

Variants in glucose- and circadian rhythm-related genes affect the response of energy expenditure to weight-loss diets: the POUNDS LOST Trial^{1–3}

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ABSTRACT

Background: Circadian rhythm has been shown to be related to glucose metabolism and risk of diabetes, probably through effects on energy balance. Recent genome-wide association studies identified variants in circadian rhythm-related genes (*CRY2* and *MTNR1B*) associated with glucose homeostasis.

Objective: We tested whether *CRY2* and *MTNR1B* genotypes affected changes in measures of energy expenditure in response to a weight-loss diet intervention in a 2-y randomized clinical trial, the POUNDS (Preventing Overweight Using Novel Dietary Strategies) LOST Trial.

Design: The variants *CRY2* rs11605924 ($n = 721$) and *MTNR1B* rs10830963 ($n = 722$) were genotyped in overweight or obese adults who were randomly assigned to 1 of 4 weight-loss diets that differed in their proportions of macronutrients. Respiratory quotient (RQ) and resting metabolic rate (RMR) were measured.

Results: By 2 y of diet intervention, the A allele of *CRY2* rs11605924 was significantly associated with a greater reduction in RQ ($P = 0.03$) and a greater increase in RMR and RMR/kg (both $P = 0.04$). The G allele of *MTNR1B* rs10830963 was significantly associated with a greater increase in RQ ($P = 0.01$) but was not related to changes in RMR and RMR/kg. In addition, we found significant gene-diet fat interactions for both *CRY2* (P -interaction = 0.02) and *MTNR1B* (P -interaction < 0.001) in relation to 2-y changes in RQ.

Conclusions: Our data indicate that variants in the circadian-related genes *CRY2* and *MTNR1B* may affect long-term changes in energy expenditure, and dietary fat intake may modify the genetic effects. This trial was registered at www.clinicaltrials.gov as NCT00072995. *Am J Clin Nutr* 2014;99:392–9.

INTRODUCTION

Compelling evidence has related disturbed circadian rhythms to obesity and diabetes—a disorder of abnormal glucose metabolism (1, 2). Recent genome-wide association studies identified a group of genetic variants determining fasting glucose concentrations. Interestingly, 2 of the glucose-associated genes—cryptochrome 2 (*CRY2*) (3) and melatonin receptor 1B (*MTNR1B*)—(4) are involved in the regulation of circadian rhythms.

One of the principal pathways that link diurnal rhythm and glucose metabolism is through alteration in balance between energy expenditure and intake. Recent evidence has shown that glucose homeostasis and energy metabolism are controlled basically along with each other through a central pacemaker in

the circadian system (5). Energy expenditure, which is under tight regulation of the circadian rhythm (6–9), plays a key role in glucose metabolism (10).

In this study, we examined the associations of the genetic variants at *CRY2* and *MTNR1B* loci, which are related to both glucose metabolism and circadian rhythms, with 2-y change in measures of energy expenditure in response to a weight-loss diet intervention in a randomized clinical trial. In addition, we assessed the potential gene-diet interactions.

SUBJECTS AND METHODS

Study population

The POUNDS (Preventing Overweight Using Novel Dietary Strategies)⁴ LOST trial was conducted from October 2004 through December 2007 at 2 sites: Harvard School of Public Health and Brigham and Women's Hospital in Boston, MA, and the Pennington Biomedical Research Center of Louisiana State University System, Baton Rouge, LA. The study design and sample collection have been described in detail previously (11). The study population included 811 overweight or obese [BMI (in kg/m²): 25–40] participants aged 30–70 y. Major criteria for exclusion were the presence of diabetes or unstable cardiovascular disease, the use of medications that affect body weight,

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⁴ Abbreviations used: POUNDS, Preventing Overweight Using Novel Dietary Strategies; RMR, resting metabolic rate; RQ, respiratory quotient; SNP, single nucleotide polymorphism.

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and insufficient motivation as assessed by interview and questionnaire (12). Individuals with type 2 diabetes controlled with diet, or with hypertension or hyperlipidemia treated with diet or drugs, were eligible to participate. Participants were randomly assigned to 1 of 4 diets; the target percentages of energy derived from fat, protein, and carbohydrate, respectively, in the 4 diets were 20%, 15%, and 65%; 20%, 25%, and 55%; 40%, 15%, and 45%; and 40%, 25% and 35%. By constituting a 2-by-2 factorial design, 2 diets were low-fat (20%), 2 were high-fat (40%), 2 were average-protein (15%), and 2 were high-protein (25%). After 2 y, 80% of the participants ($n = 645$) completed the trial.

Food provision or compliance of diets was tracked through a computer tracking system. A computer tracking system was used to self-monitor behaviors and provide feedback. Dietitians and other study staff entered the data into the computer tracking system via a World Wide Web-based application each time contact was made with a participant (individual or group sessions or via telephone, e-mail, or mail). The counselor entered objective data consisting of body weight (measured at an intervention session), attendance, intervention make-up sessions, and number of days of food diaries and physical activity records completed. We added the mean values of calorie intakes and biomarkers of adherence at 6 mo and at 2 y and at baseline for comparison.

In the current study, data were analyzed among the 721 and 722 participants with *CRY2* rs11605924 and *MTNR1B* rs10830963 genotypes, respectively. Of the study participants, 61% were women, 80% were white, 15% were African American, 3.5% were Hispanic, and 1.5% were Asian or other ethnic groups by self-report. The study was approved by the human subjects committee at each institution and by a data and safety monitoring board appointed by the National Heart, Lung, and Blood Institute. All participants provided written informed consent.

Measurements of adiposity and other variables

Body weight and waist circumference were measured in the morning before breakfast on 2 d at baseline, 6 mo, and 2 y. BMI was calculated as weight (kg)/height (m)². Dietary intake was assessed in a random sample of 50% of the participants, by a review of the 5-d diet record at baseline, and by 24-h recall during a telephone interview on 3 nonconsecutive days at 6 mo and at 2 y. Fasting blood samples were obtained on one day. Analyses of serum lipids and glucose were performed at the Clinical Laboratory at Pennington. Triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol were measured on the Synchron CX7 (Beckman Coulter). LDL cholesterol was calculated for each participant according to the following formula: LDL cholesterol = total cholesterol – HDL cholesterol – triglycerides/5 (13), except when triglyceride concentration exceeded 400 mg/dL, in which case LDL cholesterol was measured directly in all samples of the participants. Glucose and insulin were measured by using an immunoassay with chemiluminescent detection on the Immulite (Diagnostic Products Corporation). Glycated hemoglobin was measured on a Synchron CX5 (Beckman Coulter). Blood pressure was measured on 2 d at baseline and at 6, 12, and 24 mo with an automated device (IntelliSense Professional Digital Blood Pressure Monitor; HEM907XL Omron HealthCare) by using methods established in other large NIH trials (14). The calibration was evaluated at regular intervals by using a mercury manometer.

Measurements of energy expenditure

Respiratory quotient (RQ) and resting metabolic rate (RMR) were obtained by DeltaTrac II metabolic cart. RMR was used to estimate energy requirements. For the measurement of energy expenditure and substrate oxidation under resting conditions, an open-circuit ventilated-hood setup was used for indirect calorimetry. This procedure has advantages over direct calorimetry, which measures the amount of heat released by an individual. The advantage of indirect calorimetry over direct calorimetry is that the method is simpler and cheaper, the response time for changes in energy expenditure is faster, and, when carbon dioxide production and urinary nitrogen excretion are simultaneously measured, it is possible to calculate substrate oxidation rates as well. The Deltatrac II Metabolic Monitor, which has a 25-L transparent plastic canopy through which a constant ambient airflow of ~40 L/min is pulled, was placed over the volunteer's head while he or she was resting comfortably. By burning 5 mL ethanol and determining the amount of carbon dioxide recovered, the exact airflow was calculated for each system individually. Oxygen and carbon dioxide concentrations in constant airflow were continuously measured while the concentrations of the ambient air were measured every 4 min for carbon dioxide and for oxygen by using a differential oxygen sensor. Before each measurement, the analyzers were calibrated by using standard gases containing ~95% O₂ and ~5% CO₂. The accuracy of the Deltratrac is 96%.

For measurement of RMR, the participant arrived in the morning after fasting overnight for 12 h and having performed a minimal amount of physical activity. The participants were also instructed not to consume any caffeine or alcohol during the evening and morning preceding each test and to not perform any strenuous activities. On arrival, the volunteer rested for 30 min before the measurement. Oxygen consumption and carbon dioxide production were then measured for 30 min, and energy expenditure was calculated. RQ, a ratio of metabolic gas exchange, was computed as the quantity of carbon dioxide produced divided by the amount of oxygen consumed per unit time. Of the 810 (baseline), 641 (6 mo), and 497 (24 mo) participants with RMR and RQ measurements, 720, 582, and 456 were genotyped in *CRY2* rs11605924, and 721, 582, and 455 were genotyped in *MTNR1B* rs10830963.

Genotyping

DNA was extracted from the buffy-coat fraction of centrifuged blood by using the QIAamp Blood Kit (Qiagen). The single nucleotide polymorphisms (SNPs) *CRY2* rs11605924 and *MTNR1B* rs10830963 were successfully genotyped in 721 and 722 participants, respectively, by using the OpenArray SNP Genotyping System (BioTrove). Replicated quality-control samples (10%) were included in every genotyping plate with >99% concordance (15). The genotype distribution was in Hardy-Weinberg equilibrium in all participants or in whites ($P > 0.05$).

Statistical analysis

The Hardy-Weinberg equilibrium and comparison of categorical variables were assessed by using a chi-square test. Differences in continuous variables at baseline by genotype were tested by using a general linear models. Appropriate adjustments, such as age, sex, ethnicity, baseline value for the respective outcome, calorie intake, and weight change, were used in the analysis.

TABLE 1
Baseline characteristics of the study participants according to *CRY2* and *MTNR1B* genotypes¹

Characteristic	CRY2 rs11605924					MTNR1B rs10830963					P ²
	AA (n = 211)	AC (n = 342)	CC (n = 168)	Total (n = 721)	P ²	CC (n = 393)	CG (n = 273)	GG (n = 56)	Total (n = 722)		
Age (y)	49.02 ± 9.32 ³	51.41 ± 9.00	52.76 ± 8.99	51.03 ± 9.19	<0.0001	50.77 ± 9.18	51.04 ± 9.05	53.00 ± 10.28	51.04 ± 9.23	0.23	
Sex [n (%)]					0.0004					0.72	
Men	62 (29.4)	146 (42.7)	72 (42.9)	280 (38.8)		149 (37.9)	112 (41.0)	22 (39.3)	283 (39.2)		
Women	14 (70.6)	196 (57.3)	96 (57.1)	441 (61.2)		244 (62.1)	161 (59.0)	34 (60.7)	439 (60.8)		
Ethnicity [n (%)]					<0.0001					0.0001	
White	115 (54.5)	298 (87.1)	163 (97.0)	576 (79.9)		279 (71.0)	247 (90.5)	49 (87.5)	575 (79.6)		
Black	78 (37.0)	30 (8.8)	1 (0.6)	109 (15.1)		96 (24.4)	12 (4.4)	3 (5.4)	111 (15.4)		
Hispanic	10 (4.7)	12 (3.5)	3 (1.8)	25 (3.5)		13 (3.3)	10 (3.7)	2 (3.6)	25 (3.5)		
Asian or other	8 (3.8)	2 (0.6)	1 (0.6)	11 (1.5)		5 (1.3)	4 (1.5)	2 (3.6)	11 (1.5)		
Weight (kg)	93.24 ± 14.61	93.22 ± 16.05	93.21 ± 15.77	93.22 ± 15.55	NS	93.67 ± 14.96	92.84 ± 15.98	92.00 ± 17.49	93.23 ± 15.54	0.65	
WC (cm)	102.78 ± 12.05	103.93 ± 13.58	103.94 ± 13.32	103.59 ± 13.08	0.5	103.87 ± 12.99	103.17 ± 13.18	103.92 ± 13.37	103.61 ± 13.08	0.78	
BMI (kg/m ²)	33.04 ± 3.79	32.47 ± 3.94	32.54 ± 3.78	32.65 ± 3.86	0.2	32.92 ± 3.83	32.28 ± 3.91	32.54 ± 3.73	32.65 ± 3.86	0.10	
Glucose (mg/dL)	91.13 ± 12.54	92.55 ± 11.51	91.67 ± 11.49	91.93 ± 11.82	0.37	91.56 ± 12.19	91.34 ± 10.42	96.96 ± 14.33	91.90 ± 11.82	0.07	
TC (mg/dL)	199.22 ± 35.86	202.68 ± 36.64	204.67 ± 39.14	202.13 ± 37.02	0.33	199.97 ± 37.27	204.36 ± 37.24	206.16 ± 33.90	202.11 ± 37.04	0.22	
LDL-C (mg/dL)	125.25 ± 32.07	124.46 ± 31.22	127.38 ± 33.86	125.37 ± 32.08	0.62	125.01 ± 31.97	125.86 ± 32.67	126.38 ± 31.25	125.44 ± 32.14	0.92	
HDL-C (mg/dL)	49.94 ± 13.31	48.02 ± 13.98	48.87 ± 15.42	48.78 ± 14.14	0.29	48.19 ± 13.27	48.94 ± 13.81	51.20 ± 20.29	48.71 ± 14.13	0.31	
TG (mg/dL)	124.06 ± 84.98	154.40 ± 85.54	147.24 ± 84.99	143.85 ± 86.13	0.68	137.36 ± 86.80	151.43 ± 85.40	151.55 ± 84.46	143.78 ± 86.26	0.09	
Insulin (IU/mL)	11.96 ± 8.59	12.19 ± 7.26	12.37 ± 7.67	12.16 ± 7.76	0.87	12.41 ± 7.28	11.34 ± 7.99	13.93 ± 9.15	12.12 ± 7.73	0.87	
HOMA	2.75 ± 2.12	2.86 ± 1.85	2.87 ± 1.96	2.83 ± 1.96	0.77	2.87 ± 1.82	2.62 ± 2.03	3.43 ± 2.35	2.82 ± 1.96	0.56	
SBP (mm Hg)	118.94 ± 13.37	120.14 ± 13.19	119.44 ± 13.76	119.62 ± 13.37	0.58	119.89 ± 13.30	119.86 ± 13.82	116.91 ± 10.78	119.65 ± 13.33	0.27	
DBP (mm Hg)	75.34 ± 9.51	75.57 ± 9.54	75.83 ± 8.71	75.56 ± 9.33	0.88	75.73 ± 9.09	75.91 ± 9.83	72.69 ± 7.67	75.56 ± 9.30	0.054	
RMR (kcal/d)	1524.9 ± 283.6	1575.9 ± 308.0	1566.1 ± 313.8	1558.7 ± 302.8	0.35	1553.7 ± 298.2	1567.1 ± 310.8	1554.3 ± 316.6	1558.8 ± 304.1	0.85	
RQ	0.840 ± 0.049	0.840 ± 0.040	0.845 ± 0.039	0.841 ± 0.043	0.14	0.841 ± 0.042	0.843 ± 0.044	0.831 ± 0.042	0.841 ± 0.043	0.18	

¹DBP, diastolic blood pressure; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; RMR, resting metabolic rate; RQ, respiratory quotient; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WC, waist circumference.

²P values were calculated by chi-square test for categorical variables and ANOVA for continuous variables. All significant values were adjusted for age, sex, and ethnicity in the regression model.

³Mean ± SD (all such values).

Linear mixed models, with time used as a repeated measurement factor, were applied to test genetic associations with the trajectory of changes in RMR, RQ, and weight in the participants who provided measurements at baseline, 6 mo, and 2 y. Time was considered as a categorical variable in the mixed models. For genotype-diet interactions, data were pooled for 2 factorial comparisons: low fat compared with high fat or low protein compared with high protein (11). An interaction term of genotype-by-diet was included in the general linear model. All reported *P* values were 2-sided, and a *P* value of 0.05 was considered statistically significant. All data were analyzed with PASW Statistics 18 (SPSS).

RESULTS

Characteristics of the study population

The baseline characteristics of the participants, according to *CRY2* rs11605924 and *MTNR1B* rs10830963 genotypes, are shown in **Table 1**. The distributions of the 2 variants were in Hardy-Weinberg equilibrium in the study sample (*P* > 0.05), and the minor allele frequencies were 0.47 and 0.26, respectively. The distribution of *CRY2* rs11605924 was different by sex (*P* = 0.004), ethnicity, and age (*P* < 0.0001). Baseline concentrations of glucose, insulin, RMR, and RQ were not correlated with the *CRY2* genotype. The distribution of *MTNR1B* rs10830963 was different by ethnicity (*P* < 0.0001). The *MTNR1B* genotype was marginally correlated with baseline fasting glucose (*P* = 0.07) and diastolic blood pressure (*P* = 0.05), but not with insulin concentration and measures of energy expenditure.

Genotype effects on 2-y change in energy expenditure measures

We examined the genotype effects on 2-y changes in measures of energy expenditure in the whole study population. After ad-

justment for age, sex, ethnicity, diet, weight loss, and baseline values for respective outcomes, we found that carrying the A allele of *CRY2* rs11605924 was significantly associated with more reduction in RQ (*P* = 0.03), but greater increase in RMR and RMR/kg at 2 y (both *P* = 0.04). The G allele of *MTNR1B* rs10830963 was significantly associated with a greater increase in RQ (*P* = 0.01), but was not related to changes in RMR and RMR/kg (**Table 2**).

No association was found between these 2 SNPs and weight loss and changes in blood pressure, glucose, insulin, glycated hemoglobin, lipids (see Supplementary Table 1 under "Supplemental data" in the online issue), and body compositions (see Supplementary Table 2 under "Supplemental data" in the online issue). We found no significant correlation between these SNPs and changes in calorie intake and biomarkers of adherence (*P* > 0.05) (**Table 3**).

Gene-by-diet interactions on energy expenditure measures

We next tested whether the genotype effects of *CRY2* rs11605924 and *MTNR1B* rs10830963 on energy expenditure differed by diet interventions. Both genotypes showed significant interactions with dietary fat intake in relation to the 2-y change in RQ. We found that the A allele of *CRY2* rs11605924 was significantly associated with a greater reduction in RQ in the high-fat diet group as compared with the low-fat diet group (*P*-interaction = 0.02). The G allele of *MTNR1B* rs10830963 was significantly associated with a greater increase in RQ in the low-fat diet as compared with the high-fat diet (*P*-interaction < 0.001) (**Figure 1**).

The tests for the genotype-by-dietary fat interactions were not significant in relation to changes in other energy expenditure measures (RMR and RMR/kg) for both SNPs. In addition, the *CRY2* and *MTNR1B* genotypes did not interact with dietary protein intake on changes in energy expenditure measures.

TABLE 2

Genotype effect on 2-y change in energy expenditure measures after the intervention

Month 24 – baseline	<i>CRY2</i> rs11605924 (A/C)					<i>MTNR1B</i> rs10830963 (C/G)				
	Genotype	<i>N</i>	Mean ± SE	β	<i>P</i> ¹	Genotype	<i>N</i>	Mean ± SE	β	<i>P</i> ¹
Respiratory quotient	AA	115	-0.017 ± 0.005	0.047	0.03	CC	243	-0.015 ± 0.0035 ²	0.117	0.01
	AC	239	-0.012 ± 0.003			CG	168	-0.013 ± 0.0042 ²		
	CC	102	-0.006 ± 0.005			GG	44	0.015 ± 0.0075 ²		
	Total	456	-0.012 ± 0.002			Total	454	-0.011 ± 0.002		
Change in resting metabolic rate (%)	AA	115	2.15 ± 1.33 ³	-0.094	0.04	CC	243	-0.58 ± 0.89	0.019	0.65
	AC	239	-0.87 ± 0.88			CG	168	0.88 ± 1.17		
	CC	102	-2.8 ± 1.51 ³			GG	44	-3.27 ± 2.29		
	Total	456	-0.53 ± 0.66			Total	454	-0.30 ± 0.68		
Resting metabolic rate (kcal/kg)	AA	115	1.11 ± 0.22 ⁴	-0.094	0.04	CC	243	0.72 ± 0.15	0.029	0.51
	AC	239	0.89 ± 0.15			CG	168	1.17 ± 0.20		
	CC	102	0.42 ± 0.26 ⁴			GG	44	0.67 ± 0.36		
	Total	456	0.84 ± 0.11			Total	455	0.88 ± 0.11		

¹ An overall *P* value compares all 3 genotypes after adjustment for age, sex, ethnicity, diet, weight loss, and baseline value of each variable in the linear regression model.

² A post hoc analysis using Tukey's procedure for pairwise comparison showed significant differences in respiratory quotient changes between the CC and GG genotypes (*P* = 0.002) and the CG and GG genotypes (*P* = 0.005).

³ A post hoc analysis using Tukey's procedure for pairwise comparison showed significant differences in percentage change in resting metabolic rate between the AA and CC genotypes (*P* = 0.02).

⁴ A post hoc analysis using Tukey's procedure for pairwise comparison showed significant differences in resting metabolic rate/weight changes between the AA and CC genotypes (*P* = 0.03).

TABLE 3
Repeated measurements of energy intake per day and biomarkers of diet adherence in the study participants according to *CRY2* and *MTNR1B* genotypes

	<i>CRY2</i> rs11605924					<i>MTNR1B</i> rs10830963				
	AA	AC	CC	Total	<i>P</i> ¹	CC	CG	GG	Total	<i>P</i> ¹
Baseline										
Dietary intake per day										
<i>n</i>	102	186	83	371		208	133	29	370	
Energy (kcal)	1900.03 ± 509.21 ²	2006.47 ± 586.30	1947.79 ± 548.50	1964.08 ± 558.05	0.28	1945.33 ± 555.35	2016.31 ± 572.33	1883.62 ± 447.69	1966.01 ± 554.15	0.36
Biomarkers of adherence										
<i>n</i>	211	342	168	721		393	273	56	722	
Urinary nitrogen (g)	11.80 ± 3.90	12.43 ± 4.61	12.36 ± 4.66	12.23 ± 4.43	0.24	11.97 ± 4.58	12.41 ± 4.15	12.76 ± 4.67	12.20 ± 4.43	0.28
6 mo										
Dietary intake per day										
<i>n</i>	83	163	79	325		172	128	25	325	
Energy (kcal)	1511.78 ± 493.68	1690.96 ± 560.23	1582.16 ± 451.07	1618.75 ± 522.95	0.71 ²	1614.93 ± 545.94	1642.45 ± 519.52	1524.75 ± 279.73	1618.83 ± 519.43	0.58
Biomarkers of adherence										
<i>n</i>	133	269	125	527		277	202	47	526	
Urinary nitrogen (g)	11.48 ± 4.39	11.78 ± 4.69	11.29 ± 4.46	11.59 ± 4.56	0.58	11.75 ± 4.85	11.45 ± 4.24	11.14 ± 4.19	11.58 ± 4.56	0.60
2 y										
Dietary intake per day										
<i>n</i>	47	79	42	168		88	65	16	169	
Energy (kcal)	1402.07 ± 390.45	1556.53 ± 443.40	1600.74 ± 621.68	1524.37 ± 484.63	0.11	1545.77 ± 518.38	1494.30 ± 462.77	1504.37 ± 381.87	1522.05 ± 484.13	0.8
Biomarkers of adherence										
<i>n</i>	89	194	83	366		194	136	36	366	
Urinary nitrogen (g)	12.39 ± 4.10	11.90 ± 4.47	11.82 ± 5.17	12.00 ± 4.54	0.64	11.80 ± 4.38	12.12 ± 4.75	12.34 ± 4.52	11.97 ± 4.52	0.71

¹ *P* values were calculated by ANOVA. All *P* values were from models adjusted for age, sex, and ethnicity.

² Mean ± SD (all such values).

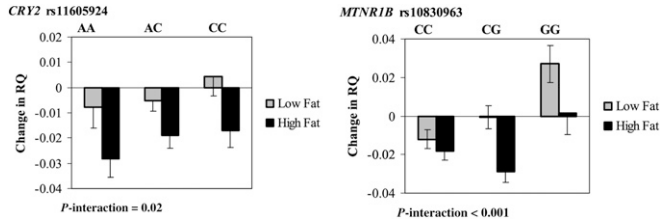


FIGURE 1. Interaction between the *CRY2* or *MTNR1B* genotype and dietary fat intervention on change in RQ at 2 y. *P*-interactions between single nucleotide polymorphism and the low-fat or high-fat diet after adjustment for age, sex, ethnicity, and baseline weight are presented. Data included 57 and 58 (AA), 122 and 117 (AC), and 53 and 49 (CC) participants in the low-fat group and the high-fat group, respectively, at 2 y (total $n = 456$) in the *CRY2* gene and 123 and 120 (CC), 86 and 82 (CG), and 24 and 20 (GG) participants in the low-fat group and the high-fat group, respectively, at 2 y (total $n = 455$) in the *MTNR1B* gene. A post hoc analysis that used Tukey's procedure showed significant differences in RQ change between the CC and GG genotypes in the low-fat diet group ($P = 0.004$). RQ, respiratory quotient.

The trajectory of changes in response to diet

In secondary analyses, we also assessed the trajectory of change patterns in energy expenditure by using repeated measures at 6 mo and 2 y. The genotype-by-dietary fat interactions on changes in RQ remained significant for both *CRY2* rs11605924 (P -interaction = 0.03; **Figure 2**) and *MTNR1B* rs10830963 (P -interaction < 0.0001; **Figure 3**). Participants with the *CRY2* rs11605924 AA genotype had a consistently greater reduction in RQ in the high-fat diet group than in the low-fat diet group across the 2-y intervention (Figure 2). Participants with the *MTNR1B* rs10830963 GG genotype had a consistently greater increase in RQ in the low-fat diet group than in the high-fat diet group across the 2-y intervention (Figure 3). The gene-by-dietary fat interactions appeared more evident at the end of the 2-y intervention than at 6 mo, especially for *MTNR1B* rs10830963.

DISCUSSION

In the 2-y randomized weight-loss diet intervention trial, we found significant associations of *CRY2* rs11605924 with changes in measures of energy expenditure, including RQ, RMR, and RMR/kg, and of the *MTNR1B* rs10830963 with changes in RQ. In addition, we observed significant interactions of dietary fat intake with *CRY2* and *MTNR1B* genotypes on changes in RQ during the 2-y intervention.

It has been estimated that ~10% of genes in a given tissue type exhibit a diurnal pattern (16). Recent genome-wide association studies identified a group of genomic loci determining blood concentrations of fasting glucose. Interestingly, 2 glucose-related loci, *CRY2* and *MTNR1B*, both located on chromosome 11, are involved in circadian pathways (17–21). *CRY2* regulates the expression of multiple clock-controlled genes (17–20) and plays a key role in feedback loops in circadian pathway (22–24). *CRY2* knockout mice showed a lengthened circadian period (25), and it was found that the gene played an important role in the direction of diurnal cycle in the suprachiasmatic nucleus (26). *MTNR1B* is also involved in the circadian pathway through cross-talk with melatonin as a circulating hormone primarily released from the pineal gland (21, 27).

Our findings provide novel and important evidence linking the circadian-related genetic variants and energy expenditure;

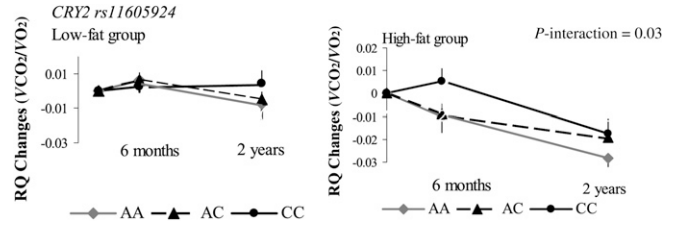


FIGURE 2. Interaction between the *CRY2* rs11605924 genotype and the dietary fat intervention on RQ changes during a 2-y intervention. *P*-interactions between single nucleotide polymorphism and the low-fat or high-fat diet after adjustment for age, sex, ethnicity, and baseline weight are presented. Data included 110 and 101 (AA), 170 and 171 (AC), and 83 and 85 (CC) participants in the low-fat group and the high-fat group, respectively, at baseline (total $n = 720$); 80 and 72 (AA), 145 and 147 (AC), and 70 and 68 (CC) participants in the low-fat group and the high-fat group, respectively, at 6 mo (total $n = 582$); and 57 and 58 (AA), 122 and 117 (AC), and 53 and 49 (CC) participants in the low-fat group and the high-fat group, respectively, at 2 y (total $n = 456$). A post hoc analysis showed no significant difference between pairwise analyses of the genotypes. RQ, respiratory quotient; VCO_2 , carbon dioxide consumption; VO_2 , oxygen consumption.

however, the results from this study suggest that these genetic variants might not have a functional effect on cardiometabolic parameters. Disturbance of energy homeostasis has been related to various health problems, including cardiovascular disease and type 2 diabetes (28). Our findings may open avenues for further investigations of the genetic variants related to circadian rhythms with these diseases, in long-term prospective studies and experimental analyses, to illustrate whether the observed genetic effects on energy expenditure and metabolic disorders are functional.

Recently, compelling evidence has shown that diurnal rhythm is closely related to glucose metabolism. Of the potential mechanisms, a prevailing explanation is that glucose metabolism is regulated (10) through circadian control of energy expenditure-related genes (6–9). It has been documented that a balance of energy expenditure and intake plays a pivotal role in the regulation of glucose homeostasis (29).

Interestingly, we found that dietary fat intake significantly modified the effect of *CRY2* and *MTNR1B* variants, especially on

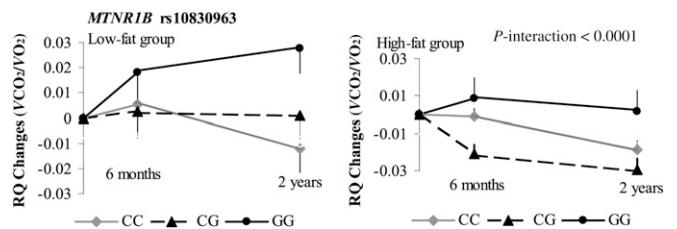


FIGURE 3. Interaction between *MTNR1B* rs10830963 genotype and the dietary fat intervention on RQ changes during the 2-y intervention. *P*-interactions between single nucleotide polymorphism and the low-fat or high-fat diet after adjustment for age, sex, ethnicity and baseline weight are presented. Data included 198 and 194 (CC), 134 and 139 (CG), and 31 and 25 (GG) participants in the low-fat group and the high-fat group, respectively, at baseline (total $n = 721$); 156 and 152 (CC), 113 and 111 (CG), and 27 and 23 (GG) participants in the low-fat group and the high-fat group, respectively, at 6 mo (total $n = 582$); and 123 and 120 (CC), 86 and 82 (CG), and 24 and 20 (GG) participants in the low-fat group and the high-fat group, respectively, at 2 y (total $n = 455$). A post hoc analysis that used Tukey's procedure showed significant differences in the 2-y RQ change between CC and GG genotypes in the low-fat diet group ($P = 0.004$) and in the 6-mo RQ change between the CC and CG ($P = 0.008$) and CG and GG ($P = 0.03$) genotypes in the high-fat diet group. RQ, respiratory quotient; VCO_2 , carbon dioxide consumption; VO_2 , oxygen consumption.

changes in RQ—a parameter that measures fuel utilization. RQ exhibits circadian rhythm in human and rodents (30–32). There is evidence that a high-fat diet might affect the circadian expression of metabolic factors and obesity (33). The effects of high-fat feeding on the rhythmic mRNA expression of clock genes (34) or circadian rhythmicity balance (35) have also been observed in animals. However, little is known about the potential mechanisms underlying the modulation of dietary fat on the *CRY2* and *MTNR1B* variants, and further experimental studies are warranted to clarify the precise mechanisms.

To our knowledge, this is the first investigation of the effects of the glucose- and circadian rhythm-related genetic variants on the response of energy expenditure to weight-loss diet interventions. However, many caveats need to be considered when interpreting our findings. Briefly, the relatively small sample size might limit the power to detect very moderate genetic effects or interactions. In addition, confounding might exist and influence our analyses. However, we did carefully adjust for the important factors that may affect energy expenditure measures, such as age, weight, dietary intake, sex, and ethnicity. We acknowledge that the measurement of clock parameters would strengthen the current study. We used genetic markers related to circadian rhythm. According to the Mendelian randomization theory, a genetic variant could be a better marker than biomarkers in causal inference, because it is less likely to be affected by confounding and reverse causation (36). We examined the exact measures of RMR and RQ only in participants who arrived in the morning after a 12-h overnight fast, had performed a minimal amount of physical activity, and did not consume any caffeine or alcohol during the evening and morning preceding each test. We acknowledge that replication in diverse populations is needed to verify our findings.

In summary, we found that genotypes of glucose- and circadian rhythm-related loci (*CRY2* and *MTNR1B*) affected long-term changes in energy expenditure in response to dietary intervention, and dietary fat intake might modify the genetic effects.

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The authors' responsibilities were as follows—KM and LQ: designed the research; KM, MX, QQ, and LQ: conducted the research; KM and LQ: analyzed the data; KM, MX, and LQ: wrote the manuscript; GAB, FS, LdJ, and GAB: provided the materials; and LQ: had primary responsibility for the final content of the manuscript. All authors read and approved the final manuscript. No potential conflicts of interest relevant to this article were reported.

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