Gene-Environment Interactions of Circadian-Related Genes for Cardiometabolic Traits

OBJECTIVE

Common circadian-related gene variants associate with increased risk for metabolic alterations including type 2 diabetes. However, little is known about whether diet and sleep could modify associations between circadian-related variants (CLOCK-rs1801260, CRY2-rs11605924, MTNR1B-rs1387153, MTNR1B-rs10830963, NR1D1-rs2314339) and cardiometabolic traits (fasting glucose [FG], HOMA-insulin resistance, BMI, waist circumference, and HDL-cholesterol) to facilitate personalized recommendations.

RESEARCH DESIGN AND METHODS

We conducted inverse-variance weighted, fixed-effect meta-analyses of results of adjusted associations and interactions between dietary intake/sleep duration and selected variants on cardiometabolic traits from 15 cohort studies including up to 28,190 participants of European descent from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.

RESULTS

We observed significant associations between relative macronutrient intakes and glycemic traits and short sleep duration (<7 h) and higher FG and replicated known MTNR1B associations with glycemic traits. No interactions were evident after accounting for multiple comparisons. However, we observed nominally significant interactions (all P < 0.01) between carbohydrate intake and MTNR1B-rs1387153 for FG with a 0.003 mmol/L higher FG with each additional 1% carbohydrate intake in the presence of the T allele, between sleep duration and CRY2-rs11605924 for HDL-cholesterol with a 0.010 mmol/L higher HDL-cholesterol with each additional hour of sleep in the presence of the A allele, and between long sleep duration (≥9 h) and MTNR1B-rs1387153 for BMI with a 0.60 kg/m² higher BMI with long sleep duration in the presence of the T allele relative to normal sleep duration (≥7 to <9 h).

CONCLUSIONS

Our results suggest that lower carbohydrate intake and normal sleep duration may ameliorate cardiometabolic abnormalities conferred by common circadian-related genetic variants. Until further mechanistic examination of the nominally significant interactions is conducted, recommendations applicable to the general population regarding diet—specifically higher carbohydrate and lower fat composition—and normal sleep duration should continue to be emphasized among individuals with the investigated circadian-related gene variants.
Gene-environment interactions can identify potential opportunities for personalized health interventions for individuals who are genetically susceptible to type 2 diabetes and related chronic diseases (1). During the past decade, researchers have examined lifestyle interventions, particularly those related to diet, physical activity, and sleep, for individuals at increased genetic risk for metabolic alterations such as type 2 diabetes (2,3). For example, dietary changes in carbohydrate (CHO) and fat intake have been shown to attenuate a genetic predisposition to elevated fasting glucose (FG) (4), insulin resistance (5), and type 2 diabetes (6). Sleep duration has also been assessed as a modifying factor in the associations between genetics and type 2 diabetes because of the association of sleep with diet and chronic disease and its effect on the genetic risk for obesity (7). Identifying optimal and personalized therapies for the primary prevention of type 2 diabetes through gene-environment investigations is critical to public health for a disease that affects an estimated 29.1 million Americans (9.3% of the U.S. population) (1,8). In addition, because only 10% of the total heritability of type 2 diabetes is accounted for by genetic variants (9), gene-environment investigations may also reveal novel biological pathways and genetic loci pertinent to this disease.

Glucose homeostasis and insulin secretion are among several biological processes that are controlled by the circadian biological clock, which is maintained endogenously through a transcription-translation feedback loop composed of clock genes (10). Glycemic control is mediated through multiple processes, including circadian regulation of hepatic glucose metabolism (11,12); secretion of adipokines, such as leptin and adiponectin (13,14); and the pancreatic secretion of insulin and glucagon (15). Experiments in clock mutant mice showing disrupted glucose homeostasis, insulin secretion and sensitivity, and other metabolic processes, along with circadian disruption in humans (16), emphasize the importance of circadian control in metabolic control (15,17).

In support of the link between the circadian system and glycemic control in humans is results from genome-wide association studies (GWAS) of FG (18,19) and type 2 diabetes (9,20) that have reported associations with clock gene CRY2, encoding cryptochrome 2, and the circadian-related melatonin receptor 1B gene MTNR1B. In addition, the circadian locomotor output of clock genes cycles kaput CLOCK and, more recently, the nuclear receptor rev-erb-α NR1D1 have been associated with related metabolic traits, including lower circulating concentrations of HDL-cholesterol (HDL-C) and elevated central adiposity (21–23). Because metabolic traits are important predictors of type 2 diabetes, these loci may also be relevant to the pathogenesis of type 2 diabetes (24). Thus, investigating whether lifestyle modifications—particularly diet, for its potent role in entraining circadian clocks in metabolic tissues (25), and sleep, for its putative effect on disease risk (7)—attenuates circadian-related genetic predispositions to metabolic disruptions may facilitate the development of personalized recommendations to improve type 2 diabetes prevention strategies.

In cross-sectional meta-analyses of large population-based cohorts from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium, we tested whether dietary intake (total CHO, total fat, polyunsaturated fatty acid [PUFA], monounsaturated fatty acid [MUFA], and saturated fatty acid [SFA]) and sleep duration (continuous and categorical) modify the associations between five common circadian-related gene variants (CLOCK-rs1801260, CRY2-rs11605924, MTNR1B-rs1387153, MTNR1B-rs10830963, and NR1D1-rs2314339) and the two glycemic traits of FG and HOMA-insulin resistance (HOMA-IR), as well as related anthropometric (BMI and waist circumference) and lipid (HDL-C) traits. These outcomes are related to cardiometabolic disease and have previously been shown to associated with the selected genetic variants.

11Global Public Health, Leiden University College, The Hague, the Netherlands
12Cardiovascular Nutrition Laboratory, Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA
13Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA
14Department of Biostatistics, University of Washington, Seattle, WA
15Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare (THL), Helsinki, Finland
16Department of Genetics, Washington University School of Medicine, St. Louis, MO
17U.S. Department of Agriculture/Agricultural Research Service Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX
18The Novo Nordisk Foundation Centre for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
19Copenhagen Prospective Studies on Asthma in Childhood, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
20Danish Pediatric Asthma Centre, Gentofte Hospital, The Capital Region, Copenhagen, Denmark
21Department of Chronic Disease Prevention, National Institute for Health and Welfare (THL), Helsinki, Finland
22Department of Nutrition and Dietetics, Harokopio University, Athens, Greece
23Department of Food and Environmental Sciences, Division of Nutrition, University of Helsinki, Helsinki, Finland
24Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland
25Department of Epidemiology, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain
26Instituto Madrileño de Estudios Avanzados en Alimentación (IMDEA-FOOD), Madrid, Spain

Corresponding author: Hassan S. Dashiti, hassan.dashiti@tufts.edu

Received 14 November 2014 and accepted 11 April 2015.

Clinical trial reg. nos. NCT00005130 (CARDIA), NCT00005133 (Cardiovascular Health Study), NCT00005136 (Family Heart Study), NCT00005121 (Framingham Offspring Study), NCT00083369 (Genetic and Environmental Determinants of Triglycerides), NCT01331512 (InCHIANTI Study), NCT00289237 (Inter99), and NCT00005487 (Multi-Ethnic Study of Atherosclerosis), clinicaltrials.gov.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc14-2709/-/DC1.

*A list of the CHARGE Nutrition Study Group Investigators can be found in the Appendix.

© 2015 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered.
RESEARCH DESIGN AND METHODS

Cohorts
The present cross-sectional meta-analyses include up to 28,190 participants of European descent from the following 15 cohort studies of the CHARGE Consortium Nutrition Working Group (Supplementary Table 1): Coronary Artery Risk Development in Young Adults Study (CARDIA); Corogene Controls; Cardiovascular Health Study (CHS); Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic syndrome (DILGOM); Family Heart Study (FamHS); Framingham Offspring Study (FOS); Genetics of Lipid Lowering Drugs and Diet Network (GOLDN); GOYA MALE; Helsinki Birth Cohort Study (HBCS); Invecchiare in Chianti (aging in the Chianti area, InCHIANTI); Inter99; Multi-Ethnic Study of Atherosclerosis (MESA); The Rotterdam Study; The Hellenic Study of Interactions between SNPs and Eating in Atherosclerosis Susceptibility (THISEAS); and the Cardiovascular Risk in Young Finns Study (YFS). Participants provided written informed consent, and the study protocols were approved by local institutional review boards and/or oversight committees.

Dietary Assessment
Dietary data were collected via validated food-frequency questionnaires (13 cohorts), dietary recall (1 cohort), and food record (1 cohort) (Supplementary Table 2). The type of food-frequency questionnaire used in each cohort differed slightly to capture the dietary habits of the population of interest. The present analysis quantified total CHO intake and total fat intake as percentages of total energy intake. Additional analyses used percentage of energy from specific fatty acids, including PUFA, MUFA, and SFA. Total energy intake from protein was not included in the present analysis because of the lack of evidence supporting protein intake in gene-environment interactions (3).

Sleep Assessment
Data on habitual weekday/weekend nighttime sleep duration in hours per night were obtained from self-reported responses to questions such as, “How many hours of sleep do you usually get at night?” or were calculated from self-reported weekday/weekend bed and rise times (Supplementary Table 3).

Responses were analyzed as continuous and categorical variables. Commonly accepted cutoffs were used to create three sleep duration categories: short (<7 h), normal (≥7 to <9 h), and long (≥9 h).

Outcome Measurements
Cohort-specific assessment methods for FG (mmol/L), BMI (kg/m²), waist circumference (cm), and HDL-C (mmol/L) are described in detail in Supplementary Table 3. HOMA-IR was estimated from fasting insulin and FG concentrations, using the previously validated equation (HOMA-IR = FG (mmol/L) × fasting insulin (mU/L)/22.5), and was natural log-transformed to reduce skew before data analysis.

Genotyping
We selected five single nucleotide polymorphisms (SNPs) in circadian-related genes based on previous reports from GWAS meta-analysis (CRY2-rs11605924, MTNR1B-rs10830963), replicated candidate gene association studies (NR1D1-rs2314339), gene-environment interaction studies, or a combination of these findings (CLOCK-rs1801260, MTNR1B-rs1387153) that showed associations with type 2 diabetes, FG, and/or BMI (4,9,18–21). SNPs and/or SNPs in linkage disequilibrium (r² > 0.80; HapMap III release 2 data set) were previously directly genotyped or imputed by participating cohorts before inclusion in this analysis (Supplementary Table 4). SNPs were assessed for quality control: genotyped SNPs were excluded on the basis of low call rate (<95%) and departure from Hardy-Weinberg equilibrium (<1E-06), and imputed SNPs were removed on the basis of low imputation quality (MACH: R² < 0.3 or IMPUTE: proper info <0.4). Not all SNPs were available in all participating cohorts (Supplementary Table 5), and therefore, total sample sizes for analyses varied.

Cohort-Specific Analyses
All participating cohort-specific statistical analyses followed a uniform analysis plan. First, main associations between dietary intake or sleep duration and all outcomes were estimated with adjustment for age, sex, BMI (except for BMI outcome), and study site (in CARDIA, CHS, FamHS, GOLDN, InCHIANTI, and MESA) using linear regression models. Second, main associations between selected SNPs and all outcomes were investigated by using linear regression models and an additive genetic model adjusted for the aforementioned covariates in addition to family or population structure (in Corogene Controls, DILGOM, FamHS, FOS, GOLDN, MESA, The Rotterdam Study, and YFS), and genotype batch (in FamHS). Third, for our primary analysis of interest, 175 interactions (7 environmental variables × 5 SNPs × 5 outcomes) between dietary intake or sleep duration (continuous and categorical) and the selected SNPs on all outcomes were tested by using intake/sleep duration × SNP cross-product terms and including main-effect terms in linear regression models adjusted for the aforementioned covariates. Participants within each cohort were excluded from the analysis if they were shift workers, on sleep medications or antidepressant medications, reported bedtimes after 5 a.m. or before 6 p.m., and/or reported sleep duration <3 or ≥16 h. For glycemic outcomes, participants with type 2 diabetes within each cohort were excluded from main association and interaction analyses.

Meta-analyses
We conducted inverse-variance weighted, fixed-effect meta-analyses using METAL (version released 2011-03-25) for 1) main associations of dietary intake/sleep duration on the outcomes, 2) main associations of the selected SNPs on the outcomes, and 3) interactions between SNPs and dietary intake/sleep duration on the outcomes. Heterogeneity across studies was tested by using Cochran’s Q statistic and quantified using the I² statistic. All association and interaction analyses with moderate heterogeneity (I² > 30%) were further assessed for potential sources of heterogeneity by conducting meta-regression and sensitivity analyses. Meta-regression analyses were conducted using the R metafor package (R version 3.1.0) to assess the effect of the following moderator variables on heterogeneity of association/interaction: geographical location (U.S. vs. northern Europe vs. Mediterranean), mean age of cohort (20–64 years vs. 65–80 years), and total energy intake (<2,000 vs. ≥2,000 kcal/day). Sensitivity analyses assessed the influence of a single cohort on the meta-analyzed estimate by
Calculations using Quanto version 1.2.4

dent SNPs). We performed power cal-

Table 6). Each additional 1% of CHO

care.diabetesjournals.org Dashti and Associates 1459

eranged between 32.6 and 70.2 years,

RESULTS

The Mediterranean cohort (i.e., Chlamy-

differences between 8 and 21, the pre-

were reported in Table 1. Mean ages

Table 1. General characteristics of participants

interactions. Statistical signi-


table 1 of CHO

Interactions between SNPs and Eating in Atherosclerosis Susceptibility (THISEAS) (Greece); Cardiovascular Risk in Young Finns Study (YFS) (Finland).

Values are means ± standard deviation.

BMI (% total energy) 11.9

BMI (kg/m2) 25.7

SFA (% total energy) 11.9

Table 1: General characteristics of participating CHARGE cohorts.
intake was associated with 0.007 mmol/L lower FG ($\beta \pm SE = -0.007 \pm 0.001$ mmol/L, $P = 2.7E-29$) and a 0.001 lower HOMA-IR ($\beta \pm SE = -0.001 \pm 0.0004$ [ln], $P = 0.002$). Each additional 1% of total fat intake was associated with a 0.01 mmol/L higher FG ($\beta \pm SE = 0.01 \pm 0.001$ mmol/L, $P = 8.36E-13$) and a 0.005 higher HOMA-IR ($\beta \pm SE = 0.005 \pm 0.0005$ [ln], $P = 2.76E-16$). Similar trends were evident for MUFA and SFA intakes: each additional 1% of MUFA intake was associated with a 0.01 mmol/L higher FG ($\beta \pm SE = 0.01 \pm 0.001$ mmol/L, $P = 4.23E-12$) and a 0.01 higher HOMA-IR ($\beta \pm SE = 0.01 \pm 0.001$ [ln], $P = 1.12E-13$), whereas each additional 1% of SFA intake was associated with a 0.001 mmol/L higher FG ($\beta \pm SE = 0.01 \pm 0.001$ mmol/L, $P = 9.26E-13$) and a 0.01 higher HOMA-IR ($\beta \pm SE = 0.01 \pm 0.001$ [ln], $P = 1.66E-19$). However, no associations were evident for PUFAs intake.

When sleep was assessed linearly, we observed a marginal association between sleep duration and FG: each additional hour of sleep was associated with a 0.01 mmol/L lower FG ($\beta \pm SE = 0.01 \pm 0.0005$ mmol/L, $P = 0.05$). However, when sleep was categorical, we observed a significant association between short sleep duration ($\leq 7$ h) and FG: short sleep duration was associated with a 0.03 mmol/L higher FG ($\beta \pm SE = 0.03 \pm 0.01$ mmol/L, $P = 0.01$) and with a 0.45 kg/m$^2$ higher BMI ($\beta \pm SE = 0.45 \pm 0.09$ kg/m$^2$, $P < 0.001$) relative to normal sleep duration ($\geq 7$ to <9 h). In addition, long sleep duration ($\geq 9$ h) was associated with a 0.33 kg/m$^2$ higher BMI ($\beta \pm SE = 0.33 \pm 0.14$ kg/m$^2$, $P = 0.02$) and a 0.76 cm higher waist circumference ($\beta \pm SE = 0.76 \pm 0.18$ cm, $P < 0.001$) and was marginally associated with a higher HOMA-IR ($\beta \pm SE = 0.03 \pm 0.02$ [ln], $P = 0.05$), relative to normal sleep duration ($\geq 7$ to <9 h).

Additional main associations of dietary intake and sleep duration on anthropometric and lipid traits are presented in Supplementary Table 6.

**Associations of SNPs With Cardiometabolic Traits**

Sensitivity analyses indicated that substantial heterogeneity ($I^2 > 30\%$) was introduced by one cohort (GOLDN) for glycemic trait outcomes; consequently, GOLDN was excluded from the association and interaction meta-analyses for glycemic traits.

Main associations of selected SNPs on cardiometabolic traits are presented in Supplementary Table 7. We replicated previously published associations between MTNR1B variants and glycemic traits in the present meta-analysis (18,19). In short, MTNR1B-rs1387153 was associated with FG ($\beta \pm SE = 0.058 \pm 0.007$ mmol/L per additional T allele, $P = 1.7E-17$), whereas MTNR1B-rs10830963 was associated with FG ($\beta \pm SE = 0.1 \pm 0.008$ mmol/L per additional G allele, $P = 4.2E-35$) and HOMA-IR ($\beta \pm SE = 0.016 \pm 0.004$ [ln] per additional G allele, $P = 0.004$). We did not replicate previous associations between CRY2-rs11605924 and FG ($P = 0.06$) (18,19). Consistent with previous findings, no associations were observed for CLOCK-rs1801260 or NR1D1-rs2314339 on glycemic traits. Main associations of selected SNPs on anthropometric and lipid traits are presented in Supplementary Table 7.

**Interactions Between Dietary Intake and Selected SNPs on Cardiometabolic Traits**

Meta-analyzed estimates of the interactions between dietary intake and selected SNPs on cardiometabolic traits are presented in Table 2. We observed no interactions at the prespecified Bonferroni-corrected significance level of $P < 0.003$. A nominal interaction was evident between sleep duration and CRY2-rs11605924 for HDL-C ($\beta \pm SE = 0.01 \pm 0.004$ mmol/L, $P = 0.005$), suggesting a 0.001 mmol/L higher HDL-C with each additional hour of sleep in the presence of the effect A allele (Supplementary Fig. 3B). That is, in the presence of each additional A allele, the protective association of higher sleep duration on HDL-C was 0.01 mmol/L stronger (per 1 h of additional sleep), such that the A allele appears to strengthen the positive association observed with longer sleep duration. No interactions were evident between categories of sleep duration and this variant for HDL-C (short sleep duration, $P = 0.15$; long sleep duration, $P = 0.21$).

Finally, we observed a nominal interaction between short sleep duration ($<7$ h) and MTNR1B-rs1387153 for BMI ($\beta \pm SE = 0.25 \pm 0.12$ kg/m$^2$, $P = 0.04$) (Supplementary Fig. 2F) and a stronger interaction between long sleep duration ($\geq 9$ h) and the same variant for BMI ($\beta \pm SE = 0.60 \pm 0.20$ kg/m$^2$, $P = 0.003$) (Supplementary Fig. 2G); these interactions suggest 0.25 and 0.60 kg/m$^2$ higher BMIs with short and long sleep durations, respectively, in the
<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Effect</th>
<th>n 1</th>
<th>n 2</th>
<th>p (2-tailed)</th>
<th>p (1-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10830963</td>
<td>MTNR1B</td>
<td>T/C</td>
<td>25,016</td>
<td>0.0017</td>
<td>6</td>
<td>0.0001</td>
<td>6.0E-05</td>
<td>0.82</td>
</tr>
<tr>
<td>rs11605924</td>
<td>MTNR1B</td>
<td>T/C</td>
<td>18,401</td>
<td>0.0002</td>
<td>6</td>
<td>0.0045</td>
<td>5.2E-05</td>
<td>0.08</td>
</tr>
<tr>
<td>rs2314339</td>
<td>MTNR1B</td>
<td>T/C</td>
<td>18,401</td>
<td>0.0003</td>
<td>6</td>
<td>0.0010</td>
<td>5.2E-05</td>
<td>0.01</td>
</tr>
<tr>
<td>rs10830963</td>
<td>MTNR1B</td>
<td>C/T</td>
<td>26,532</td>
<td>0.0010</td>
<td>6</td>
<td>0.0010</td>
<td>5.2E-05</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 2—Meta-analyzed interactions between dietary intake and SNPs on cardiometabolic traits.

**Note:** Alleles presented as effect/noneffect alleles.
Table 3—Meta-analyzed interactions between sleep duration (continuous and categorical) and SNPs on cardiometabolic traits

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Alleles†</th>
<th>n</th>
<th>Glycemic traits</th>
<th>BMI (kg/m²)</th>
<th>Waist circumference (cm)</th>
<th>HDL-C (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FG (mmol/L) β ± SE</td>
<td>In-HOMA-IR β ± SE</td>
<td>BMI (kg/m²) β ± SE</td>
<td>Waist (cm) β ± SE</td>
</tr>
<tr>
<td>Continuous, h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1801260</td>
<td>CLOCK</td>
<td>C/T</td>
<td>1707</td>
<td>0.005 ± 0.02</td>
<td>0.67</td>
<td>-0.004 ± 0.01</td>
<td>0.61</td>
</tr>
<tr>
<td>rs11605924</td>
<td>CY2</td>
<td>A/C</td>
<td>1076</td>
<td>-0.001 ± 0.01</td>
<td>0.85</td>
<td>-0.004 ± 0.01</td>
<td>0.52</td>
</tr>
<tr>
<td>rs1387153</td>
<td>MTNR1B</td>
<td>T/C</td>
<td>19,913</td>
<td>0.006 ± 0.01</td>
<td>0.42</td>
<td>0.007 ± 0.01</td>
<td>0.23</td>
</tr>
<tr>
<td>rs10830963</td>
<td>MTNR1B</td>
<td>G/C</td>
<td>19,913</td>
<td>0.002 ± 0.01</td>
<td>0.859</td>
<td>-0.004 ± 0.01</td>
<td>0.60</td>
</tr>
<tr>
<td>rs2314339</td>
<td>NR1D1</td>
<td>T/C</td>
<td>18,404</td>
<td>0.007 ± 0.02</td>
<td>0.64</td>
<td>0.015 ± 0.01</td>
<td>0.19</td>
</tr>
<tr>
<td>Short (&lt;7 h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1801260</td>
<td>CLOCK</td>
<td>C/T</td>
<td>2,158</td>
<td>-0.010 ± 0.03</td>
<td>0.709</td>
<td>0.005 ± 0.23</td>
<td>0.82</td>
</tr>
<tr>
<td>rs11605924</td>
<td>CY2</td>
<td>A/C</td>
<td>3,294</td>
<td>-0.002 ± 0.02</td>
<td>0.929</td>
<td>0.006 ± 0.01</td>
<td>0.666</td>
</tr>
<tr>
<td>rs1387153</td>
<td>MTNR1B</td>
<td>T/C</td>
<td>2,158</td>
<td>0.010 ± 0.02</td>
<td>0.49</td>
<td>-0.001 ± 0.01</td>
<td>0.929</td>
</tr>
<tr>
<td>rs10830963</td>
<td>MTNR1B</td>
<td>G/C</td>
<td>3,525</td>
<td>0.020 ± 0.02</td>
<td>0.285</td>
<td>0.020 ± 0.02</td>
<td>0.139</td>
</tr>
<tr>
<td>rs2314339</td>
<td>NR1D1</td>
<td>T/C</td>
<td>3,525</td>
<td>0.0004 ± 0.04</td>
<td>0.88</td>
<td>-0.030 ± 0.03</td>
<td>0.26</td>
</tr>
<tr>
<td>Long (≥9 h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1801260</td>
<td>CLOCK</td>
<td>C/T</td>
<td>522</td>
<td>-0.006 ± 0.04</td>
<td>0.89</td>
<td>-0.030 ± 0.04</td>
<td>0.48</td>
</tr>
<tr>
<td>rs11605924</td>
<td>CY2</td>
<td>A/C</td>
<td>906</td>
<td>0.002 ± 0.03</td>
<td>0.95</td>
<td>0.010 ± 0.02</td>
<td>0.68</td>
</tr>
<tr>
<td>rs1387153</td>
<td>MTNR1B</td>
<td>T/C</td>
<td>522</td>
<td>0.020 ± 0.03</td>
<td>0.54</td>
<td>0.010 ± 0.02</td>
<td>0.70</td>
</tr>
<tr>
<td>rs10830963</td>
<td>MTNR1B</td>
<td>G/C</td>
<td>961</td>
<td>0.008 ± 0.03</td>
<td>0.79</td>
<td>0.030 ± 0.03</td>
<td>0.30</td>
</tr>
<tr>
<td>rs2314339</td>
<td>NR1D1</td>
<td>T/C</td>
<td>961</td>
<td>0.070 ± 0.06</td>
<td>0.286</td>
<td>-0.040 ± 0.05</td>
<td>0.506</td>
</tr>
</tbody>
</table>

*Additive allele mode, adjusted for age, sex, BMI (except when assessing BMI outcome), study site (in CHS, InCHIANTI, MESA), family or population structure (in CoroGene Controls; DILGOM; FOS; MESA; YFS), and genotype batch (in FamHS). Interaction coefficients are shown as β ± SE. β represents the direction and magnitude of the change in outcome trait with each additional minor allele, per each additional hour of sleep or compared with the reference sleep group (≥7 to <9 h). Boldface type indicates nominally significant values (P < 0.05). †Alleles presented as effect/noneffect alleles. ‡The number of independent observations in each interaction analysis. Exact numbers of observations vary depending on availability of phenotype and genotype information and are presented in Supplementary Table 8. §I² > 30%, where I² represents the heterogeneity statistic presented as percent. Exact I² is presented in Supplementary Table 8.
duration (<7 h) and higher FG supports previously reported associations in single
cohorts (28) and supports previously reported associations between short
sleep duration and type 2 diabetes (7). As such, lifestyle recommendations
should include dietary modifications related to higher CHO and lower fat com-
position and achieving normal sleep durations (7 to <9 h).

Our evaluation of gene-environment interactions suggest novel putative inter-
actions that fell short of our Bonferroni-corrected cut point but are supported by
biological plausibility and may be important for understanding the etiology of
type 2 diabetes. The strongest nominal interaction for glycemic traits was an
interaction of CHO intake and the MTNR1B:rs1387153 variant on FG, which suggests
that every 1% increase in CHO intake exacerbates the FG-raising effect of the T
allele that interacts similarly with CHO as another clock variant in CRY1 (5). Along
with other nominal interactions observed for MTNR1B:rs1387153, these findings
suggest lower CHO and higher MUFA in-
takes for lower FG among those with the effect
T allele. The high frequency of the effect
T allele among individuals of Euro-
pesian descent (minor allele frequency = 0.28) and the consistency of the
rs1387153-FG association across dif-
ferent ethnicities (29) warrant further
investigation of this interaction and
examination of the potential role of
CHO quality in the FG-raising effect of the T allele. These nominal findings, in
conjunction with confirmed associations between two common MTNR1B variants
and FG of effect sizes similar to those re-
ported earlier (18,30), indicate that con-
tinuing efforts to identify lifestyle modifications that offset this genetic risk
should remain an important area of active
research. Consistent with previous find-
ings, no interactions were evident be-
tween sleep duration and the selected
variants on glycemic traits (7).

Our previous work suggests that var-
iants identified through GWAS or can-
didate gene association studies for type 2
diabetes may show gene-environment interactions for related metabolic traits
beyond glycemic traits (3). We identified
three nominal interactions that are sup-
ported by previous reports and biologi-
cal plausibility. The first is a nominal interaction between SFA intake and
NR1D1:rs2314339 on BMI. The obesity-
associated NR1D1 gene encodes the nu-
clear receptor rev-erb-α, which plays a
critical role in metabolism and was
reported to respond to dietary MUFA for
the outcome of BMI (21,31,32). The sec-
ond is a nominal interaction between
FG-associated CRY2:rs11605924 and
sleep duration for HDL-C. We observed a
positive association between HDL-C and
sleep only when considered in the con-
text of CRY2, a clock gene that inhibits
CLOCK:BMAL1-mediated transcription of
genes involved in lipid metabolism (33). Supporting the circadian control
of HDL-C are results from the Global
Lipids Genetics Consortium GWAS for
HDL-C for this CRY2 variant (β ± SE =
0.0004 ± 0.0001 mmol/L per additional
A allele, P < 0.001) (34), which suggest
marginal associations between CRY2 and
HDL-C (Supplementary Fig. 4). The
CRY2 variant is in linkage disequilibrium
with rs6843722 (r² = 1.00 in the 1000
Genomes Project data set), a CRY2 in-
trinsic variant that was shown to abolish
the upregulation of CRY2 expression in
human peripheral blood mononuclear
 cells after sleep restriction and has func-
tional evidence to affect transcriptional
regulation of CCCTC-binding factor and
glucocorticoid receptor, two transcription
factors associated with HDL-C (35–37).
Therefore, it is possible that short
sleep duration results in differences in
CRY2 expression, influencing CRY2
control of downstream pathways,
namely HDL-C. Finally, we observed
nominal interactions between short
and long sleep duration, both of which
are associated with higher BMI, and
MTNR1B:rs1387153 on BMI, suggesting
even higher BMI with short and long
sleep duration among carriers of the ef-
f ect T allele. This interaction provides
additional support for the potential
role of sleep duration in modifying the
associations between circadian-related
 genetic variants and cardiometabolic
outcomes (38) and the importance of
normal sleep duration (7 to <9 h)
for optimal health.

The strengths of the present observa-
tional study from 15 cohorts include
a large sample size necessary to detect
gene-environment interactions. Our col-
aborative approach enabled us to stan-
dardize our analytic approach across
cohorts, and despite the wide range of
cohorts included in the study, we ob-
served little evidence of heterogeneity
in our overall analysis. However the
present investigation also has limita-
tions. The reported findings are limited
to individuals of European descent, and
exploring the interaction in other popu-
lations is warranted considering replica-
tion of the SNP associations in different
ethnicities (29,39).

Our use of self-reported dietary in-
take and sleep duration was susceptible
to reporting bias, and the use of differ-
ent assessment tools across cohorts
could have increased measurement
error, biasing our results toward the null
(40). In addition, we failed to replicate a
previously reported significant associa-
tion between CRY2 variant and FG,
although we observed an effect size
similar to that of the discovery GWAS
of up to 46,000 individuals (18); it is
possible our sample size was too small
to replicate the significant associations.
Although we have selected circadian-
related gene variants showing strong
 associations with metabolic traits from
GWAS and candidate-gene studies, it is
possible that interactions might be
observable for other circadian-related
variants.

Lastly, these cross-sectional meta-
analyses of observational studies can
only lead us toward hypotheses rather
d than demonstrate the temporal rela-
tionships or causal pathways linking
clock genes, diet, or sleep, with glycémic,
anthropometric, and lipid traits.
Other studies are required to establish
these mechanistic links, including stud-
ies of genetic modification of the effects
of experimental changes in diet compo-
sition or sleep duration.

Our findings contribute to the under-
standing of how lifestyle can reduce
the risk of type 2 diabetes and cardiomet-
bolic disorders in genetically susceptible
individuals. Results from the present
large observational study from 15 co-
horts suggest the potential presence of
selected common circadian-related
gene-environment interactions on me-
tabolic traits. The nominal interaction
between CHO intake and the MTNR1B
variant on FG, suggesting that CHO in-
take could exacerbate the FG-raising
effects of this well-studied MTNR1B
variant, the evidence supporting the
role for CRY2 in HDL-C control and its
responsiveness to sleep duration, and
the interaction between long sleep du-
ratio and MTNR1B variant on BMI,
suggesting that the association between long sleep duration and higher BMI is exacerbated among carriers of the MTNR1B variant, are interesting and merit further study. Mechanistic examinations of the novel nominal interactions and further investigations in larger cohorts are necessary before personalized recommendations are framed for individuals at genetic risk for metabolic disruption. Moreover, the observed associations between diet—specifically, higher CHO and lower fat composition—and normal sleep duration ($\geq 7$ to $< 9$ h) on glycemic traits—particularly FG—suggest that emphasis should be placed on these modifiable lifestyle factors to offset the growing prevalence of type 2 diabetes and cardiovascular disease.

**Funding.** Infrastructure for the CHARGE Consortium is supported in part by the National Heart, Lung, and Blood Institute grant HHSN268200800007C. Support was provided by the U.S. Department of Agriculture, under agreement no. 58-1950-0-014, American Heart Association (grant 14BGIA18740001), Academy of Finland (grant 117787), Italian Ministry of Health (grant IC5110:RF97.71), National Institute of Diabetes and Digestive and Kidney Diseases (grant DK063491), National Center for Advancing Translational Sciences (grant UL1TR000124). C.E.S. is supported by K08-HL-112845-01. F.A.J.L.S. was supported in part by National Institutes of Health grants R21-DK-089378 and R01-HL-094806. Cohort-specific sources of support and acknowledgments are presented in Supplementary Table 1. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

**Author Contributions.** H.S.D., C.E.S., T.T., M.G., D.J.G., F.A.J.L.S., and J.M.O. designed the study. H.S.D., J.L.F., T.T., T.M.B., L.K., M.K.W., A.C.F.-W., T.S.A., M.-M.P., A.J., T.M., I.P.K., and V.M. conducted research and contributed to statistical analyses. H.S.D., C.E.S., M.G., D.J.G., A.H., P.F.J., I.C.K.-d.I., S.L.F., F.A.J.L.S., and J.M.O. wrote the manuscript. All authors read and approved the final version of the manuscript. H.S.D. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Prior Presentation.** Preliminary results were presented at the 78th Scientific Sessions and Annual Meeting of the American Society for Nutrition in conjunction with Experimental Biology 2014, San Diego, CA, 26–30 April 2014, and at the 2014 Society for Research on Biological Rhythms Meeting, Big Sky, MT, 14–18 June 2014.

**Appendix**

**Members of the CHARGE Nutrition Study Group.** Hassan S. Dashti, PhD, Nutrition and Genomics Laboratory, Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA; Jack L. Follis, PhD, Department of Mathematics, Computer Science, and Cooperative Engineering, University of St. Thomas, Houston, TX; Caren E. Smith, MS, DVM, Nutrition and Genomics Laboratory, Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA; Toshiko Tanaka, PhD, Translational Gerontology Branch, National Institute on Aging, Baltimore, MD; Marta Garaulet, PhD, Department of Physiology, University of Murcia, Murcia, Spain; Daniel J. Gottlieb, MD, MPH, Division of Sleep and Circadian Disorders, Brigham and Women’s Hospital, Boston, MA, Division of Sleep Medicine, Harvard Medical School, Boston, MA, and Sleep Disorders Center, VA Boston Healthcare System, Boston, MA; Adela Hruby, PhD, MPH, Department of Nutrition, Harvard School of Public Health, Boston, MA; Paul F. Jacques, ScD, Nutritional Epidemiology Laboratory, Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA; Frank A.J.L. Scheer, PhD, Division of Sleep and Circadian Disorders, Brigham and Women’s Hospital, Boston, MA, and Division of Sleep Medicine, Harvard Medical School, Boston, MA; Traci M. Bartz, MS, Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA, and Department of Bioengineering, University of Washington, Seattle, WA; Leena Kovanen, MS, Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare (THL), Helsinki, Finland; Maria Perälä, MSc, Department of Chronic Asthma Centre, Gentofte Hospital, Copenhagen, Denmark; Taulant Muka, MD, MSc, Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; Joanna P. Kalafati, MSc, Department of Nutrition and Dietetics, Harokopio University, Athens, Greece; Vera Mikkilä, PhD, Department of Food and Environmental Sciences, Division of Nutrition, University of Helsinki, Helsinki, Finland, and Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland; Rozenn N. Lemaitre, PhD, MPH, Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA; Timo Partonen, MD, PhD, Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare (THL), Helsinki, Finland; Tapani Ebeling, MD, PhD, Oulu University Hospital, Department of Internal Medicine, Division of Endocrinology, Oulu, Finland; Paul N. Hopkins, MD, MSPH, School of Medicine, University of Utah, Salt Lake City, UT; Lavina Paternoster, PhD, MRC Integrative Epidemiology Unit, School of Social and Community Medicine, University of Bristol, Bristol, U.K.; Jari Lahti, PhD, Institute of Behavioural Sciences, University of Helsinki, Helsinki, Finland, and Folkhalsan Research Centre, Helsinki, Finland; Dena G. Hernandez, MS, Laboratory of Neurogenetics, National Institute on Aging, Baltimore, MD; Ulla Toft, PhD, Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark; Richa Saxena, PhD, Division of Sleep and Circadian Disorders, Brigham and Women’s Hospital, Boston, MA, and Center for Human Genetic Research and Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA; Anna Vitezova, PharmD, MSc, Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; Stavroula Kanoni, PhD, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, U.K.; Olli T. Raitakari, PhD, Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland, and Department of Clinical Physiology and Nuclear Medicine, University of Turku and Turku University Hospital, Turku, Finland; Bruce M. Psaty, MD, PhD, Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA, Departments of Epidemiology and Health Services, University of Washington, Seattle, WA, and Group Health Research Institute, Group Health, Seattle, WA; Markus Perola, MD, PhD, Department of Chronic Disease Prevention, National Institute for Health and Welfare (THL), Helsinki, Finland; Satu Mannisto, PhD, Department of Chronic Disease Prevention, National Institute for Health and Welfare (THL), Helsinki, Finland; Robert J. Straka, BSc, PharmD, FCCP, Experimental and Clinical Pharmacology, College of Pharmacy, University of Minnesota, MN; Torben Hansen, MD, PhD, The Novo Nordisk Foundation Centre for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; Katri Raikkonen, PhD, Institute of Behavioural Sciences, University of Helsinki, Helsinki, Finland; Luigi Ferrucci, MD, PhD,
Translational Gerontology Branch, National Institute on Aging, Baltimore, MD; Niels Grarup, MD, PhD, The Novo Nordisk Foundation Centre for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; W. Craig Johnson, MS, Department of Biostatistics, University of Washington, Seattle, WA; Loukianos Rallidis, MD, Second Department of Biostatistics, University of Washington, Seattle, WA; Aki S. Havulinna, DSc, Department of Chronic Disease Prevention, National Institute for Health and Welfare (THL), Helsinki, Finland; Sina A. Gharib, MD, Department of Clinical Epidemiology, Tampere University Hospital and University of Tampere, Tampere, Finland; David S. Siscovick, MS, Department of Nutrition and Dietetics, Children’s Hospital of Philadelphia, Philadelphia, PA; Wanja H. Hofman, MD, PhD, Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; Kari Saasen Wilhelmsen, MD, PhD, Helsinki Regional Hospital, Helsinki, Finland; Gianfranco Cresci, MD, Department of Clinical Experimental Research, GluEbro University Hospital, GluEbro, Denmark, and Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; Ilkka Seppälä, PhD, Department of Clinical Experimental Research, Centre for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Sciences, University of Turku, Turku, Finland, and Faculty of Medicine, University of Aalborg, Aalborg, Denmark; Tuu-An Chen, PhD, U.S. Department of Agriculture/Agricultural Research Service, Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX; Albert Hofman, MD, PhD, Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; Panos Deloukas, PhD, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK, and Princess Al-Jawhara Al-Br&mash;m Centre of Excellence in Research of Hereditary Disorders (PARC-HD), King Abdulaziz University, Jeddah, Saudi Arabia; Jorma S.A. Viikari, PhD, Department of Medicine, University of Turku, Turku, Finland, and Division of Medicine, Turku University Hospital, Turku, Finland; Dariusz Motyl, MD, PhD, DrPh, Friedman School of Nutrition Science and Policy, Tufts University, Boston, MA; Oluf Pedersen, MD, PhD, The Novo Nordisk Foundation Centre for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; Jerome I. Rotter, MD, Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; André G. Utterlinden, PhD, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands; Ilkka Seppälä, MSc, Department of Clinical Chemistry, Finlab Laboratories, University of Tampere School of Medicine, Tampere, Finland; Henning Tiemeier, MD, PhD, Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; Lars Kähönen, MD, PhD, Department of Chronic Disease Prevention, National Institute for Health and Welfare (THL), Helsinki, Finland; Sina A. Gharib, MD, Department of Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA, and Computational Medicine Core, Center for Lung Biology, UW Medicine Sleep Center, University of Washington, Seattle, WA; Ingrid B. Borecki, PhD, Department of Genetics, Washington University School of Medicine, St. Louis, MO; Donna K. Arnett, PhD, Department of Epidemiology, School of Public Health, University of Alabama at Birmingham, Birmingham, AL; Thorklid I.A. Sørensen, DrMedSci, The Novo Nordisk Foundation Centre for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, and Institute of Preventive Medicine, Bispebjerg and Frederiksberg Hospitals, The Capital Region, Copenhagen, Denmark; Johan G. Eriksson, PhD, MD, Department of Chronic Disease Prevention, National Institute for Health and Welfare (THL), Helsinki, Finland, Folkhälsoins Research Centre, Helsinki, Finland, Department of General Practice and Primary Health Care, University of Helsinki, Helsinki, Finland, Helsinki University Central Hospital, Unit of General Practice, Helsinki, Finland, and Vasa Central Hospital, Vasa, Finland; Stefania Bandinelli, MD, Geriatric Unit, Azienda Sanitaria Firenze (ASF), Florence, Italy; Allan Linneberg, MD, PhD, Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark, and Department of Clinical Experimental Research, Glostrup University Hospital, Glostrup, Denmark, and Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; Stephen S. Rich, PhD, Center for Public Health Genomics, University of Virginia, Charlottesville, VA; Oscar H. Franco, MD, PhD, Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; George Dedoussis, PhD, Department of Nutrition and Dietetics, Harokopio University, Athens, Greece; Terho Lehtimäki, PhD, Department of Clinical Chemistry, Finlab Laboratories, University of Tampere School of Medicine, Tampere, Finland; Jose M. Ordovás, PhD, Nutrition and Genomics Laboratory, Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA, Department of Epidemiology, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain, and Instituto Madrileño de Estudios Avanzados en Alimentación (IMDEA-FOOD), Madrid, Spain.

References