, was used as the reference group. All analyses were carried out by a statistical Package for Social Science (Version 22.0; SPSS Inc., Chicago IL, US). A p value<0.05 was considered statistically significant, and for interactions $0.05 \leq p < 0.07$ was considered marginally significant.

Results

Study population characteristics

The general characteristics and biochemical measurements of subjects across tertiles of LCD scores are shown in Table 1. A total of 228 women were divided into tertiles of LCD scores. The mean (SD) age, height and weight were 36.4±8.42 years, 161±5.65 cm and 78.9±10.5 kg, respectively. The mean (SD) of glycaemic parameters such as HOMA-IR and ISQUCKI was 3.33(0.158) and 0.497(0.002) mg/l, respectively.

Participants' age, physical activity, BMI, WC, WHR, body weight, height, FBS, LDL cholesterol, insulin and ISQUKI did not differ across tertiles of the LCD scores.

In the crude model, significant differences were found in education across tertiles of LCD scores (p<0.026). Also, there was a significant difference across tertiles of LCD for HOMA-IR after adjustment for potential confounders (p=0.035). Subjects in the third tertile of LCD score had lower levels of HOMA-IR. After controlling for confounding variables, a significant trend toward decreased TG was found with increases in the LCD score. In comparison with the first tertile, participants in the third tertile of the LCD score had lower levels of TG (p=0.018). Besides that, a significant trend toward increased HDL cholesterol was found with increases in the LCD score in the adjusted model (p=0.036).

Although no difference was found between *Cry1* genotypes across LCD tertiles, the frequency of genotypes which included no risk allele (GG) was more in the third tertile (24%); by contrast the. majority of genotypes containing one risk allele (CG) were found in the first tertile (31%) (p>0.05) (Table 1).

Study participant characteristics among genotypes of *Cry1*

The general characteristics and biochemical measurements of subjects according to genotypes of *Cry1* are shown in Table 2. Participants were categorized based on *Cry1* genotypes and divided into three groups: GG genotype (n=80), CG genotype (n=73) and CC genotype (n=75). After genotype classification, significant differences in the crude model were found among genotypes for age (p=0.011), weight (p=0.003), BMI (p=0.015), WC (p=0.048) with a lowest mean in low-risk allele (GG), and HDL (p=0.036) with the highest mean in the CG group. After adjustment for age, physical activity, BMI and total energy intake, only weight (p=0.038) and BMI (p=0.032) with higher means in the group with two allele risk (CC), remained significant. For other variables, no difference was observed (p>0.05) (Table 2).

Dietary intakes of participants and LCD components

Dietary intakes of the participants across LCDs tertiles are shown in Table 3. Intake of food groups such as vegetables, legumes, dairy, meat and seafood was higher in the highest tertile compared to the lowest tertile of LCD score (p<0.05). Total energy intake was significantly higher in the lowest LCD tertile compared to the highest tertile (2828 vs. 2402 kcal/day), and a similar result was found for carbohydrate intake (p<0.001). Protein, total fat, MUFA and PUFA increased significantly from the first tertile to the third tertile (p<0.001). After adjusting for energy intake, there were significant differences among tertiles of LCD score for all micronutrients except iron and magnesium. In the top tertile of the LCD score consumption of calcium and zinc was greater than in the first tertile (p<0.05) whereas intakes of selenium (p=0.011) and chromium (p=0.002) were significantly lower (Table 3).

The interaction of *Cry1* genotypes and LCDs on insulin, HOMA-IR index and ISQUKI

In Table 4, the GG genotype was considered as the reference. In the crude model, increased adherence to LCD (T3 vs T1) in the interaction with the CC genotype led to a decrease in both insulin level (β: -0.013, CI: -0.025, -8.02, p=0.049) (Figure 1) and HOMA-IR (β: -0.003, CI: -0.006, -7.48, p=0.049) (Figure 2). However, after controlling for confounding variables such as age, physical activity, BMI and total energy intake, the results for insulin level only remained marginally significant (β: -0.012, CI: 0-0.024, 0.001, p=0.054). Additionally, in the crude model, increased adherence to LCD (T3 vs T1) in the interaction with genotypes including two risk alleles (CC) yielded an increase in ISQUKI (β: 0.001, CI: -1.43, 0.002, p=0.053) (Figure 3). After adjusting for confounders, a significant positive interaction between genotypes including one risk allele (CG) with increased adherence to LCD and ISQUKI was found (β: 0.001, CI: 0.00, 0.002, p=0.041) (Table 4).

Table 1 Study participant characteristics and frequency of *Cry1* genotypes across tertiles (T) of LDCs

Variables	LCDs			P value	P value*
	T1≤32 (n=80)	33<T2<36 (n=73)	T3≥37 (n=75)		
Age (years)	34.4±1.42	33.5±1.19	34.0±1.53	0.900	0.137
Physical activity (min/week)	1031±185	1292±310	735±143	0.211	0.217
Anthropometric measurements					
Height (cm)	160±0.674	160±0.694	160±0.700	0.439	0.365
Weight (kg)	78.9±0.739	78.7±0.741	78.0±0.774	0.750	0.743
BMI (kg/m^2)	30.1±0.674	29.4±0.502	29.6±0.633	0.614	0.396
WC (cm)	95.6±1.58	96.7±1.58	95.1±1.70	0.774	0.345
WHR	0.912±0.008	0.926±0.008	0.914±0.008	0.473	0.557
Blood parameters					
Cholesterol (mg/dl)	184±4.14	182±4.28	186±4.31	0.616	0.795
TG (g/dl)	124±7.42	104±7.81	117±7.72	0.923	**0.018**
HDL (mg/dl)	45.3±1.35	44.9±1.39	49.6±1.40	0.262	**0.036**
LDL (mg/dl)	93.0±3.94	100±4.21	91.6±4.02	0.327	0.309
FBS (mmol/l)					
Crude model	87.3±1.05	87.2±1.08	87.1±1.19	0.817	
Model 1	86.3±1.54	86.2±1.65	86.0±1.64		0.988
HOMA-IR					
Crude model	3.19±0.142	3.19±0.138	3.16±0.178	0.068	
Model 1	2.92±0.195	2.86±0.208	2.17±0.206		**0.035**
Insulin (mIU/ ml)					
Crude	1.23±0.025	1.19±0.25	1.20±0.028	0.510	
Model 1	1.22±0.042	1.23±0.045	1.16±0.044		0.620
ISQUKI (mg/l)					
Crude	0.795±0.002	0.499±0.002	0.499±0.003	0.545	
Model 1	0.497±0.004	0.497±0.004	0.504±0.004		0.469
Qualitative variables					
Education				**0.026**	0.381
Illiterate	0	0	3 (100)		
Beneath diploma	9 (32.1)	12 (42.9)	7 (25)		
Diploma	34 (39.1)	32 (36.8)	21(24.1)		
Master and upper	37 (33.6)	28 (25.5)	45 (40.9)		
Marital status				0.725	0.704
Single	16 (32)	15 (30)	19 (38)		
Married	64 (36)	57 (32)	57 (32)		
Cry1 genotypes				0.975	0.617
GG	30 (37)	23 (28.4)	27 (34.6)		
CG	27 (37)	22 (30.1)	24 (32.9)		
CC	28 (37.3)	22 (29.3)	25 (33.4)		

LCD, low carbohydrate diet; BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio; TG, triglyceride; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; FBS, fasting blood sugar; HOMA, homeostatic model assessment; ISQUICKI, insulin sensitivity quantitative insulin sensitivity check index; T, tertile; n, number of subjects.

Data are presented as n (%) or mean ± standard error. The one-way analysis of variance and the chi-square test were used for comparison of continuous and categorical variables, respectively, among tertiles of LCDs.

Significance of variance analysis test.

*Significant level after adjustment for age, physical activity, BMI and total energy intake. p<0.05 was considered significant.

Table 2 Demographic and blood parameters of study participants across *Cry1* genotype

Variables	*Cry1* genotypes			P value	P value*
	GG (n=80)	CG (n=73)	CC (n=75)		
Age (years)	34.1±0.848	37.1±0.904	37.9±1.03	**0.011**	0.387
Physical activity (min/week)	922±101	1275±265	1625±276	0.223	0.809
Anthropometric measurements					
Height (cm)	161±0.771	160±0.673	1610±0.762	0.219	0.144
Weight (kg)	78.3±0.829	77.1±0.724	79.9±0.720	**0.003**	**0.038**
BMI (kg/m^2)	29.9±0.376	29.9±0.352	31.4±0.480	**0.015**	**0.032**
WC (cm)	97.1±0.612	96.7±0.535	98.1±0.605	**0.048**	0.413
WHR	0.927±0.006	0.926±0.005	0.935±0.006	0.139	0.490
Blood parameters					
Cholesterol (mg/dl)	187±4.61	178±4.56	180±4.03	0.745	0.333
TG (mg/dl)	113±8.33	116±7.27	111±8.24	0.618	0.916
HDL (mg/dl)	45.9±1.48	48.5±1.29	45.0±1.46	**0.036**	0.169
LDL (mg/dl)	92.7±3.21	92.7±2.80	94.8±3.17	0.806	0.952
FBS (mmol/l)					
Crude	85.7±0.926	87.6±0.952	89.3±1.35	0.078	
Model 1	85.3±1.28	87.7±1.11	88.5±1.26		0.149
HOMA-IR					
Crude	3.36±0.127	3.20±0.129	3.46±0.189	0.429	
Model 1	3.23±0.168	3.29±0.147	3.24±0.166		0.946
Insulin (mIU/ ml)					
Crude	1.24±0.031	1.17±0.021	1.23±0.028	0.120	
Model 1	1.22±0.032	1.17±0.028	1.21±0.032		0.403
ISQUKI (mg/l)					
Crude	0.497±0.003	0.499±0.002	0.493±0.002	0.227	
Model 1	0.499±0.003	0.500±0.003	0.496±0.003		0.572

BMI, body mass index; WC, waist circumference; WHR, waist hip ratio; TG, triglyceride; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA, homeostatic model assessment; ISQUKI, Insulin sensitivity quantitative insulin sensitivity check index; T, tertile; n, number of subjects.

Data are presented as mean ± standard error. The one-way analysis of variance and covariance (ANCOVA) was used for the comparison of continuous variables among tertiles of CRY1 genotypes.

Model 1 adjusted for age, physical activity, BMI and total energy intake

Significance of variance analysis test

*Significant level after adjustment for age, physical activity, BMI, and total energy intake. $p < 0.05$ was considered significant.

Table 3 Components of LCD and micronutrient and macronutrient intakes of study participants across LCD score tertiles

Variables	LCD			P value*
	T1≤32 (n=80)	33<T2<36 (n=73)	T3≥37 (n=75)	
	Mean±SE			
Components of LCD				
GL	638±57.2[7,9]	372±59.8[9]	261±59.8[7]	**<0.001**
Carbohydrates (% energy)	60.9±0.642[7]	56.4±0.662	53.3±0.659[7]	**<0.001**
Refined grains (% energy)	22.1±0.705[7,9]	18.1±0.728[8,9]	13.5±0.729[7,8]	**<0.001**
MUFA (% energy, g/d)	1.05±0.032[7,9]	1.24±0.033[9]	1.30±0.033[7]	**<0.001**
PUFA (% energy, g/d)	6.08±0.270[7,9]	7.36±0.278[9]	7.45±0.277[7]	**0.001**
N3/N9	0.073±0.004	0.080±0.004	0.104±0.004	**<0.001**
Vegetable protein intake (% energy)	22.1 ±0.705[7]	18.1±0.728[8]	13.8±0.729[7,8]	**<0.001**
Total fibre (g/1000Kcal)	16.2±0.628	17.7±0.648	18.1±0.649	0.097
Food groups				
Whole grains (g/d)	5.96±1.20	8.32±1.24	9.06±1.24	0.183
Fruits (g/d)	541±34.1	547±35.5	531±35.5	0.949
Nuts (g/d)	13.8±1.82	14.3±1.88	18.6±1.88	0.141
Legumes	40.4±4.65[7]	57.9±4.84	70.0±4.81[7]	**<0.001**
Sweet desserts	68.5±14.5	77.2±15.0	51.0±15.5	0.460
Vegetables (g/d)	346±25.8[7]	421±26.68	575±26.6[7,8]	**<0.001**
Dairy (g/d)	345±25.6[7]	384±26.4	440±26.5[7]	**0.039**
Vegetable oils (g/d)	19.0±2.08	25.8±2.15	22.8±2.15	0.076
Animal fats (g/d)	3.48±0.858	4.38±0.88[6]	5.57±0.887	0.247
Meat (g/d)	47.0±4.78[7,9]	66.0±4.94[9]	81.7±4.94[7]	**<0.001**
Seafood (g/d)	8.10±1.28[7]	12.2±1.32	13.9±1.32[7]	**0.006**
Macronutrients and energy				
Energy (kcal)	638±57.9[7]	372±59.8	261±59.8[7]	**0.002**
Carbohydrate (g/d)	401±4.27[7,9]	371±4.38[8,9]	351±4.35[7,8]	**<0.001**
Protein (g/d)	82.4±1.75[7]	87.4±1.80[8]	96.4±1.79[7,8]	**<0.001**
Protein (% energy)	13.6±0.255[7]	13.3±0.264[8]	14.8±0.262[7,8]	**<0.001**
Total fat (g/d)	84.5±1.87[7,9]	95.9±1.92[9]	101±1.91[7]	**<0.001**
Fat (% energy)	29.1±0.631[7]	33.0±0.651	34.9±0.648	**<0.001**
Micronutrients				
Vitamin E (mg/d)	15.0±0.925[9]	19.1±0.949[9]	17.2±0.944	**0.010**
Vitamin A (mg/d)	640±39.0[7]	760±40.3[8]	974±40.1[7,8]	**<0.001**
Thiamin (mg/d)	2.19±0.037[7]	2.06±0.038	1.96±0.038[7]	**<0.001**
Riboflavin (mg/d)	2.03±0.064[7]	2.20±0.065	2.41±0.065[7]	**<0.001**
Niacin (mg/d)	24.0±0.639[7]	24.7±0.656	26.7±0.652[7]	**0.015**
Vitamin B6 (mg/d)	2.07±0.047[7]	2.21±0.48[8]	2.31±0.048[7,8]	**<0.001**
Folate (mcg/d)	719±15.4[7,9]	661±15.8[9]	652±15.8[7]	**0.006**
Vitamin B12 (mcg/d)	3.71±0.223[7]	4.38±0.229	5.09±0.228[7]	**<0.001**
Calcium (mg/d)	1092±33.1[7]	1134±34.0[8]	1262±33.8[7,8]	**0.001**
Iron (mg/d)	18.8±0.319	18.6±0.327	18.7±0.326	0.929
Zinc (mg/d)	12.5±0.264[7]	12.8±0.271[8]	13.7±0.269[7,8]	**0.005**
Selenium (mg/d)	128±3.31[7]	118±3.39	113±3.37[7]	**0.011**
Magnesium (mg/d)	463±9.80	451±10.0	478±10.0	0.151
Chromium (µg/d)	0.135±0.009[7]	0.110±0.009	0.090±0.009[7]	**0.002**

GL, glycemic load; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; T, tertile; n, number of subjects.

Data are presented as mean ± standard error. Analysis of covariance (ANCOVA) was used for the comparison of continuous variables among tertiles of LCDs.

*Significant level after adjustment for age, physical activity, BMI and total energy intake. $p<0.05$ was considered significant.

Macronutrient and micronutrients adjusted with energy.

The numbers 7, 8 and 9 are used to present a significant difference between *Cry1* genotypes between tertile 1 and 3, 2 and 3, and 1 and 2, respectively by Bonferroni analysis.

Discussion

It is becoming more and more obvious that disruption in the circadian rhythm raises the risk of some diseases like cancer,[38] diabetes,[39] mood disorders and sleep disorders.[40] Multiple polymorphisms have been found in clock genes, which might have an impact on disease development via several physiological systems.

To the best of our knowledge, the present study represents the first cross-sectional study to investigate the interaction of LCDs and *Cry1* genotypes on insulin and two insulin-related factors, HOMA-IR and ISQUKI. The current study found that, after controlling for confounding variables, LCDs were associated with lower HOMA-IR and TG, and with higher HDL-cholesterol. However, we found no association between LCDs and insulin sensitivity. The crude model results suggest that, in comparison with

Table 4 The interaction of *Cry1* genotypes and LCDs on insulin, HOMA-IR index and ISQUICKI

Interaction genotypes * LCD		B	(95%) CI	P value*
Insulin (mIU/ ml)				
Crude	CC*LCD	-0.013	-0.025, -8.02	**0.049**
	CG*LCD	-0.004	-0.017, 0.009	0.559
	GG*LCD	. ref		
Model 1	CC*LCD	-0.012	-0.024, 0.001	**0.054**
	CG*LCD	-0.005	0.008, 0.631	0.438
	GG*LCD	. ref		
HOMA-IR				
Crude	CC*LCD	-0.003	-0.006, -7.48	**0.049**
	CG*LCD	-0.002	-0.005, 0.001	0.158
	GG*LCD	. ref		
Model 1	CC*LCD	-0.002	-0.005, 0.001	0.106
	CG*LCD	-0.002	-0.005, 0.001	0.157
	GG*LCD	. ref		
ISQUKI (mg/lit)				
Crude	CC*LCD	0.001	-1.43, 0.002	**0.053**
	CG*LCD	0.001	0.00, 0.002	0.166
	GG*LCD	. ref		
Model 1	CC*LCD	0.001	0.00, 0.002	0.144
	CG*LCD	0.001	0.00, 0.002	**0.041**
	GG*LCD	. ref		

HOMA, homeostatic model assessment; ISQUICKI: insulin sensitivity quantitative insulin sensitivity check index.

Model 1: additionally adjusted for BMI and physical activity.

GG genotype has no risk alleles, CG genotype has one and CC genotype has two risk alleles

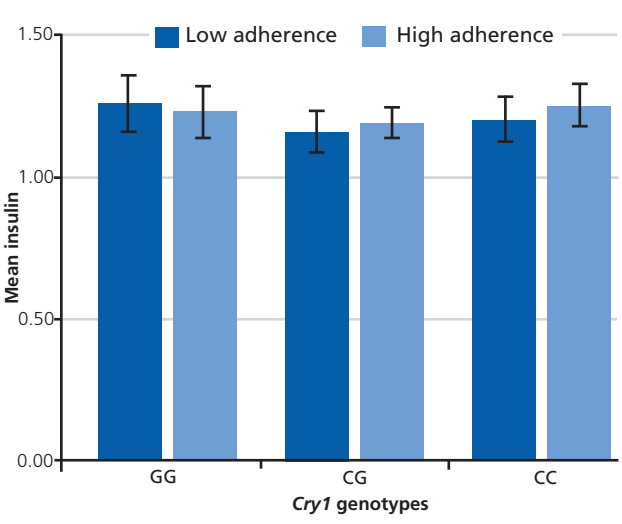
GG genotype is considered as a reference.

The generalised linear model (GLM) was used.

*Significant level after adjustment for age, physical activity, BMI and total energy intake.

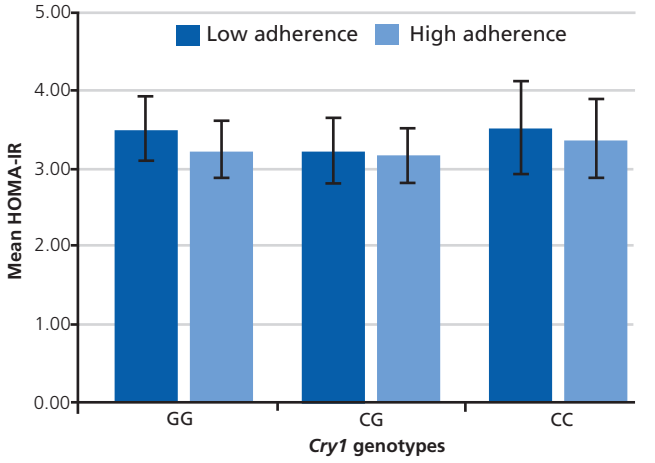
For interactions, 0.05≤P<0.07 was considered marginally significant.

Figure 1. The interaction between *Cry1* genotype and adherence to LCDs on insulin (mIU/ ml).



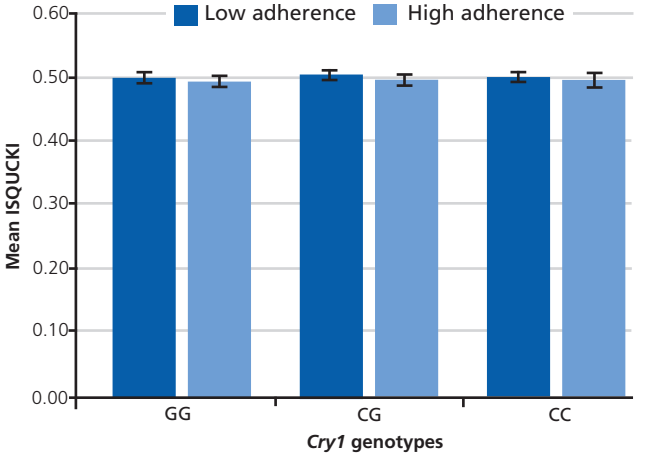
P value for adherence to LCD: 0.387; p value for CC genotype: 0.069; p value for interaction: 0.049.

Figure 2. The interaction between *Cry* genotypes and adherence to LCDs on HOMA-IR.



P value for adherence to LCD: 0.151; p value for CC genotype: 0.051; p value for interaction: 0.049.

Figure 3. The interaction between *Cry1* and adherence to low carbohydrate diet score (LCDs) on ISQUKI (mg/l).



P value for adherence to LCD: 0.160; p value for CC genotype: 0.050; p value for interaction: 0.053.

poor adherence, those in the third tertile of LCD score in the interaction with CC *Cry1* genotypes may have decreases in insulin and HOMA-IR and an increase in ISQUKI. A strong relationship was found between *Cry1* genotypes and some anthropometric variables (weight, BMI and WC).

The present study indicated that participants with higher adherence to LCD diets had a significantly lower HOMA-IR. However, increasing numbers of risk alleles among participants were not associated with insulin and its relative or other biochemistry factors, such as LDL and FBS. The absence of a direct correlation between these qualities and *Cry* genotypes points to possible interactions with environmental factors.

Moreover, genetic factors have been recognised as causes of variability in insulin sensitivity when consuming carbohydrate.[41] Similar to the present study, Dashti *et al*. found an interaction between *Cry1* genotypes and carbohydrate consumption for fasting glucose and insulin level.[42] According to a review study, it was hypothesized that circadian disruption was related to the increase of insulin resistance in humans.[43] Indeed, accumulating evidence suggests that circadian clocks have a vital role and are a decisive factor in the regulation of mammalian metabolic physiology.[44] In human studies, it has been shown that disruption of circadian rhythms is related to metabolic dysregulation and risk for obesity and type 2 diabetes. Further evidence suggests that insulin resistance and diabetes mellitus are induced by dysregulation of the circadian clock system.[45] Core clock genes, like cryptochromes, constituting the negative limb of the circadian system, decrease glucose output by the liver and insulin secretion from the pancreas.[46] *Cry1* and *Cry2* genes have important roles in hepatic gluconeogenesis.[31] Consequently, factors that affect the expression of *Cry1* and *Cry2* could conceivably damage hepatic regulation of glucose homeostasis by regulation of these regulatory genes, thus increasing the risk for diabetes in humans.[31]

In this study, the low-calorie diets included a large amount of fat and protein. According to the majority of current observations, dietary proteins stimulate insulin secretion, which leads to greater glucose clearance from the blood via some tissues.[47] Amino acids play a decisive role in facilitating insulin and glucagon secretion.[48] In our study, consumption of fat and protein intake increased in moving from the first to the third tertile. Additionally, the average intake of vegetables increased, which might affect FBG and insulin levels. Our findings were consistent with those from some studies that suggested a positive association between a high fat and high protein diet and decreased insulin resistance because its lower GI and GL leads to decreased glucose and insulin responses.[49-53] High-fibre diets, because of their greater vegetable and legume content, tend to have a lower GI and GL. In several investigations the importance of vegetable and legume consumption in lowering insulin resistance and decreased diabetes has been highlighted.[54-58]

According to data from prospective cohort studies, certain fats may be especially helpful in lowering the risk for type 2 diabetes. In a cross-over intervention, a diet rich in unsaturated fat with a focus on MUFAs significantly improved insulin sensitivity compared to a diet high in carbohydrates like the Dietary Approaches to Stop Hypertension (DASH).[59] A meta-analysis of feeding trials revealed that MUFAs and PUFAs had some effects in improving insulin resistance.[60] This is congruent with our results, which showed that subjects in the third tertile of LCD consumed higher amounts of MUFAs and PUFAs. Another study conducted on *Cry1* polymorphism demonstrated that SFA intake was significantly lower in the non-risk allele genotype group compared to the group with risk allele present.[61]

In this study, subjects in the third tertile of LCD consumed higher amounts of some elements which are necessary for insulin modulation such as calcium and zinc. One of the possible reasons for the interaction of LCD and *Cry1* on insulin resistance and insulin sensitivity might be the intake or level of minerals. Any increase in body fat is related to increased risk of insulin resistance.[62] A potential mechanism through which calcium regulates body fat is suggested by the fact that higher calcium consumption promotes fat cell death. This effect is produced through the suppression of a regulator of apoptosis, uncoupling protein 2 (UCP2).[63] Zinc ions play a central role in insulin biosynthesis, especially in crystallization, which presumably reduces the rate of proteolysis, especially by the converting enzyme(s).[64] Hence higher consumption of important minerals can result in improved diabetes-related factors, such as insulin resistance and insulin sensitivity.

Key messages

- Higher adherence to low carbohydrate diet (LCD) and an interaction with the *Cry1* genotype containing two risk alleles (CC) led to lower insulin levels.
- Higher adherence to LCD score in the interaction with genotypes which included one risk allele (CG) showed an increase in ISQUKI in adjusted models.
- Participants with higher adherence to LCD had a significantly lower HOMA-IR.

The major strength of this investigation is that it is the first study from Middle-Eastern countries to examine the interaction of LCDs and *Cry1* genotypes on insulin, HOMA-IR and ISQUKI. These interactions remained significant following Bonferroni correction for multiple testing, despite the high correlations among the outcomes of interest. Moreover, our findings may be applied to clinical practice and contribute to personalised therapies for the prevention and treatment of metabolic disorders. Limitations of this investigation include the use of a cross-sectional design, which limits the interpretation of the causal link between the *Cry1* variant and glucose metabolism. Longitudinal epidemiologic studies and biochemical experimentally-based research are required to elucidate and strengthen the findings from this study. It has not been explored whether this interaction relates to changes in circadian rhythmicity. The pathway linking dietary carbohydrate intake to this circadian gene remains unknown. Also, a retrospective FFQ questionnaire was used for recording the subject's dietary intake, calculating LCD score and glycaemic load; these questionnaires can be subject to recall bias.

Conclusion

This study revealed an interaction between *Cry1* genotypes, insulin and ISQUKI. Higher adherence to LCD and an interaction with the *Cry1* genotype containing two risk alleles (CC) led to lower insulin levels. Also, higher adherence to LCD score in the interaction with genotypes which included one risk allele (CG) showed an increase in ISQUKI in adjusted models. However, the mechanism of interac-

tion between LCDs and *Cry1* genotypes remains unclear. Future studies need to clarify the exact function of the *Cry1* variant in disrupting the transcription factor binding region, and the role of carbohydrate intake, which might contribute to the development of future targets for intervention.

Conflict of interest All authors declare that they have no competing interests.

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Authors' contributions KhM designed the study, MF and HT drafted the first manuscript, FSH performed the statistical analyses. AM, SH, HT and CC contributed to the interpretation of the data and revised the article, and KhM had full access to all of the data in the study and took responsibility for the integrity and accuracy of the data. All authors read and approved the final manuscript.

Ethics approval and consent This study was conducted according to the guidelines laid down in the Declaration of Helsinki. Ethics approval for the study protocol was confirmed by the Human Ethics Committee of Tehran University of Medical Sciences (Ethics Number: IR.TUMS.VCR.REC.1398.051). All participants signed a written informed consent form that was approved by the ethics committee.

Availability of data and materials Participants of this study did not agree for their data to be shared publicly, so supporting data are not available.

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