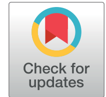

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Interplay of Dinner Timing and *MTNR1B* Type 2 Diabetes Risk Variant on Glucose Tolerance and Insulin Secretion: A Randomized Crossover Trial

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OBJECTIVE

We tested whether the concurrence of food intake and elevated concentrations of endogenous melatonin, as occurs with late eating, results in impaired glucose control, in particular in carriers of the type 2 diabetes–associated G allele in the melatonin receptor-1B gene (*MTNR1B*).

RESEARCH DESIGN AND METHODS

In a Spanish natural late-eating population, a randomized, crossover study was performed. Each participant (*n* = 845) underwent two evening 2-h 75-g oral glucose tolerance tests following an 8-h fast: an early condition scheduled 4 h prior to habitual bedtime ("early dinner timing") and a late condition scheduled 1 h prior to habitual bedtime ("late dinner timing"), simulating an early and a late dinner timing, respectively. Differences in postprandial glucose and insulin responses between early and late dinner timing were determined using incremental area under the curve (AUC) calculated by the trapezoidal method.

RESULTS

Melatonin serum levels were 3.5-fold higher in the late versus early condition, with late dinner timing resulting in 6.7% lower insulin AUC and 8.3% higher glucose AUC. The effect of late eating impairing glucose tolerance was stronger in the *MTNR1B* G-allele carriers than in noncarriers. Genotype differences in glucose tolerance were attributed to reductions in β-cell function (*P* for interaction, P_{int} glucose area under the curve = 0.009, P_{int} corrected insulin response = 0.022, and P_{int} disposition index = 0.018).

CONCLUSIONS

Concurrently high endogenous melatonin and carbohydrate intake, as typical for late eating, impairs glucose tolerance, especially in *MTNR1B* G-risk allele carriers, attributable to insulin secretion defects.

The timing of food intake is a synchronizer of the peripheral circadian clocks (1–3). Behaviors misaligned with the central clock may contribute to chronodisruption, as observed in night shift workers and, to a lesser extent, in natural late-night eaters (4). Consequently, eating at times that likely conflict with normal physiology, such

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as late at night or very early morning, is associated with increased risk for type 2 diabetes (T2D) and other cardiometabolic diseases (5,6).

Variation in meal timing appears to influence glucose metabolism (7). Studies performed in highly controlled laboratory conditions have found that eating during the biological night causes metabolic changes, including increased postprandial glucose (3,8,9). The postprandial responses of some healthy individuals during the biological night are equivalent to the responses of individuals with prediabetes (9). So far, no large population-based studies in natural physiological conditions have been conducted to show changes in glucose metabolism during the biological evening.

We and others have proposed that high endogenous levels of melatonin contribute to the impairment of glucose tolerance during the biological night (10). The discovery of melatonin receptor-1B gene (*MTNR1B*) as a T2D-associated gene suggests that beyond sleep and circadian regulation, melatonin plays a key role in glucose metabolism. The *MTNR1B* risk variant rs10830963 has a strong association with fasting glucose and confers increased expression of the melatonin receptor in human pancreatic islets (11). This melatonin receptor is a G-protein–coupled receptor that mainly acts via interfering with the formation of cAMP through inhibitory G proteins, inhibiting adenylate cyclase and subsequently inhibiting insulin release (11). Our pilot data investigating dinner timing in 40 habitual late eaters (those who self-reported dinner within 2.5 h of their bedtime) demonstrated that the concurrence of food intake with elevated endogenous melatonin concentrations can result in impaired glucose tolerance, particularly among carriers of the G risk allele at this locus (10); however, the physiological mechanism underlying these findings was not investigated.

There exist conflicting data regarding the effects of melatonin and *MTNR1B* genotype on glucose control (12–14) and disagreement on whether melatonin increases or decreases fasting glucose, glucose tolerance, and T2D risk. While some studies show that elevated melatonin concentrations are associated with improved glucose control (15–17), others show associations with impaired glucose control (18–20). Furthermore, most studies have suggested an inhibitory effect of melatonin on insulin secretion (21,22), while some studies performed in isolated human pancreatic islets have shown stimulatory effects (15,17,23).

The seemingly contradictory effects of melatonin on glucose metabolism may appear difficult to reconcile, but they are consistent with the "timing model" (14), in which we hypothesized that the concurrence of elevated melatonin concentrations and food intake adversely influences glucose tolerance in humans. This concurrence can be due to eating at night or elevated melatonin levels during the day. Elevated melatonin concentrations in both states may suppress insulin release and/or decrease insulin sensitivity, resulting in impaired glucose tolerance.

To our knowledge, no large-scale studies have examined the impact of concurrent high melatonin levels, food intake, and *MTNR1B* risk variant on glucose tolerance. Furthermore, no study has shown whether the decrease in glucose tolerance at night is due to a decrease of insulin secretion, insulin sensitivity, or both.

In the Obesity, Nutrigenetics, Timing, Mediterranean–Melatonin (ONTIME-MT) study, a randomized crossover trial, we compared two evening oral glucose tolerance tests (OGTTs) simulating early and late dinner timing. We hypothesized that the concurrence of food intake with elevated endogenous melatonin concentrations (simulated by the late dinner timing) impairs glucose tolerance and alters the insulin response to glucose. In this study, we test the hypothesis that this effect is stronger in *MTNR1B* G (T2D risk) allele carriers versus noncarriers. Results will help to better understand: *1*) the effects of melatonin on glucose metabolism; and *2*) the influence of *MTNR1B* genetic variant on diabetes risk in a physiological context in which the ligand (melatonin) is present. This new knowledge will help to develop individualize interventions based on the interaction between *MTNR1B* genotype and the concurrence of food timing and melatonin on glucose tolerance and diabetes risk.

RESEARCH DESIGN AND METHODS

Cohort

Participants in the ongoing ONTIME-MT study (ClinicalTrials.gov NCT03036592), a Spanish natural late-eating population, are male and female of European descent, between 18 and 70 years of age, free of diabetes, and without any relevant medication use, including diabetes-related medications, growth hormones, anticoagulants, β-blockers, hypnotics, and any melatonin use or other sleep-related medications. At baseline, height (in meters) and weight (in kilograms) were measured, and BMI was calculated. The protocol was approved by the ethics committee of the University of Murcia and the Virgen de la Arrixaca University hospital (ID: 1188/2015). Written informed consent was obtained from all study participants upon recruitment.

In a randomized, crossover study design, following an 8-h fast, each participant underwent two evening 2-h 75-g OGTTs: an early and a late simulated dinner timing individualized according to participants' habitual bedtime (Supplementary Fig. 1). The early condition was scheduled 4 h prior to habitual bedtime, and the late condition was scheduled 1 h prior to habitual bedtime. Habitual bedtime was determined using 1 week of data derived from a study-specific smartphone application, and habitual dinner timing was determined using time-stamped photographs in the same smartphone application and further confirmed with 7-day sleep logs reflecting the week prior to the first OGTT. Dinner timing was further confirmed using complementary 7-day dietary records. The order of the OGTTs was randomized, and OGTTs were conducted on the same day of the week with a 1-week washout period. Light intensity was kept bright (≥450 lux) during the early condition and kept dim (0–25 lux) during the late condition to capture habitual light intensity for this population.

Serum glucose and insulin were measured at fasting (T0) and subsequently every 30 min during the 2-h tests (T30, T60, T90, and T120) (Fig. 1). Melatonin was assessed at the start and end of each OGTT (T0 and T120). Glucose was measured using the hexokinase test, GLUC3, Cobas c-702 (Roche Diagnostics GmbH, Mannheim, Germany) with a sensitivity of 2 mg/dL and an intra- and interassay precision of 0.8% and 1.3%, respectively. Insulin was measured using the ECLIA Elecsys test, Cobas e-801 (Roche Diagnostics GmbH) with a sensitivity of 0.2 μIU/mL and an intra- and interassay precision of 0.8–1.5% and 3.2–3.7%, respectively. Serum melatonin concentrations were measured by radioimmunoassay (IBL

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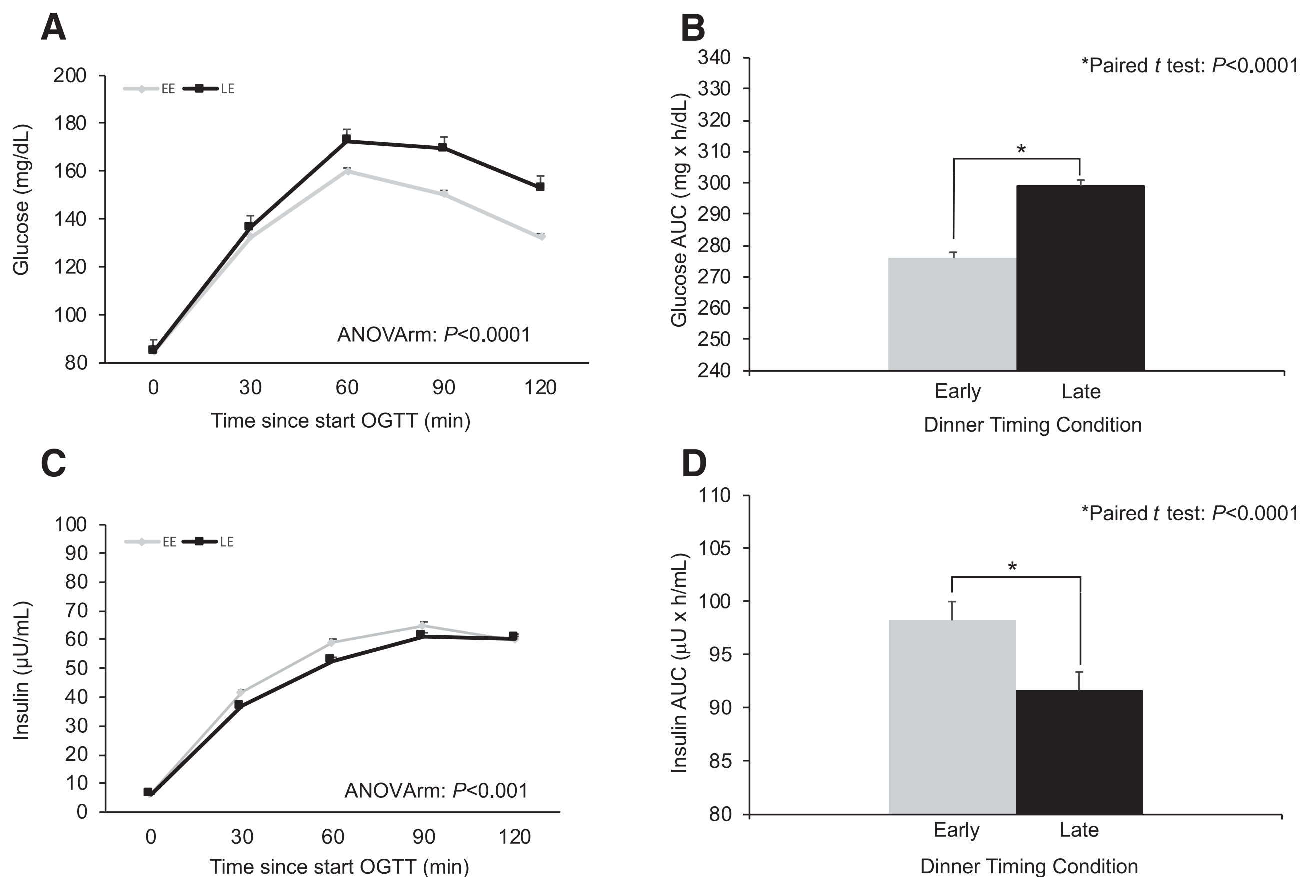

Figure 1—Comparison of OGTTs simulating EE and LE timing (n = 845). Comparison between EE and LE simulated dinner timing conditions of serum glucose (*A*) and insulin (*C*) concentrations. Time 0 min is fasting. ANOVArm represents the time since OGTT start and dinner timing condition. *B* and *D* represent the glucose and insulin AUC, respectively, for the EE and LE timing conditions. Paired t test was used to compare 120-min glucose AUC (*B*) and insulin AUC (*D*) between EE and LE timing conditions. Figures depict the mean ± SEM. *$P < 0.05$.

International GmbH, Hamburg, Germany). The intra- and interassay precision was 6.7% and 10.4%, respectively. Melatonin samples that were below the detection limits of the assay were reported as the lower limit of detection of 0.5 pg/mL.

DNA was isolated from blood samples using standard procedures (Qiagen, Valencia, CA). Genotyping of the *MTNR1B* SNP was performed using a TaqMan assay with allele-specific probes on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) according to standardized laboratory protocols.

Statistical Analyses

Postprandial glucose and insulin responses were estimated during the 120-min incremental area under the curve (AUC) and were calculated by the trapezoidal method (24). In the total population, a repeated-measures ANOVA (ANOVArm) was used to compare glucose and insulin responses between late and early dinner timing conditions (Fig. 1*A* and *C*) across time, while a paired t test was used to compare glucose and insulin AUCs between dinner timing conditions) (Fig. 1*B* and *D*).

To determine potential differences across genotypes (GG, CG, and CC) in the Δ between late and early conditions for the different glucose metabolism outcomes, a one-way ANOVA test was used; if significant, a Fisher least significant difference (LSD) post hoc test was done for pairwise comparisons (Table 2). A two-way ANOVA test was also performed to test whether there was an interaction between meal timing (early eating [EE] vs. late eating [LE]) and genotype (GG, CG, and CC) for postprandial glucose and insulin responses (Supplementary Table 5). Linear regression models were used for testing associations between the *MTNR1B* risk variant and difference in glycemic outcomes between EE and LE timing with an additive genetic model (Supplementary Table 6).

Insulin sensitivity was evaluated using the Insulin Sensitivity Index (ISI) calculated from the OGTT data as 10,000/(fasting glucose × fasting insulin × mean OGTT glucose × mean OGTT insulin)$^{1/2}$. β-Cell function was assessed as the corrected insulin response (CIR) during the OGTT (100 × insulin30/[glucose30 × (glucose30 − 70)]) (25). Finally, the disposition index (DI) was calculated as CIR × ISI (26). Insulin AUC (µU × h/mL), insulin/glucose AUC, ISI, CIR, and DI were logarithmically transformed before statistical analyses. Data were not stratified by sex because no effect modification ($P > 0.05$). Data were further adjusted for randomization order, and no effect modification was found. Goodness of fit was assessed for all models.

RESULTS

Participants

A total of 845 participants (mean ± SD; aged 38 ± 14 years; BMI 25.67 ± 4.69 kg/m^2; 71% female) underwent two

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evening OGTTs in randomized order simulating EE and LE timing (3 h apart) (Supplementary Fig. 1); 50% carriers of at least one copy of the G risk allele (CG, 40% and GG, 10%) and 50% carriers of two copies of the nonrisk C allele (CC); a distribution consistent with the 30% known allele frequency of this variant in the European population (Table 1) (27). The early dinner timing condition (early evening OGTT or EE) was scheduled 4 h before habitual bedtime and ranged from 1800 to 2300 h (mean time 1949 h), and the late dinner timing condition (late evening OGTT or LE) was scheduled 1 h before habitual bedtime and ranged from 2100 to 0200 h (mean time 2248 h). Average serum melatonin values were 3.5-fold higher in the LE compared with the EE timing condition (i.e., 66.18 ± 44 pg/mL LE and 22.76 ± 13.07 pg/mL EE) (Table 1). Habitual self-reported dinner timing, as derived from 7-day time-stamped photographs, ranged from 1900 to 2321 h. No significant differences were found in age, sex, adiposity, habitual bedtime, or habitual dinner timing across the genotype groups (Table 1).

LE Timing Condition Decreases Glucose Tolerance and Reduces Insulin Secretion

In the total population, glucose AUC was 8.3% higher during the LE timing condition when compared with the EE timing condition ($P < 0.0001$) (Fig. 1*A* and *B*). While fasting glucose was similar in the two conditions (*t* test, $P = 0.519$), postload glucose levels were consistently higher in the late OGTT, with relative differences rising over the 2 h (ANOVArm; $P < 0.0001$) (Fig. 1*A*). The higher postprandial glucose concentrations in the LE timing condition were concurrent with an overall 6.7% decrease in insulin AUC (*P t* test AUC <0.0001) (Fig. 1*D*). Indeed, insulin levels were lower in the LE compared with the EE timing condition (ANOVArm, $P < 0.001$) (Fig. 1*C*).

MTNR1B Risk Allele Carriers Have Worse Glucose Tolerance and Reduced Insulin Secretion in LE Versus EE Timing Condition Compared With Nonrisk Allele Carriers

When stratified by *MTNR1B* risk variant (rs10830963), significant interactions were found between *MTNR1B* and the dinner timing condition on glucose AUC (*P* for interaction, $P_{int} = 0.009$) (Supplemental Table 5). We observed a dose-dependent increase in the change in glucose AUC (LE minus EE timing) with increasing numbers of G-risk allele, with a β-effect per G allele of 12.53 mg × h/dL (Supplementary Table 6). In all three genotype groups (CC, CG, and GG), the LE timing condition showed consistently higher glucose AUC compared with the EE timing condition (Fig. 2*A–C*). However, differences in glucose AUC between the LE and EE timing condition (Δ glucose AUC) were greatest in GG carriers, followed by CG carriers, and least in CC carriers (*P* for global ANOVA <0.0001) (Fig. 2*D* and Table 2).

With respect to insulin, a significant interaction was found between *MTNR1B* and the dinner timing condition for insulin AUC ($P_{int} = 0.035$) (Supplemental Table 5). We observed a decrease in the change in insulin AUC (LE minus EE timing) in G carriers, with a β-effect per G allele of -9.56 μU × h/mL (Supplementary Table 6). As shown in Fig. 2*E–H*, among the three genotypes, G carriers had the largest decrease in insulin AUC in the LE timing condition compared with the EE timing (Supplemental Table 5) (*P* for global ANOVA <0.01) (Table 2).

To delineate whether differences between both dinner timing conditions were due to defects in insulin sensitivity or secretion, we compared the ratio of insulin/glucose AUC, ISI, CIR, and DI between the two conditions and across genotype groups (Supplemental Table 5). Significant interactions were found between genotype and the dinner timing condition for the ratio of insulin/glucose AUC, CIR, and DI ($P_{int} < 0.05$) (Supplemental Table 5).

The ratio of insulin/glucose AUC, an integrated measure of insulin response over the 2-h OGTT, was significantly lower in the LE than in the EE timing condition in the total

Table 1—General characteristics of the ONTIME-MT study population (*n* = 845)

	Total population	*MTNR1B* genotype			*P* value‡
		CC genotype	CG genotype	GG genotype	
n (%)	845 (100)	423 (50)	342 (40)	80 (10)	—
Age, years	38 ± 14	37 ± 14	39 ± 14	37 ± 15	0.192
Sex, % female (*n*)	71 (600)	70 (295)	71 (243)	78 (62)	0.375
BMI, kg/m^2	25.67 ± 4.69	25.94 ± 4.91	25.41 ± 4.36	25.40 ± 4.90	0.264
Body fat, %	28.93 ± 9.86	29.16 ± 9.87	28.74 ± 9.93	28.57 ± 9.64	0.794
Obesity, % (*n*)	17.8 (150)	18.9 (80)	15.8 (54)	20 (16)	0.468
Habitual dinner timing, h	2138 ± 0034	2138 ± 0033	2138 ± 0036	2135 ± 0032	0.803
Habitual bedtime, h	2432 ± 0057	2432 ± 0058	2433 ± 0059	2430 ± 0052	0.955
Melatonin EE timing condition (pg/mL)*†	22.76 ± 13.07	23.03 ± 12.72	21.91 ± 12.43	25.15 ± 17.18	0.246
Melatonin LE timing condition (pg/mL)*†	66.18 ± 44	65.9 ± 41.96	65.32 ± 47.4	71.47 ± 39.32	0.643
Habitual dinner energy intake (kcal)	434.42 ± 248.65	425.77 ± 238.08	434.76 ± 256.57	477.88 ± 266.12	0.234
Habitual dinner carbohydrate intake (g)	40.13 ± 23.33	39.47 ± 22.59	40.28 ± 23.95	42.93 ± 24.52	0.477

Data are mean ± SD unless otherwise indicated. **n* = 588. †Mean of T0 and T120. ‡ANOVA among three genotypes.

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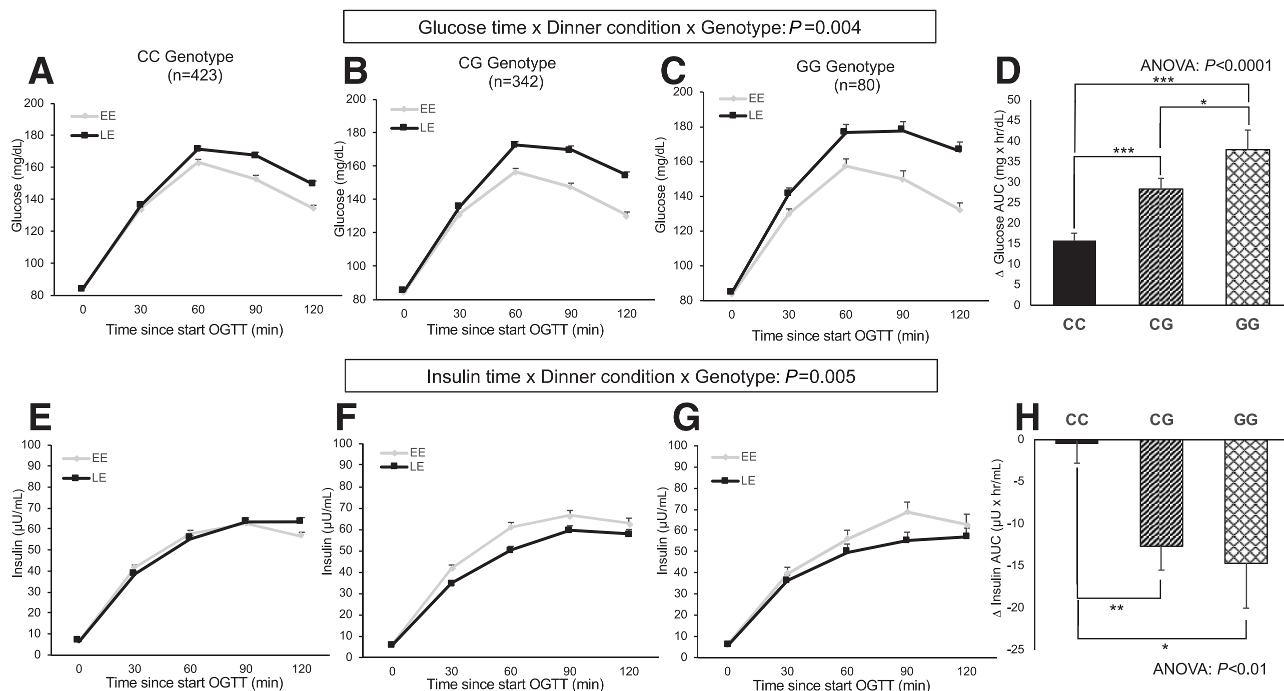

Figure 2—Comparison of OGTTs among *MTNR1B* genotypes simulating EE and LE timing. Comparison between EE and LE simulated dinner timing conditions of serum glucose (*A*–*C*) and insulin (*E*–*G*) concentrations. Time 0 min is fasting. ANOVArm represents the time since OGTT start and dinner timing condition. *D* and *H* represent the Δ between LE and EE of glucose and insulin AUC, respectively. A one-way ANOVA test was used to determine potential differences across genotypes (GG, CG, and CC) in the Δ between LE and EE conditions for glucose and insulin AUC ($P < 0.0001$, and $P < 0.01$, respectively), and Fisher LSD tests were used for pairwise comparisons among genotypes (*$P < 0.05$; **$P < 0.01$; ***$P < 0.0001$).

population. When stratified by *MTNR1B* risk variant, significant differences were found among genotypes with the largest decrease in G carriers ($P < 0.0001$) (Table 2). Furthermore, the CIR (representing the first-phase insulin response to glucose) was lower in the LE than in the EE timing condition in the total population (Supplemental Table 5), and the Δ CIR differed among genotypes (Table 2), with the largest decrease in GG carriers (Supplementary Fig. 2). Notably, the ISI (an integrated measure of insulin sensitivity) remained similar in the two dinner timing conditions and did not differ across the genotype groups (Table 2), while the DI (a measure of β-cell function that accounts for concomitant insulin sensitivity) was significantly lower in the LE than in the EE timing condition, with the largest decrease in GG homozygotes.

Melatonin Concentration in the Late Evening OGTT Modulated Insulin Secretion Among MTNR1B G Carriers

We stratified the population by high and low melatonin levels based on the median serum melatonin concentrations in the late evening OGTT ($n = 588$; 67.61 pg/mL concentration). The level of melatonin in the LE OGTT modulated insulin secretion across genotype groups. With respect to insulin, a significant interaction was found between *MTNR1B* and the dinner timing condition for insulin AUC ($P_{int} = 0.036$). When melatonin concentration was high, G carriers, but not CC carriers, had a decrease in insulin secretion in the late evening compared with the early evening OGTT (Supplementary Fig. 3). However, when melatonin concentration was low, surprisingly, only CG carriers appeared to have a decrease in insulin secretion in the LE versus EE condition (and not GG). Nevertheless, this should be interpreted with caution because no significant interaction was found between *MTNR1B* and the dinner timing condition for insulin AUC ($P_{int} = 0.144$), and the Δ insulin AUC was not significantly different across genotype groups in those with low melatonin concentrations.

In addition, with respect to glucose tolerance, a single copy of the G risk allele was sufficient to increase glucose OGTT in the late evening compared with the early evening in the presence of high melatonin levels (Supplementary Fig. 4*A*–*D*). In the setting of low melatonin levels, this difference was only evident in GG carriers (Supplementary Fig. 4*E*–*H*).

CONCLUSIONS

In this randomized, crossover study, a glucose challenge simulating late dinner impaired glucose tolerance relative to a glucose challenge simulating early dinner, and this effect was significantly greater among carriers of the *MTNR1B* risk variant. These results support the hypothesis that the concurrence of elevated melatonin concentrations (during dinner close to bedtime) and food intake decreases glucose tolerance. Our results suggest that differences in glucose tolerance are primarily attributed to decreased insulin secretion and β-cell function in the late condition, particularly in risk allele carriers at this locus. Indeed, we observed significantly lower CIR and DI, but not ISI, in the LE timing condition compared with the EE as determined by two OGTTs. This is the largest study to date to showcase

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Table 2—Differences in glucose metabolism between two evening OGTTs simulating EE and LE timing in the total population and across *MTNR1B* genotypes

		MTNR1B genotype						
						Post hoc LSD test		
Differences between late and early condition, Δ	Total population (*n* = 845)	CC genotype (*n* = 423)	CG genotype (*n* = 342)	GG genotype (*n* = 80)	Global *P* value	CC-CG *P* value	CC-GG *P* value	CG-GG *P* value
Test start time, h	2.97 ± 0.73	2.95 ± 1.03	2.99 ± 0.09	2.99 ± 0.23	0.701	—	—	—
Fasting glucose, mg/dL	0.16 ± 7.51	−0.45 ± 7.67	0.68 ± 7.24	1.22 ± 7.64	**0.049**	**0.038**	0.068	0.563
Postprandial glucose (30 min), mg/dL	3.84 ± 26.37	1.64 ± 25.24	4.59 ± 26.65	12.24 ± 29.35	**0.003**	0.123	**0.001**	**0.019**
Postprandial glucose (2 h), mg/dL	20.36 ± 53.54	15.04 ± 32.08	23.67 ± 36.79	34.35 ± 41.94	**<0.0001**	**0.001**	**<0.0001**	**0.014**
Fasting insulin, μU/mL	−0.39 ± 4.83	−0.38 ± 5.33	−0.37 ± 4.47	−0.59 ± 3.45	0.933	—	—	—
Postprandial insulin (30 min), μU/mL	−4.73 ± 28.78	−2.96 ± 29.64	−7.10 ± 28.23	−3.98 ± 26.94	0.138	—	—	—
Glucose AUC (mg × h/dL)	22.92 ± 43.96	15.48 ± 39.28	28.36 ± 47.89	38.95 ± 42.66	**<0.0001**	**<0.0001**	**<0.0001**	**0.049**
Insulin AUC (μU × h/mL)*	−6.58 ± 53.71	−0.19 ± 55.16	−12.67 ± 52.36	−14.31 ± 47.81	**0.004**	**0.002**	**0.049**	0.914
Insulin/glucose AUC*	0.62 ± 3.20	0.28 ± 3.28	0.98 ± 3.15	0.93 ± 2.82	**<0.0001**	**<0.0001**	**0.002**	0.608
ISI*	0.42 ± 6.72	0.28 ± 7.09	0.55 ± 6.55	0.67 ± 5.15	0.761	—	—	—
CIR*	−0.13 ± 0.82	−0.06 ± 0.70	−0.18 ± 0.91	−0.31 ± 1.02	**0.011**	0.061	**0.006**	0.106
DI*	−0.74 ± 4.91	−0.23 ± 3.99	−1.21 ± 5.57	−1.46 ± 5.97	**0.039**	0.126	**0.018**	0.148

*Values of these parameters were logarithmically transformed before statistical analyses. *P* values were calculated using a one-way ANOVA. Fisher LSD tests were used for those parameters with a statistically significant ANOVA. Boldface values represent significant differences.

intraindividual glucose tolerance and insulin profiles in two dinner timing conditions individualized according to bedtime and examining the interaction of dinner timing with the *MTNR1B* risk variant.

Our results confirm that late eating acutely impairs glucose tolerance through a defect in insulin secretion. We observed an impairment of glucose tolerance and a decrease in insulin secretion during the LE timing condition, despite only a 3-h difference in glucose challenge start time. This difference might be partly due to the 3.5-fold higher melatonin concentrations, as supported by our earlier trial in which acute melatonin administration impaired glucose tolerance and in vitro β-cell experiments showing decreased insulin secretion due to melatonin (10,11,18,28).

Given that melatonin is the natural ligand of the melatonin receptor and typically rises ∼2 h before bedtime, we further studied the *MTNR1B* rs10830963 genotype across the population to test the involvement of melatonin in these results. We based our hypothesis on previous results showing that the G risk variant of *MTNR1B* confers increased expression of the receptor in human pancreatic islets (11) and that the rs10830963 T2D risk variant is associated with impaired fasting glucose and measures of decreased insulin secretion in daytime OGTT assessments (26). However, this is the first large population-based study that investigates the influence of *MTNR1B* genetic variant on diabetes risk during the biological evening in a physiological context in which the ligand (melatonin) is present.

As expected, the genotype distribution in the ONTIME population mirrored that in previous genome-wide association study of European ancestry (G allele frequency = 0.30 with CC, 50%; CG, 40%; and GG, 10%) (26). Importantly, we found genotype-driven differences in glucose AUC, reflecting greater impairment of glucose tolerance in G risk allele carriers. Of relevance, this effect was dose-dependent and largest among GG carriers and was accompanied by a significant decrease in indices of insulin secretion but not in indices of insulin sensitivity. Thus, our study supports the hypothesis that melatonin contributes to impaired glucose homeostasis by inhibiting insulin secretion and is in agreement with results shown in insulin-secreting cells (11) that

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demonstrated that *MTNR1B* overexpression augmented the inhibition of insulin release exerted by melatonin.

These results are also in concordance with previous studies that support the "equilibrium hypothesis," which states that either exaggerated or dampened melatonin signaling in common and rare variant carriers, respectively, becomes detrimental for glucose homeostasis (in this case, enhanced melatonin signaling in *MTNR1B* G risk allele carriers becomes detrimental for glucose homeostasis) (29,30). Of note, in the current study, melatonin concentrations were 3.5-fold higher during the LE than during the EE timing condition.

Behavioral cycles, the circadian system, and melatonin levels may all contribute to glucose control during LE timing. Previous highly controlled laboratory experimental studies have reported that under normally entrained conditions (i.e., sleep at night and evening dinner), glucose tolerance, β-cell responsivity, and insulin sensitivity peak in the morning and deteriorate as the day progresses (31). Furthermore, it has been shown that such a decrease in insulin sensitivity is mostly caused by the behavioral cycle, while the circadian system dominates the deterioration in glucose tolerance and β-cell responsivity (8,32). The circadian effect appeared to be independent of melatonin signaling, because melatonin levels were low during the circadian evening (~2000–2200 h) in the previous studies (8,32).

Our results suggest a new role of melatonin in attenuating glucose tolerance and β-cell function in the late evening in addition to its known role in endogenous circadian effects and effects on the behavioral cycle. This new role, however, requires future studies with a direct manipulation of melatonin levels. Thus, in addition to the behavioral cycle influences—the contributors that are varied and differ greatly from study to study—there appear to be two fundamental biological drivers: circadian phase (even across times of low melatonin) and melatonin.

While in the current study the late dinner timing was simulated as a glucose challenge during 1 h before habitual sleep timing, we predict that any dinner that permits concurrence between glucose and elevated physiological melatonin concentrations may be considered as a late dinner. In healthy individuals, melatonin levels usually rise about 2 h before bedtime (33), while postchallenge glucose needs ~2 h to recover. Although the regular postprandial glucose dynamics is complex and dependent on many factors, such as the amount and the composition of the meal, a linear relationship between postprandial and postchallenge (after a 75-g oral glucose load) glucose at 2 h has been established in laboratory conditions (34). Therefore, a late dinner could be understood as a dinner taken within roughly 2.5 h before bedtime (10). Nevertheless, results were obtained in response to an OGTT so that it may be difficult to extrapolate to what may happen with a regular dinner, as other factors may be involved. However, we observed similar effects when mixed meals were given instead of an OGTT (10). In that pilot study, we used identical mixed meals under the two dinner conditions (EE and LE) and found similar results.

Key strengths of our study include the crossover design, intraindividual comparison in a large study population, identical fasting conditions and prechallenge meals in the two tests, use of 7-day food and objective sleep logging to ascertain habitual behavior, and measurements relative to individual bedtime to examine impact relative to endogenous physiology rather than external clock timing only. Whereas our study was limited to 2-h assessments, it is possible that glucose impairments persist for several hours in the LE condition (9). However, our study also has some limitations. First, the study has been performed in participants free of diabetes; further studies should be performed in people with diabetes. New crossover studies should be developed alternating chronic exposures of LE and EE relative to sleep timing for longer periods in order to test the chronic impact of the concurrence of melatonin and food intake, especially in *MTNR1B* G-allele carriers. Furthermore, while previous studies have shown that the association with fasting glucose during the day (typically morning) is robust and reproducible (11), in our study, fasting glucose measurements were similar in the two dinner timing conditions following an 8-h fast. It is therefore plausible that our sample size did not have enough power to replicate the association or that differences in the time of collection (morning in published reports vs. evening in this study) explain our inability to detect the association.

This study has implications for reducing risk of cardiometabolic diseases. Postprandial hyperglycemia is an independent factor contributing to cardiovascular complications and increased mortality, especially in people with T2D (35). Indeed, epidemiologic and pathophysiological studies have shown that excessive postload glucose excursions have acute and chronic harmful effects on the endothelium and vessel wall (36). Our study findings are applicable to the 35% of the population in the U.S. who consume food close to bedtime (37). In our cohort, our findings are relevant to 34% of the population who reported to consume dinners close to bedtime (during the 2.5 h before bedtime). The current population is a large population of natural late eaters; they may have already developed adapting responses of the circadian timing system that may affect the response to an overall slight modification of the time of the OGTT administration, which may limit the generalizability of the results to other populations with different eating habits. However, data show no significant interaction between habitual dinner timing and *MTNR1B* genotype group for Δ glucose or insulin AUC ($P = 1.000$ for both). Furthermore, the proportions of individuals within each genotype were consistent between habitual EE and LE eaters (data not shown). Therefore, these results suggest that the conclusions of the current study are applicable for other populations who habitually have dinner earlier.

Future intervention studies based on advancing dinner to early timing over longer periods are necessary to examine whether there is an improvement in glucose tolerance, cardiovascular complications, or the risk for T2D, particularly in *MTNR1B* risk carriers.

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