Habitual sleep duration is associated with BMI and macronutrient intake and may be modified by *CLOCK* genetic variants[1–5]

Hassan S Dashti, Jack L Follis, Caren E Smith, Toshiko Tanaka, Brian E Cade, Daniel J Gottlieb, Adela Hruby, Paul F Jacques, Stefania Lamon-Fava, Kris Richardson, Richa Saxena, Frank AJL Scheer, Leena Kovanen, Traci M Bartz, Mia-Maria Perälä, Anna Jonsson, Alexis C Frazier-Wood, Ioanna-Panagiota Kalafati, Vera Mikkilä, Timo Partonen, Rozenn N Lemaitre, Jari Lahti, Dena G Hernandez, Ulla Toft, W Craig Johnson, Stavroula Kanoni, Olli T Raitakari, Markus Perola, Bruce M Psaty, Luigi Ferrucci, Niels Grarup, Heather M Highland, Loukianos Rallidis, Mika Kähönen, Aki S Havulinna, David S Siscovick, Katri Räikkönen, Torben Jørgensen, Jerome I Rotter, Panos Deloukas, Jorma SA Viikari, Dariush Mozaffarian, Allan Linneberg, Ilkka Seppälä, Torben Hansen, Veikko Salomaa, Sina A Gharib, Johan G Eriksson, Stefania Bandinelli, Oluf Pedersen, Stephen S Rich, George Dedoussis, Terho Lehtimäki, and José M Ordovás

ABSTRACT

Background: Short sleep duration has been associated with greater risks of obesity, hypertension, diabetes, and cardiovascular disease. Also, common genetic variants in the human Circadian Locomotor Output Cycles Kaput (*CLOCK*) show associations with ghrelin and total energy intake.

Objectives: We examined associations between habitual sleep duration, body mass index (BMI), and macronutrient intake and assessed whether *CLOCK* variants modify these associations.

Design: We conducted inverse-variance weighted, fixed-effect meta-analyses of results of adjusted associations of sleep duration and BMI and macronutrient intake as percentages of total energy as well as interactions with *CLOCK* variants from 9 cohort studies including up to 14,906 participants of European descent from the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium.

Results: We observed a significant association between sleep duration and lower BMI ($\beta \pm$ SE = 0.16 $\pm$ 0.04, $P < 0.0001$) in the overall sample; however, associations between sleep duration and relative macronutrient intake were evident in age- and sex-stratified analyses only. We observed a significant association between sleep duration and lower saturated fatty acid intake in younger (aged 20–64 y) adults (men: 0.11 $\pm$ 0.06%, $P = 0.03$; women: 0.10 $\pm$ 0.05%, $P = 0.04$) and with lower carbohydrate ($-0.31 \pm 0.12\%$, $P < 0.01$), higher total fat (0.18 $\pm$ 0.09%, $P = 0.05$), and higher PUFA (0.05 $\pm$ 0.02%, $P = 0.02$) intakes in older (aged 65–80 y) women. In addition, the following 2 nominally significant interactions were observed: between sleep duration and rs12649507 on PUFA intake and between sleep duration and rs6858749 on protein intake.

Conclusions: Our results indicate that longer habitual sleep duration is associated with lower BMI and age- and sex-specific favorable dietary behaviors. Differences in the relative intake of specific macronutrients associated with short sleep duration could, at least in part, explain previously reported associations between short sleep duration and chronic metabolic abnormalities. In addition, the influence of obesity-associated *CLOCK* variants on the association between sleep duration and macronutrient intake suggests that longer habitual sleep duration could ameliorate the genetic predisposition to obesity via a favorable dietary profile. Trials related to this study were registered at clinicaltrials.gov as NCT00005133 (Cardiovascular Health Study), NCT00005121 (Framingham Offspring Study), NCT01331512 [Invecchiare in Chianti (Aging in the Chianti Area) study], NCT00289237 (Inter99), and NCT00005487 (Multi-Ethnic Study of Atherosclerosis). *Am J Clin Nutr* 2015;101:135–43.

INTRODUCTION

In 2013, approximately one-third of U.S. workers reported sleep duration ≤ 6 h/night and fewer than 21% of U.S. adults met NIH recommendation of 7–8 h sleep/night (1). The increasing prevalence of short sleep duration raises public health concerns related to both safety and health (2). Short sleep duration has been associated with greater risk of obesity (3), hypertension (4), diabetes (5), cardiovascular disease (6), and all-cause mortality (7). Because cardiometabolic conditions have strong nutritional determinants, links between insufficient sleep and metabolic disruption could be mechanistically mediated through changes in dietary intake.

Studies of experimental sleep restriction showed unfavorable impacts on the appetite-related hormones ghrelin and leptin and on hunger, total energy intake, and weight (8, 9). In short-term, crossover, inpatient trials in adults, sleep restriction was associated with increased energy intake (10, 11), increased total fat (11), and SFA (10) intakes and excess consumption of carbohydrate-rich snacks (12). However, habitual sleep duration assesses long-term sleep patterns, which may affect risk for chronic diseases. Self-reported habitual short sleep duration is associated with elevated ghrelin and decreased leptin concentrations in the general population (13), and a recent cross-sectional analysis reported higher energy intake in individuals with self-reported short sleep duration (14). Whether chronic metabolic abnormalities associated with short sleep duration result from differences in relative intakes of macronutrients in addition to reported higher energy intake is not known.

In addition to sleep, the circadian system, which is comprised of transcription factors including Circadian Locomotor Output

Cycles Kaput (*CLOCK*)[6] and governs the sleep-wake cycle, also influences diet through changes in appetite mediated by endocrine hormones such as leptin, ghrelin, and insulin (15). Common genetic variants in the human *CLOCK* show associations with ghrelin (16), whereas other *CLOCK* variants situated in the 3′-untranslated region show associations with total energy intake (17). These observations are in line with genome-wide association studies that identified 3 *CLOCK* variants rs4864548, rs3736544, and rs1801260 to be associated with obesity and metabolic syndrome (18). Observed differences in appetite-related endocrine hormones and total energy intake related to *CLOCK* variants suggest that *CLOCK* could modify associations between habitual sleep duration and dietary intake.

We hypothesized that habitual short sleep duration is associated with macronutrient composition, specifically with diets higher in relative carbohydrate and SFAs and lower in PUFAs and MUFAs. On the basis of the influence of *CLOCK* on appetite and total energy intake, we further hypothesized that *CLOCK* variants modify the association between habitual short sleep duration and relative macronutrient intake. These hypotheses were tested in large cross-sectional meta-analyses of population-based cohorts from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.

SUBJECTS AND METHODS

Cohorts

The cross-sectional meta-analyses included up to 14,906 participants of European descent from the following 9 cohort studies of the CHARGE Consortium Nutrition Working Group (**Supplemental Table 1**): Corogene Controls, the Cardiovascular Health Study (CHS), the Framingham Offspring Study (FOS); the Helsinki Birth Cohort Study (HBCS), Invecchiare in Chianti (aging in the Chianti area, InCHIANTI), Inter99, Multi-Ethnic Study of Atherosclerosis (MESA), The Hellenic Study of Interactions between SNPs and Eating in Atherosclerosis Susceptibility (THISEAS), and the Cardiovascular Disease Risk in Young Finns Study (YFS). Participants provided written informed consent, and the protocol was approved by local institutional review boards and oversight committees.

Dietary assessment and BMI

Habitual dietary intake data were collected via validated food-frequency questionnaires (**Supplemental Table 2**) (19–24). The type of food-frequency questionnaire used in each cohort differed slightly to capture the dietary habits of the population of interest. The current analysis focused on intake as percentages of total energy of protein, carbohydrate, and total fat. In addition, we examined PUFA, MUFA, and SFA intakes as percentages of total energy. Cohort-specific assessment methods for BMI are provided in **Supplemental Table 3**.

Sleep

Data on habitual weekday/workday nighttime sleep duration in hours 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/workday bed and rise times (Supplemental Table 3). Responses were analyzed as

continuous variables. Participants within each cohort were excluded from the analysis if they were shift workers, on a sleep or depression medication, reported bedtimes after 0500 or before 1800, or reported sleep duration ≤3 or ≥16 h/night.

Genotyping

We selected 9 tagging single nucleotide polymorphisms (tSNPs) that capture variations for *CLOCK* gene and flanking regulatory regions (±20 kb) by using the web-based service of the Tagger option (aggressive tagging approach) within Haploview software (version 4.2; Broad Institute) (25) with variables on the basis of the Caucasian European Utah analysis panel (HapMap III release 2) with a minor allele frequency ≥0.10 and $r^2 \geq 0.80$. Selected tSNPs and single nucleotide polymorphisms (SNPs) in linkage disequilibrium ($r^2 \geq 0.80$) were previously directly genotyped or imputed by participating cohorts before inclusion in this analysis (**Supplemental Table 4**). Not all SNPs were available in all participating cohorts (**Supplemental Table 5**), and as a result, total sample sizes for analyses varied accordingly.

Cohort-specific analyses

All participating cohort-specific statistical analyses followed a uniform analysis plan. First, main associations between sleep duration and BMI were estimated by using linear regression models with adjustment for age, sex, and, when relevant, study site. Main associations between sleep duration and relative dietary intake were estimated similarly with adjustment for age, sex, BMI, and, when relevant, study site. Because sex and age have been reported to modify the association of sleep duration with BMI and total energy intake (11, 26, 27), associations were further explored in stratified age [20–64 y (younger) compared with 65–80 y (older)] and sex groups. Second, main associations between *CLOCK* tSNPs and dietary intake were investigated by using linear fixed-effect regression models or linear mixed-effects models for cohorts with family data in an additive genetic model adjusted for age, sex, BMI, and, when relevant, study site and family or population substructure. Third, the sleep duration × tSNP effect modification on dietary intake was investigated by including an interaction term in a model adjusted for the aforementioned covariates.

Meta-analyses

We conducted inverse-variance weighted, fixed-effect meta-analyses by using the METAL program (version released 25 March 2011; University of Michigan, Center for Statistical Genetics) (28) for *1*) main associations of sleep duration on both BMI and macronutrient intake, *2*) main associations of tSNPs on macronutrient intake, and *3*) interactions between tSNPs and sleep duration on macronutrient intake.

The heterogeneity across studies was tested by using Cochran's *Q* statistic and quantified by using the I^2 statistic (29). To assess potential sources of heterogeneity, we conducted a meta-regression of all association and interaction analyses with moderate heterogeneity ($I^2 > 30\%$) to assess the impact of moderator variables on heterogeneity by using the R metafor package (version 1.9-4; Maastricht University) (30). Meta-regression moderators considered included geographic location (United States compared with northern Europe compared with Mediterranean),

TABLE 1
General characteristics of participants by cohort[1]

Characteristics	Corogene Controls	CHS	FOS	HBCS	InCHIANTI	Inter99	MESA	THISEAS	YFS
n	571	1322	898	1184	912	6514	1670	420	1415
Age, y	53.4 ± 13.5[2]	70.8 ± 3.8	57.7 ± 9.1	69.0 ± 2.9	64.4 ± 14.2	46.2 ± 7.9	65.1 ± 8.8	58.4 ± 12.4	37.8 ± 5.1
Female, %	50.1	62.5	53.3	60.6	53.5	51.4	51.6	39.0	53.6
BMI, kg/m²	26.7 ± 4.5	26.6 ± 4.2	28.1 ± 5.2	27.5 ± 4.6	27.3 ± 4.2	26.3 ± 4.6	27.8 ± 5.1	28.3 ± 4.5	26.6 ± 4.4
Waist circumference, cm	90.2 ± 13.1	93.3 ± 12.5	97.5 ± 13.6	94.4 ± 13.0	91.5 ± 11.2	86.7 ± 13.4	98.2 ± 14.5	96.5 ± 13.4	87.8 ± 13.0
Sleep duration,[3] h	7.2 ± 1	7.3 ± 1.3	7.1 ± 1.1	7.4 ± 1.1	6.8 ± 1.4	7.3 ± 0.9	7.1 ± 1.1	7.1 ± 1.5	7.4 ± 0.8
Dietary intake									
Total energy, kcal/d	2314 ± 793	2007 ± 610	1854 ± 610	2212 ± 807	2066 ± 603	2330 ± 870	1612 ± 714	2172 ± 1057	2396 ± 772
Total fat, % of total energy	31.0 ± 4.9	32.0 ± 6.1	29.5 ± 6.5	33.4 ± 5.3	31.1 ± 5.2	32.7 ± 7.1	32.1 ± 7.3	35.7 ± 6.0	32.9 ± 4.9
PUFA, % of total energy	5.5 ± 1.1	7.5 ± 2.2	5.7 ± 1.6	5.3 ± 1.3	3.4 ± 0.7	5.3 ± 1.5	6.3 ± 1.8	5.2 ± 1.5	5.3 ± 1.1
MUFA, % of total energy	11.1 ± 2.2	11.5 ± 2.4	11.0 ± 2.6	11.1 ± 2.1	15.7 ± 3.1	10.5 ± 2.7	12.3 ± 3.0	15.7 ± 3.4	11.1 ± 2.0
SFA, % of total energy	11.2 ± 2.4	10.1 ± 2.2	10.1 ± 2.8	12.1 ± 2.6	10.4 ± 2.2	12.3 ± 3.5	10.8 ± 3.4	11.9 ± 3.3	11.8 ± 2.3
Carbohydrate, % of total energy	48.3 ± 6.3	52.1 ± 8.0	51.3 ± 8.3	47.0 ± 6.5	51.3 ± 6.7	47.5 ± 7.9	50.6 ± 9.1	45.0 ± 8.6	44.8 ± 5.8
Protein, % of total energy	17.6 ± 2.6	19.5 ± 3.0	17.2 ± 3.2	17.1 ± 2.5	15.7 ± 2.2	15.1 ± 2.6	15.7 ± 3.4	18.3 ± 3.6	17.5 ± 2.4
Caffeine, mg/d	NA	165.1 ± 177	258.7 ± 207	NA	170.0 ± 143	NA	265.2 ± 232	88.1 ± 53.0	473.9 ± 261

[1] Cohort study countries are as follows: Corogene Controls, Finland; CHS, United States; FOS, United States; HBCS, Finland; InCHIANTI, Italy; Inter99, Denmark; MESA, United States; THISEAS, Greece; YFS, Finland. CHS, Cardiovascular Health Study; FOS, Framingham Offspring Study; HBCS, Helsinki Birth Cohort Study; InCHIANTI, Invecchiare in Chianti (Aging in the Chianti Area); MESA, Multi-Ethnic Study of Atherosclerosis; NA, not available; SNP, single nucleotide polymorphism; THISEAS, The Hellenic Study of Interactions between SNPs and Eating in Atherosclerosis Susceptibility; YFS, Cardiovascular Disease Risk in Young Finns Study.

[2] Mean ± SD (all such values).

[3] Sleep duration was defined as weekday/workday self-reported sleep duration as usual hours of sleep per night.

mean age of cohort (20–64 compared with 65–80 y), and total energy intake (<2000 compared with ≥2000 kcal/d). In addition, we conducted sensitivity analyses to assess the influence on the meta-analyzed estimate of any single cohort study by repeating analyses with the removal of one cohort study at a time in both association and interaction analyses. Statistical significance was defined at $\alpha = 0.002$ on the basis of Bonferroni correction for 27 total independent interaction tests (3 independent macronutrients × 9 independent tSNPs).

RESULTS

General characteristics of participants are shown in **Table 1**. Average habitual sleep duration was consistent in the 9 cohorts and remained similar when stratified by sex and age group [20–64 y (younger) compared with 65–80 y (older)] (**Supplemental Figure 1**). In older adults, however, we observed that women had lower sleep duration than that of men ($P < 0.0001$). Mean dietary intakes were not significantly different across studies and differed only for MUFA intake; the Mediterranean cohorts (InCHIANTI and THISEAS) had higher mean MUFA intakes than northern European and U.S. cohorts ($P < 0.0001$).

Associations of sleep duration with BMI and macronutrient intake

After adjustment for age, sex, and study site, we identified a significant association between sleep duration and BMI (**Table 2**, **Supplemental Table 6**). Each additional hour of sleep was associated with 0.16 lower BMI ($\beta \pm SE = -0.16 \pm 0.04$, $P < 0.0001$). In sex-stratified analyses, the magnitude of this association was approximately twice as great in men as women and was significant in men only with slight differences by age group; each additional hour of sleep was associated with 0.23 ± 0.07 lower BMI ($P < 0.001$) and 0.19 ± 0.07 lower BMI ($P < 0.01$) in younger and older men, respectively.

There were no significant associations between sleep duration and relative macronutrient intake adjusted for age, sex, BMI, and study site in the overall sample. However, associations between sleep duration and intake were evident in age- and sex-stratified analyses (Table 2). We observed a significant association between sleep duration and SFA intake in younger adults whereby, per each additional hour of sleep, SFA intake was $0.11 \pm 0.06\%$ ($P = 0.03$) and $0.10 \pm 0.05\%$ ($P = 0.04$) lower in younger men and women, respectively. In addition, in older women, each additional hour of sleep was associated with $-0.31 \pm 0.12\%$ ($P < 0.01$), $0.18 \pm 0.09\%$ ($P = 0.05$), and $0.05 \pm 0.02\%$ ($P = 0.02$) differences in percentages of energy from carbohydrate, total fat, and PUFAs, respectively. Results from both meta-regressions and sensitivity analyses did not substantively affect these results or reveal any clear sources of heterogeneity between sleep duration and BMI and dietary intake (results not shown).

Associations of *CLOCK* variants with macronutrient intake

Meta-analyzed estimates of SNP associations with macronutrient intake are presented in **Table 3**. No associations met the prespecified Bonferroni-corrected significance level of $P < 0.002$. A nominally significant (i.e., $P < 0.05$) association with the percentage of energy from MUFAs was observed at rs10462028 ($\beta \pm SE = -0.07 \pm 0.03\%$ per additional C allele,

TABLE 2
Meta-analyzed associations between sleep duration, BMI, and macronutrient intake and stratified by age and sex[1]

Participants	N[2]	BMI, kg/m² β ± SE	P	PUFA, % of total energy β ± SE	P	MUFA, % of total energy β ± SE	P	SFA, % of total energy β ± SE	P	Total fat, % of total energy β ± SE	P	Total carbohydrate, % of total energy β ± SE	P	Total protein, % of total energy β ± SE	P
All	14,906	−0.16 ± 0.04	2.06 ×10⁻⁵	0.01 ± 0.01	0.37*	0.01 ± 0.02	0.54*	−0.02 ± 0.02	0.40*	0.02 ± 0.05	0.99*	−0.07 ± 0.06	0.23*	0.001 ± 0.02	0.96
M, aged 20–64 y	5022	−0.23 ± 0.07	0.0007	−0.01 ± 0.02	0.64	−0.05 ± 0.04	0.30	−0.11 ± 0.05	0.03	−0.17 ± 0.11	0.11	0.08 ± 0.12	0.52	−0.04 ± 0.05	0.32
F, aged 20–64 y	5297	−0.10 ± 0.08	0.26*	0.01 ± 0.02	0.83*	0.01 ± 0.04	0.88*	−0.10 ± 0.05	0.04	−0.09 ± 0.10	0.38	0.04 ± 0.12	0.76	−0.03 ± 0.05	0.54
M, aged 65–80 y	1958	−0.19 ± 0.07	0.005	−0.02 ± 0.02	0.42	0.03 ± 0.05	0.60*	0.03 ± 0.05	0.47	0.02 ± 0.10	0.85*	−0.13 ± 0.14	0.36	0.01 ± 0.05	0.80
F, aged 65–80 y	2629	−0.11 ± 0.08	0.16	0.05 ± 0.02	0.02	0.05 ± 0.04	0.15*	0.05 ± 0.04	0.20*	0.18 ± 0.09	0.05*	−0.31 ± 0.12	0.007	0.07 ± 0.04	0.09

[1]Adjusted for age, sex, BMI (except for BMI outcome), and study site (in the CHS, InCHIANTI, and MESA). Sleep duration defined as weekday/workday self-reported sleep duration as usual hours of sleep per night. Association coefficients are shown as βs ± SEs. β represents the change in BMI (in kg/m²) or macronutrient intake (percentage of total energy) per each additional hour of sleep. Heterogeneity statistics (I²) are presented in Supplemental Table 6. *I² > 30%. CHS, Cardiovascular Health Study; InCHIANTI, Invecchiare in Chianti (Aging in the Chianti Area); MESA, Multi-Ethnic Study of Atherosclerosis.
[2]Number of independent observations in each association analysis.

TABLE 3
Meta-analyzed associations between SNPs and macronutrient intake[1]

SNP	Alleles, minor/major	Position	Location	Minor allele frequency	N[2]	PUFA, % of total energy		MUFA, % of total energy		SFA, % of total energy		Total fat, % of total energy		Total carbohydrate, % of total energy		Total protein, % of total energy	
						$\beta \pm SE$	P	$\beta \pm SE$	P	$\beta \pm SE$	P	$\beta \pm SE$	P	$\beta \pm SE$	P	$\beta \pm SE$	P
rs504836	C/T	55980062	Intronic[3]	0.26	7417	−0.002 ± 0.03	0.96	0.01 ± 0.06	0.92*	0.01 ± 0.05	0.93	0.06 ± 0.13	0.63	0.17 ± 0.15	0.25*	0.13 ± 0.06	0.04*
rs6858749	T/C	55985194	Intronic[3]	0.41	8316	0.02 ± 0.02	0.43	0.02 ± 0.04	0.63	0.003 ± 0.04	0.95	0.09 ± 0.095	0.33	0.05 ± 0.11	0.62*	0.06 ± 0.05	0.18*
rs1047354	G/A	55990340	Intergenic	0.34	14,274	−0.03 ± 0.02	0.15	−0.003 ± 0.03	0.92	0.01 ± 0.04	0.84	−0.03 ± 0.08	0.71	0.08 ± 0.09	0.38	0.12 ± 0.03	0.0002*,[4]
rs10462028	G/A	55993057	Downstream	0.34	14,305	−0.02 ± 0.02	0.25*,[5]	−0.07 ± 0.03	0.05	−0.02 ± 0.04	0.64	−0.12 ± 0.08	0.13	0.09 ± 0.09	0.34	0.02 ± 0.04	0.69
rs1801260	C/T	55996126	3'UTR	0.31	8316	0.02 ± 0.02	0.34	0.04 ± 0.04	0.33	0.03 ± 0.04	0.54	0.10 ± 0.11	0.33	−0.09 ± 0.11	0.41	0.09 ± 0.05	0.85
rs3792603	G/A	55996815	Intronic	0.18	8313	0.002 ± 0.03	0.93	−0.02 ± 0.06	0.78	0.01 ± 0.06	0.86	0.02 ± 0.12	0.88	−0.10 ± 0.14	0.47	−0.10 ± 0.06	0.09
rs1932595	C/T	56018354	Intronic	0.36	7007	0.01 ± 0.02	0.79*	0.003 ± 0.05	0.95	−0.01 ± 0.05	0.90	0.08 ± 0.11	0.50	−0.17 ± 0.14	0.22	−0.04 ± 0.05	0.51
rs12649507	A/G	56075241	Intronic	0.17	7748	−0.03 ± 0.02	0.16	−0.02 ± 0.05	0.66	0.02 ± 0.05	0.71	−0.03 ± 0.11	0.80	−0.04 ± 0.12	0.74	−0.03 ± 0.05	0.62*
rs11726609	A/T	56095626	Intronic	0.38	14,215	−0.001 ± 0.02	0.97*	−0.06 ± 0.03	0.09	−0.03 ± 0.04	0.49	−0.11 ± 0.08	0.19	0.06 ± 0.09	0.54	0.03 ± 0.04	0.47

[1] Additive allele model was adjusted for age, sex, BMI, study site (in the CHS, InCHIANTI, and MESA) and family or population structure (in the Corgene Controls, FOS, and MESA). Association coefficients are shown as βs ± SEs. β represents the change in macronutrient intake (percentage of total energy) per each additional minor allele. Prespecified Bonferroni-corrected significance level of $P < 0.002$. Heterogeneity statistics (I^2) are presented in Supplemental Table 8. *$I^2 > 30\%$. CHS, Cardiovascular Health Study; FOS, Framingham Offspring Study; HBCS, Helsinki Birth Cohort study; InCHIANTI, Invecchiare in Chianti (Aging in the Chianti Area); MESA, Multi-Ethnic Study of Atherosclerosis; SNP, single nucleotide polymorphism; 3' UTR, 3'-untranslated region.
[2] Number of independent observations in each association analysis. Exact numbers of observations varied for each outcome and are presented in Supplemental Table 8.
[3] Transmembrane protein 165, TMEM165.
[4] Sensitivity analysis (removal of the HBCS); $\beta \pm SE = 0.03 \pm 0.03\%$, $P = 0.46$, $I^2 = 52\%$.
[5] Sensitivity analysis (removal of the InCHIANTI); $\beta \pm SE = -0.05 \pm 0.02\%$, $P = 0.03$, $I^2 = 23\%$.

$P = 0.05$), and percentage of energy from protein at rs504836 ($\beta \pm SE = 0.13 \pm 0.06\%$ per additional C allele, $P = 0.04$). Although overall meta-analyzed estimates of SNP associations show little evidence of heterogeneity (**Supplemental Table 7**), results of the meta-regression revealed that geographic location ($P < 0.05$) might be a source of heterogeneity for several of the associations between tSNPs and PUFAs; these subgroup meta-analyzed estimates by geographic location (United States compared with northern Europe compared with Mediterranean) are presented in **Supplemental Table 8**. Sensitivity analyses showed that the association for rs1047354 with protein ($I^2 = 96\%$) was driven by one cohort (HBCS); the removal of the cohort from the meta-analysis resulted in a weaker and nonsignificant association ($\beta \pm SE = 0.03 \pm 0.03\%$ per additional G allele, $P = 0.46$, $I^2 = 52\%$). Sensitivity analyses also altered the association of rs10462028 with PUFA such that the association became nominally significant when the outlying cohort (InCHIANTI) was excluded ($\beta \pm SE = -0.05 \pm 0.02\%$ per additional C allele, $P = 0.03$, $I^2 = 23\%$).

Sleep duration × *CLOCK* variants on macronutrient intake

Meta-analyzed estimates of interactions between sleep duration and tSNPs on macronutrient intake are presented in **Table 4**. There were no significant interactions evident on intake after correction for multiple testing (i.e., at corrected $P < 0.002$). The following 2 nominally significant interactions were observed: *1*) between sleep duration and rs12649507 for PUFA ($\beta \pm SE = 0.05 \pm 0.02\%$, $P = 0.01$), which suggested higher PUFA intake with each additional hour of sleep in the presence of the minor G allele, and *2*) between sleep duration and rs6858749 for energy from protein ($\beta \pm SE = -0.08 \pm 0.04\%$, $P = 0.04$), which suggested lower protein intake with each additional hour of sleep in the presence of the minor T allele. Meta-analyzed estimates of interactions showed little evidence of heterogeneity (**Supplemental Table 9**), and results of meta-regressions and the sensitivity analyses did not substantively affect our results or reveal any clear sources of heterogeneity (results not shown).

DISCUSSION

In meta-analyses of 9 cohorts, we showed that sleep duration was associated with BMI in the overall sample and relative macronutrient intake in specific age and sex groups. We also identified several nominal associations between *CLOCK* variants and intake, some of which appeared to be region specific. Finally, we observed that *CLOCK* variants could modify the associations between sleep duration and dietary intake; however, these results were only nominally significant.

Our large-scale, multinational assessment of habitual sleep duration and BMI was consistent with previous literature whereby adults who reported habitually longer sleep durations had significantly lower BMI than that of those with habitually shorter sleep durations (31). No associations were evident between sleep duration and relative macronutrient intake in the overall sample; however, stratified exploratory analyses indicated that associations between sleep duration and intake tended to be age- and sex-specific; longer sleep duration was associated with lower SFA intake in younger adults and higher total fat intake, primarily driven by higher PUFA intake, as well as lower

TABLE 4
Meta-analyzed interactions between sleep duration and SNPs on macronutrient intake[1]

SNP	Alleles, minor/major	N[2]	PUFA, % of total energy β ± SE	P	MUFA, % of total energy β ± SE	P	SFA, % of total energy β ± SE	P	Total fat, % of total energy β ± SE	P	Total carbohydrate, % of total energy β ± SE	P	Total protein, % of total energy β ± SE	P
rs504836	C/T	7417	−0.02 ± 0.02	0.35	−0.07 ± 0.05	0.14	0.03 ± 0.05	0.58	−0.80 ± 0.11	0.47	0.12 ± 0.12	0.32	−0.04 ± 0.05	0.44
rs6858749	T/C	8316	−0.03 ± 0.02	0.12	−0.06 ± 0.08	0.08	0.02 ± 0.03	0.50	−0.09 ± 0.08	0.27	0.08 ± 0.09	0.37	−0.08 ± 0.04	0.04
rs1047354	G/A	14,274	0.03 ± 0.01	0.06	0.02 ± 0.03	0.55	−0.04 ± 0.03	0.24	0.04 ± 0.07	0.63	−0.11 ± 0.08	0.17	0.02 ± 0.03	0.59
rs10462028	G/A	14,305	0.01 ± 0.02	0.41	0.02 ± 0.03	0.54	−0.03 ± 0.04	0.41	0.03 ± 0.08	0.75	0.04 ± 0.09	0.69	−0.04 ± 0.04	0.21
rs1801260	C/T	8316	−0.01 ± 0.02	0.52	−0.04 ± 0.04	0.37	0.02 ± 0.04	0.60	−0.05 ± 0.10	0.64	0.05 ± 0.10	0.65	−0.03 ± 0.05	0.46
rs3792603	G/A	8313	−0.002 ± 0.02	0.89	0.04 ± 0.05	0.45	0.03 ± 0.05	0.60	0.09 ± 0.10	0.37	−0.14 ± 0.11	0.22	−0.05 ± 0.05	0.32
rs11932595	C/T	7007	−0.0002 ± 0.02	0.99	−0.02 ± 0.04	0.72	−0.002 ± 0.04	0.96*	−0.02 ± 0.09	0.86*	0.06 ± 0.11	0.61	−0.04 ± 0.04	0.35
rs12649507	A/G	7748	0.05 ± 0.02	0.01	0.07 ± 0.04	0.07	−0.03 ± 0.04	0.40	0.14 ± 0.09	0.12	−0.07 ± 0.09	0.45	−0.01 ± 0.04	0.88*
rs1726609	A/T	14,215	−0.03 ± 0.02	0.05*	−0.02 ± 0.02	0.53	0.02 ± 0.03	0.54*	−0.05 ± 0.08	0.53	0.11 ± 0.08	0.19	−0.03 ± 0.03	0.45

[1] Additive allele model was adjusted for age, sex, BMI, study site (in the CHS, InCHIANTI, and MESA) and family or population structure (in the Corogene Controls, FOS, and MESA). Interaction coefficients are shown as βs ± SEs. β represents the direction and magnitude of the change in macronutrient intake (percentage of total energy) with each additional hour of sleep per each additional minor allele. Prespecified Bonferroni-corrected significance level of $P < 0.002$. Heterogeneity statistics (I^2) are presented in Supplemental Table 9. *$I^2 > 30\%$. CHS, Cardiovascular Health Study; FOS, Framingham Offspring Study; InCHIANTI, Invecchiare in Chianti (Aging in the Chianti Area); MESA, Multi-Ethnic Study of Atherosclerosis; SNP, single nucleotide polymorphism.
[2] Number of independent observations in each interaction analysis. Exact numbers of observations varied for each outcome and are presented in Supplemental Table 9.

carbohydrate intake in older women. The mechanisms that underlie these sex-specific associations were unclear but could have included sex-specific hormonal differences, differences in self-reporting behaviors (32), or differences in sleep duration between older adult men and women identified in the current study. Sex-specific associations of short sleep duration were also shown for other outcomes such as hypertension (4).

The *2010 Dietary Guidelines for Americans* emphasized the cardioprotective benefits of consuming diets lower in SFAs by replacing them with MUFAs and PUFAs (33). On the basis of our observation of associations between habitual sleep duration and dietary fat intake in younger adults and older females, our findings suggest that longer sleep duration may facilitate compliance to the current recommendations for healthier eating behaviors. Together with previously reported differences in total energy intake with sleep duration (9), our results may support the hypothesis that metabolic differences associated with sleep duration can be mediated in part by differences in dietary intake, specifically differences in SFA, PUFA, and carbohydrate intakes.

For associations between *CLOCK* variants and macronutrient intake, results from both sensitivity and meta-regression analyses indicated various sources of heterogeneity. Meta-regression results indicated that geographic location could have influenced these associations, particularly between *CLOCK* and PUFA. When stratified by geographic location, meta-analyses results showed nominally significant SNP-PUFA associations. Although region-specific observations could result from mechanistically plausible effects of geographic variation on the entrainment of circadian rhythms (34), whether the SNP-diet associations are truly region specific or confounded by other factors such as differences in dietary assessment tools and dietary patterns across the meta-analyzed cohorts could not be elucidated in our analysis. Likewise, by the sequential removal of single cohorts from the meta-analyses, sensitivity analyses suggested the strong influence of single cohorts on some of these associations. Single cohort–driven results are possibly attributable to differences in intakes across cohorts as evident for the association between rs10462028 and PUFAs for which heterogeneity was substantively reduced after the removal of InCHIANTI, which was a Mediterranean cohort with the lowest PUFA intake. Overall, we report no associations between *CLOCK* variants and macronutrient composition; however, our results suggest that differences in intakes across geographic regions should be accounted for in future studies of the genetic component of dietary intake.

A potential mechanistic overlap between effects of *CLOCK* and sleep duration on dietary intake led us to investigate potential gene × sleep duration interactions. Our interaction analyses failed to identify interactions that met prespecified criteria for statistical significance. However, they did identify potentially interesting and nominally significant interactions. One of these interactions was between sleep duration and rs12649507, which is an intronic variant, that indicated that, in individuals with the minor A allele, longer sleep duration was associated with a more favorable dietary profile through greater increases in PUFA intake. The minor allele was associated with shorter sleep duration in an earlier meta-analysis (35). In addition, this variant is in linkage disequilibrium with rs6843722 ($r^2 = 1.00$ in Caucasian European Utah by using the HapMap II dataset), which is a variant previously associated with obesity

(36). Taken together, the interaction suggests that longer sleep duration could result in an increase in PUFA consumption in individuals at risk for obesity.

Our meta-analysis of data from 9 cohort studies had several strengths. To our knowledge, this was the largest observational study to investigate sleep duration with both BMI and dietary composition and the largest meta-analysis to investigate associations of *CLOCK*, as well as its interactions with sleep duration, with dietary intake. With the investigation of habitual lifestyle, including sleep and diet, our findings are potentially relevant to chronic diseases (14). Our standardized analytic plan and uniform analysis across all participating cohorts reduced errors and biases often associated with meta-analyses, including selection and publication biases. Although the associations we observed between sleep duration and BMI and dietary behavior were small in terms of the size, they were consistent in direction with those reported in single cohorts that investigated similar relations, but more importantly, they are relevant to elucidating the potential underlying biology of very complex human dietary and sleep behaviors. The small size of these associations could have also resulted from a potential U-shaped relation between sleep duration and dietary intake, which was not investigated in the current analysis. Finally, we observed, overall, little evidence of heterogeneity in our analyses despite the wide range of cohorts investigated.

The implications of our current study were limited to individuals of European descent, and additional analysis in other ethnic groups is warranted to generalize these findings. Although our analysis examined associations and interactions of a single circadian gene on dietary intake because of well-established biological pathways linking *CLOCK* to dietary intake, it is likely there are potentially important interactions elsewhere in the genome. Our use of self-reported habitual sleep duration and dietary intake was susceptible to reporting bias, and objective measures of sleep duration may be required for future studies (37). Likewise, the use of different assessment tools across cohorts could affect these findings. Although our investigation focused on nighttime sleep duration, which is a commonly surveyed sleep variable in large observational studies, the assessment of other dimensions of sleep, such as sleep quality, in addition to napping, which may have important effects on dietary intake, should also be considered (38–40). The current cross-sectional meta-analyses of observational studies could not inform us about the temporal relation or the causal pathway linking *CLOCK*, sleep, and diet. Therefore, whether sleep duration directly influenced dietary intake or indirectly moderated macronutrient effects on BMI, potentially through changes in substrate utilization, cannot be inferred from this investigation, and other studies are necessary to establish these mechanistic links.

In conclusion, given the declining trends in habitual sleep duration and rising trends in metabolic abnormalities, our findings may have public health implications by providing recommendations to individuals at risk for chronic diseases. Our results indicate that an increase in habitual sleep duration is associated with desirably lower BMI and age- and sex-specific favorable dietary behaviors. Furthermore, differences in the relative intake of specific macronutrients associated with short sleep duration could provide a partial basis for previously reported associations between short sleep duration and chronic metabolic abnormalities. In addition, the nominal evidence for the influence of an obesity-associated *CLOCK* variant on the association between sleep duration and macronutrient intake suggests that an increase in habitual sleep duration could ameliorate a genetic predisposition to obesity via a more favorable dietary profile. The identified interaction provides preliminary findings for personalized sleep recommendations for individuals at increased genetic risk for obesity aimed at attenuating this risk, and additional exploration of these interactions in age- and sex-specific groups is warranted.

REFERENCES

1. Buman MP, Phillips BA, Youngstedt SD, Kline CE, Hirshkowitz M. Does nighttime exercise really disturb sleep? Results from the 2013 National Sleep Foundation Sleep in America Poll. Sleep Med 2014;15: 755–61.
2. Institute of Medicine (US) Committee on Sleep Medicine and Research, Colten HR, Altevogt BM. Sleep disorders and sleep deprivation: an unmet public health problem. Washington, DC: National Academies Press; 2006.
3. Cappuccio FP, Taggart FM, Kandala N-B, Currie A, Peile E, Stranges S, Miller MA. Meta-analysis of short sleep duration and obesity in children and adults. Sleep 2008;31:619–26.
4. Stranges S, Dorn JM, Cappuccio FP, Donahue RP, Rafalson LB, Hovey KM, Freudenheim JL, Kandala NB, Miller MA, Trevisan M. A population-based study of reduced sleep duration and hypertension: the strongest association may be in premenopausal women. J Hypertens 2010;28: 896–902.
5. Ayas NT, White DP, Al-Delaimy WK, Manson JE, Stampfer MJ, Speizer FE, Patel S, Hu FB. A prospective study of self-reported sleep duration and incident diabetes in women. Diabetes Care 2003;26:380–4.
6. Cappuccio FP, Cooper D, D'Elia L, Strazzullo P, Miller MA. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. Eur Heart J 2011;32:1484–92.
7. Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Sleep duration and all-cause mortality: a systematic review and meta-analysis of prospective studies. Sleep 2010;33:585–92.
8. Spiegel K, Tasali E, Penev P, Van Cauter E. Brief communication: sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. Ann Intern Med 2004;141:846–50.
9. Markwald RR, Melanson EL, Smith MR, Higgins J, Perreault L, Eckel RH, Wright KP Jr. Impact of insufficient sleep on total daily energy expenditure, food intake, and weight gain. Proc Natl Acad Sci USA 2013;110:5695–700.
10. St-Onge M-P, Roberts AL, Chen J, Kelleman M, O'Keeffe M, RoyChoudhury A, Jones PJ. Short sleep duration increases energy intakes but does not change energy expenditure in normal-weight individuals. Am J Clin Nutr 2011;94:410–6.
11. Spaeth AM, Dinges DF, Goel N. Sex and race differences in caloric intake during sleep restriction in healthy adults. Am J Clin Nutr 2014; 100:559–66.
12. Nedeltcheva AV, Kilkus JM, Imperial J, Kasza K, Schoeller DA, Penev PD. Sleep curtailment is accompanied by increased intake of calories from snacks. Am J Clin Nutr 2009;89:126–33.
13. Taheri S, Lin L, Austin D, Young T, Mignot E. Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. PLoS Med 2004;1:e62.
14. Patterson RE, Emond JA, Natarajan L, Wesseling-Perry K, Kolonel LN, Jardack P, Ancoli-Israel S, Arab L. Short sleep duration is associated with higher energy intake and expenditure among African-American and Non-Hispanic white adults. J Nutr 2014;144:461–6.

15. Scheer FAJL, Morris CJ, Shea SA. The internal circadian clock increases hunger and appetite in the evening independent of food intake and other behaviors. Obesity (Silver Spring) 2013;21:421–3.

16. Garaulet M, Sánchez-Moreno C, Smith CE, Lee Y-C, Nicolás F, Ordovas JM. Ghrelin, sleep reduction and evening preference: relationships to CLOCK 3111 T/C SNP and weight loss. PLoS ONE 2011;6:e17435.

17. Garaulet M, Lee Y-C, Shen J, Parnell LD, Arnett DK, Tsai MY, Lai CQ, Ordovas JM. Genetic variants in human CLOCK associate with total energy intake and cytokine sleep factors in overweight subjects (GOLDN population). Eur J Hum Genet 2010;18:364–9.

18. Scott EM, Carter AM, Grant PJ. Association between polymorphisms in the Clock gene, obesity and the metabolic syndrome in man. Int J Obes (Lond) 2008;32:658–62.

19. Paalanen L, Männistö S, Virtanen MJ, Knekt P, Räsänen L, Montonen J, Pietinen P. Validity of a food frequency questionnaire varied by age and body mass index. J Clin Epidemiol 2006;59:994–1001.

20. Toft U, Kristoffersen L, Ladelund S, Bysted A, Jakobsen J, Lau C, Jørgensen T, Borch-Johnsen K, Ovesen L. Relative validity of a food frequency questionnaire used in the Inter99 study. Eur J Clin Nutr 2008;62:1038–46.

21. Posner BM, Martin-Munley SS, Smigelski C, Cupples LA, Cobb JL, Schaefer E, Miller DR, D'Agostino RB. Comparison of techniques for estimating nutrient intake: the Framingham Study. Epidemiology 1992; 3:171–7.

22. Nettleton JA, Rock CL, Wang Y, Jenny NS, Jacobs DR. Associations between dietary macronutrient intake and plasma lipids demonstrate criterion performance of the Multi-Ethnic Study of Atherosclerosis (MESA) food-frequency questionnaire. Br J Nutr 2009;102:1220–7.

23. Bartali B, Turrini A, Salvini S, Lauretani F, Russo CR, Corsi AM, Miller DR, D'Agostino RB. Dietary intake estimated using different methods in two Italian older populations. Arch Gerontol Geriatr 2004; 38:51–60.

24. Kumanyika S, Tell GS, Fried L, Martel JK, Chinchilli VM. Picture-sort method for administering a food frequency questionnaire to older adults. J Am Diet Assoc 1996;96:137–44.

25. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–5.

26. Storfer-Isser A, Patel SR, Babineau DC, Redline S. Relation between sleep duration and BMI varies by age and sex in youth age 8-19. Pediatr Obes. 2012;7:53–64.

27. Tatone-Tokuda F, Dubois L, Ramsay T, Girard M, Touchette E, Petit D, Montplaisir JY. Sex differences in the association between sleep duration, diet and body mass index: a birth cohort study. J Sleep Res 2012;21:448–60.

28. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 2010;26:2190–1.

29. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.

30. Viechtbauer W. Conducting meta-analyses in R with the metafor package. J Stat Softw 2010;36:1–48.

31. Watson NF, Buchwald D, Vitiello MV, Noonan C, Goldberg J. A twin study of sleep duration and body mass index. J Clin Sleep Med 2010;6: 11–7.

32. Baldwin CM, Kapur VK, Holberg CJ, Rosen C, Nieto FJ, Sleep Heart Health Study Group. Associations between gender and measures of daytime somnolence in the Sleep Heart Health Study. Sleep 2004;27: 305–11.

33. Flock MR, Kris-Etherton PM. Dietary Guidelines for Americans 2010: implications for cardiovascular disease. Curr Atheroscler Rep 2011;13: 499–507.

34. Roenneberg T, Kumar CJ, Merrow M. The human circadian clock entrains to sun time. Curr Biol 2007;17:R44–5.

35. Allebrandt KV, Teder-Laving M, Akyol M, Pichler I, Müller-Myhsok B, Pramstaller P, Merrow M, Meitinger T, Metspalu A, Roenneberg T. CLOCK gene variants associate with sleep duration in two independent populations. Biol Psychiatry 2010;67:1040–7.

36. Sookoian S, Gemma C, Gianotti T-F, Burgueño A, Castaño G, Pirola C-J. Genetic variants of Clock transcription factor are associated with individual susceptibility to obesity. Am J Clin Nutr 2008;87: 1606–15.

37. Zhang L, Zhao Z-X. Objective and subjective measures for sleep disorders. Neurosci Bull 2007;23:236–40.

38. Jennings JR, Muldoon MF, Hall M, Buysse DJ, Manuck SB. Self-reported sleep quality is associated with the metabolic syndrome. Sleep 2007;30:219–23.

39. Tasali E, Leproult R, Ehrmann DA, Van Cauter E. Slow-wave sleep and the risk of type 2 diabetes in humans. Proc Natl Acad Sci USA 2008; 105:1044–9.

40. Grandner MA, Kripke DF, Naidoo N, Langer RD. Relationships among dietary nutrients and subjective sleep, objective sleep, and napping in women. Sleep Med 2010;11:180–4.