Citation: Clin Transl Sci (2016) 9, 114–122; doi:10.1111/cts.12389

ARTICLE

Melatonin Pathway and Atenolol-Related Glucose Dysregulation: Is There a Correlation?

SW Chang[1], Y Gong[1], CW McDonough[1], TY Langaee[1], N Nasiri Kenari[2], AL Beitelshees[3], JG Gums[4], AB Chapman[5], ST Turner[6], JA Johnson[1,7] and RM Cooper-DeHoff[1,7,*]

Lower melatonin level, melatonin receptor gene variations, and atenolol treatment are associated with glucose dysregulation. We investigated whether atenolol-related glucose and melatonin changes are correlated, and whether single nucleotide polymorphisms (SNPs) in melatonin candidate genes contribute to interindividual variation in glucose change. Hypertensive Caucasians ($n = 232$) from the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study treated with atenolol for 9 weeks were studied. Urinary 6-sulfatoxymelatonin (aMT6s) was measured pre- and posttreatment and normalized to urinary creatinine. Pharmacogenetic effects on glucose change of 160 SNPs in 16 melatonin candidate genes were assessed with multiple linear regression. Atenolol was associated with increased glucose (1.8 ± 10.1mg/dl, $P = 0.02$) and decreased aMT6s (–4.5 ± 10.1 ng/mg, $P < 0.0001$). However, the aMT6s change was not correlated with post-atenolol glucose change. SNP rs11649514 in *PRKCB* was associated with glucose change ($P = 1.0 \times 10^{-4}$). *PRKCB* is involved in the melatonin-insulin regulatory pathway, and may be important in mediating clinically meaningful atenolol-related hyperglycemia.
Clin Transl Sci (2016) **9**, 114–122; doi:10.1111/cts.12389; published online on 7 March 2016.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔ Atenolol treatment both suppresses melatonin secretion and increases glucose, and the melatonin pathway is associated with glucose homeostasis.

WHAT QUESTION DID THIS STUDY ADDRESS?
✔ We sought to determine the correlation between atenolol-related glucose dysregulation and change in melatonin level, and to determine the association between melatonin candidate genes and susceptibility to glucose dysregulation after atenolol treatment.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
✔ We demonstrated that the atenolol-related decrease in melatonin metabolite is not correlated with the increase in glucose. Additionally, we identified a gene, *PRKCB,* involved in the melatonin-insulin regulatory pathway that may be important in mediating atenolol-related hyperglycemia.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS
✔ A better understanding of potential mechanisms and genetic predictors of atenolol-related glucose dysregulation could allow for a precision approach to individualizing risk assessment, and avoid use of medications with diabetogenic potential in individuals at high risk for diabetes mellitus.

The pineal hormone melatonin, which serves as a mediator of circadian rhythm, is primarily synthesized and released into the circulatory system under regulation of pineal β1-adrenergic receptors.[1,2] Accumulating evidence, beginning in the 1930s, documents an association between pineal gland function and glucose homeostasis.[3] More recently, a lower melatonin level has been associated with higher risk for developing type 2 diabetes mellitus (DM) and insulin resistance,[4,5] and disturbance of the circadian rhythm is linked with hyperglycemia, insulin resistance, and type 2 DM.[6–8] Interestingly, repeated findings from large-scale genome-wide association studies have demonstrated that variants in the melatonin receptor 1B gene (*MTNR1B*), which is expressed in pancreatic β-cells, are associated with the risk for increased fasting glucose, impaired insulin secretion, and type 2 DM.[9–11] Whether lower melatonin secretion or impaired melatonin signal transduction directly causes glucose dysregulation in humans is not completely

[1]Department of Pharmacotherapy and Translational Research and Center of Pharmacogenomics, College of Pharmacy, University of Florida, Gainesville, Florida, USA; [2]Department of Pharmacotherapy and Translational Research, College of Pharmacy, University of Florida, Gainesville, Florida, USA; [3]Division of Endocrinology, Diabetes & Nutrition, School of Medicine, University of Maryland, Baltimore, Maryland, USA; [4]Department of Community Health and Family Medicine, College of Medicine, University of Florida, Gainesville, Florida, USA; [5]Renal Division, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, USA; [6]Division of Nephrology and Hypertension, Department of Internal Medicine, Mayo Clinic College of Medicine, Rochester, Minnesota, USA; [7]Division of Cardiology, Department of Medicine, College of Medicine, University of Florida, Gainesville, Florida, USA. *Correspondence: RM Cooper-DeHoff (dehoff@cop.ufl.edu)
Received 23 November 2015; accepted 29 January 2016; published online on 7 March 2016. doi:10.1111/cts.12389

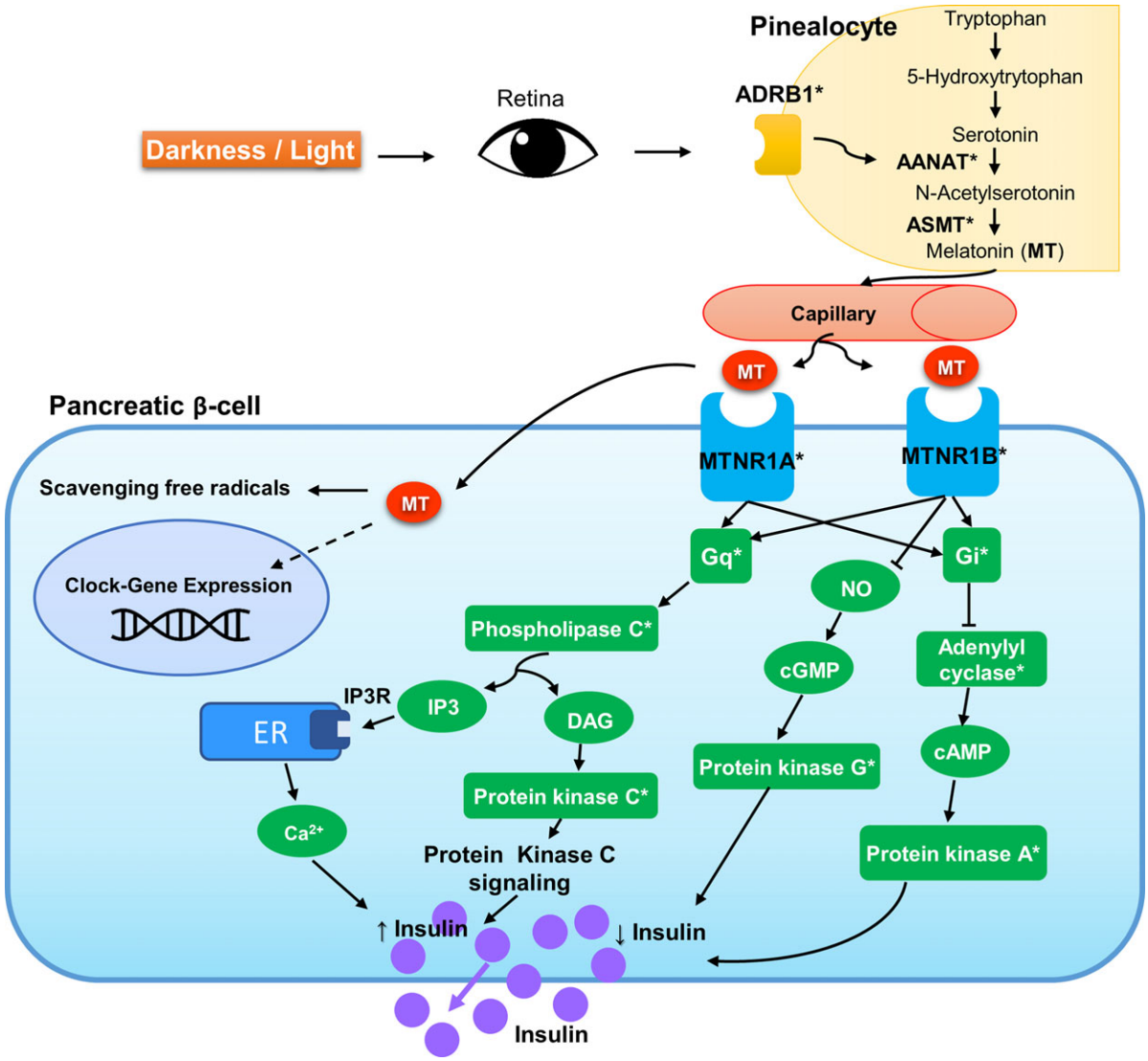

Figure 1 Melatonin pathway and melatonin candidate genes. Squared symbol in the melatonin signaling cascade represents the involved proteins and the * symbol indicates protein encoded by melatonin candidate genes. Subtypes of the same protein family are not shown individually in the figure. AANAT, Aralkylamine N-Acetyltransferase; ADRB1, Adrenoceptor Beta 1; ASMT, Acetylserotonin O-Methyltransferase; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; DAG, diacylglycerol; ER, endoplasmic reticulum; IP3, Inositol trisphosphate; IP3R, Inositol trisphosphate receptor; MT, melatonin; MTNR1A, melatonin receptor 1A; MTNR1B, melatonin receptor 1B.

understood. However, the cumulative evidence indicates a strong correlation between the melatonin pathway and glucose dysregulation.

Atenolol is a β1-selective β-blocker, which is one of the most commonly prescribed medication classes in the world, used for a variety of cardiovascular diseases, including hypertension. However, consistent evidence from randomized clinical trials has demonstrated the adverse glucose effects of atenolol and other β-blockers.[12–14] Some β-blockers are associated with reduced melatonin secretion,[15–17] and atenolol treatment suppresses nocturnal melatonin secretion in a dose-dependent fashion.[16,18,19] Despite the overwhelming evidence suggesting an interplay between the melatonin pathway and glucose regulation, no study to our knowledge has investigated the association between atenolol-related glucose dysregulation and the melatonin pathway.

Since altered melatonin levels and melatonin receptor signal transduction are associated with impaired glucose homeostasis, and atenolol treatment affects both melatonin and glucose, we aimed to investigate the association between the melatonin pathway and atenolol-related glucose dysregulation. We sought first to determine if atenolol-related glucose dysregulation is correlated with change in melatonin level, and second to determine the association between genetic variation in 16 melatonin candidate genes that are involved in melatonin synthesis, melatonin receptor, or the melatonin receptor signaling pathway (**Figure 1**) and susceptibility to glucose dysregulation after atenolol treatment. We integrated genetic, glucose, and urine melatonin metabolite

[6-sulfatoxymelatonin (aMT6s)] information from atenolol-treated hypertensive Caucasians in the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study.[20]

METHODS

PEAR study design and melatonin study participants

PEAR (clinicaltrials.gov: NCT00246519) is a prospective, randomized, multicenter clinical trial evaluating the pharmacogenomic effects of hydrochlorothiazide (HCTZ), atenolol, and their combination on blood pressure response and adverse metabolic effects. The PEAR study design was described previously.[20] Briefly, individuals aged 17–65 years with mild-to-moderate essential hypertension were recruited at the University of Florida (Gainesville, FL), Mayo Clinic (Rochester, MN), and Emory University (Atlanta, GA). Individuals with a history of heart disease, DM, renal disease, or impaired renal function (serum creatinine $>$1.5 mg/dl in males, $>$1.4 mg/dl in females) were excluded. The study protocol was approved by the Institutional Review Board at all study sites and all participants provided written informed consent. After an initial 3–8 week washout period, participants were randomized to receive HCTZ 12.5 mg or atenolol 50 mg daily monotherapy for 9 weeks, which included dose titration to HCTZ 25 mg or atenolol 100 mg daily in individuals whose blood pressure remained over 120/70 mmHg. After the monotherapy period, the other agent was added and a similar titration strategy followed to form the combination treatment period. Blood pressure and laboratory results were acquired at baseline and after each treatment period. During the study period, participants were advised to maintain their lifestyle behaviors.

Our study focused on 234 Caucasians from the PEAR study who received atenolol monotherapy. To prevent confounding from participants who were nonfasting at the time of blood draw, two outliers for change in glucose (described below) were excluded from all analyses involving glucose data. To prevent potential confounding from participants who had altered circadian rhythms due to night work, or who did not report using melatonin supplement at either visit, self-reported night workers ($n = 14$), and outliers for change in aMT6s ($n = 1$) were excluded from analysis using aMT6s data. Additionally, individuals with a missing urine sample at either study visit ($n = 23$) were excluded due to lack of aMT6s data. No individual reported using melatonin supplements in the relevant study periods. After applying the exclusion criteria, 194 individuals were eligible for examining the correlation between glucose and aMT6s changes, and 232 individuals were included for the pharmacogenetics analysis. An additional 207 PEAR Caucasians (not included in the atenolol monotherapy group) who received atenolol as add-on therapy were available for examination of the genetic associations identified in the monotherapy group.

Fasting glucose, clinical variables, and 6-sulfatoxymelatonin (aMT6s)

Fasting glucose and insulin levels were measured from blood samples collected before and after completion of treatment with atenolol. A change in glucose was defined as the glucose level immediately after atenolol monotherapy minus the level at baseline. Plasma glucose levels were measured spectrophotometrically at a central laboratory located at the Mayo Clinic (Rochester, MN) using an automated enzymatic assay on a Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Plasma insulin levels were determined using the Access Ultrasensitive Insulin immunoassay system (Beckman Coulter, Brea, CA). All measurements presented were the average of duplicate runs. Body mass index (BMI) was calculated by dividing body weight (kilograms) by the squared value of height (centimeters) measured at baseline.

Circulating melatonin is rapidly metabolized into aMT6s in the liver, then excreted into urine as the major melatonin metabolite. Measurement of aMT6s normalized to creatinine from the morning urine has been shown as a simple and reliable surrogate for endogenous plasma melatonin levels in human studies for practical considerations.[21,22] We measured urinary aMT6s concentrations in unpreserved urine using an enzyme-linked immunosorbent assay (ALPCO, Salem, NH). Interassay coefficient of variation was 10%, which is consistent with reported assay performance.[23] Urine creatinine levels were photometrically measured using an enzymatic assay on a Hitachi 911 Chemistry Analyzer (Roche Diagnostics). All aMT6s levels were normalized to urine creatinine at the same visit for each individual to account for differences in urine volume. Both the aMT6s and creatinine concentrations were measured in duplicate and reported as the mean of the duplicates. The change in aMT6s was defined as the normalized aMT6s level measured after atenolol monotherapy minus the normalized aMT6s level at baseline.

Genotyping, quality control, and candidate gene selection

DNA were genotyped on the 50K Illumina HumanCVD beadchip (Illumina, San Diego, CA) and standard quality control procedures were conducted using PLINK (Broad Institute, Cambridge, MA).[24] A detailed description is provided in the **Supplementary Information**. Principal component (PC) analysis with quality controlled single nucleotide polymorphisms (SNPs) as input was performed using EIGENSTRAT to determine genetic continental ancestry.[25] The PC that best summarized the genetic structure and separated the ancestry clusters in the Caucasian ancestry group was identified.

Since the melatonin pathway is the focus of this study, we selected only candidate genes that were suggested to have involvement in the melatonin pathway by the literature or the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway database, and were present on the HumanCVD chip. We ended up with 16 candidate genes including, 1) genes involved in regulating melatonin synthesis: *AANAT, ASMT*, and *ADRB1*[16,18,19,26]; 2) melatonin receptor genes: *MTNR1A* and *MTNR1B*[27,28]; and 3) genes involved in melatonin receptor downstream signal transduction: *ADCY6, GNAI1, GNAI2, GNB3, PLCB1, PLCB3, PLCB4, PRKACG, PRKCA, PRKCB*, and *PRKCG.*[27,28] There were 160 SNPs with a minor allele frequency (MAF) $\geqq$0.03 and within 1 kb upstream and downstream of the candidate genes. Genotype data of these SNPs were extracted from the PEAR HumanCVD genetic data set. Among the 160 SNPs included, 108 were considered independent signals ($r^2 < 0.5$). The average genotyping call rate of the 160 SNPs in individuals included in this study was 99.95%.

Statistical analysis

Continuous characteristics are presented as mean and standard deviation (SD) or median and interquartile range (IQR) when appropriate, and categorical characteristics are presented as frequency and percentages. To identify outliers of glucose change, the change in glucose was first regressed to the previously defined clinical variables that are associated with glucose change, including age, gender, BMI, baseline glucose, and baseline insulin, and then residuals were standardized to a mean of 0 and SD of 1.[29] Participants with a standardized residual outside of 4 SDs from the mean were defined as glucose change outliers.[29] Outliers of the change in aMT6s level were defined in a similar manner, and excluded those with a standardized mean (mean of 0 and SD of 1) greater than 4 SDs from the mean of the entire cohort.

Correlation between atenolol-related glucose and aMT6s change

The paired *t*-test was used to compare the glucose and normalized aMT6s levels before and after atenolol therapy. The Pearson correlation coefficient was calculated to determine the correlation between changes in aMT6s and glucose levels. Pearson's partial correlation controlling for clinical variables (age, gender, BMI, and baseline levels of glucose and insulin) was conducted to determine the adjusted correlation. Additional analysis investigating correlation between the log-transformed glucose and aMT6s ratios, aMT6s tertiles and glucose change, and baseline log-transformed aMT6s level and glucose change were also performed. The glucose ratio was defined as the ratio of posttreatment glucose to pretreatment glucose levels, and the aMT6s ratio was defined as the ratio of posttreatment aMT6s to pretreatment aMT6s levels. These analyses were conducted using SAS 9.3 (SAS Institute, Cary, NC), and a P-value <0.05 was considered statistically significant.

Pharmacogenetics analysis

Linear regression modeling was first conducted for each SNP assuming an additive genetic model for association with change in glucose level. Follow-up analysis using a dominate model was conducted when appropriate. In addition to controlling for the clinical variables, the first PC for ancestry was included to adjust for population substructure. To account for multiple comparisons, SNPs with $P < 3.1\times10^{-4}$ were deemed statistically significant (defined by applying a Bonferroni correction accounting for 160 SNPs tested). For SNPs that achieved statistical significance for association with glucose change, association with baseline glucose, aMT6s change, and baseline aMT6s level were examined. Additionally, subgroup analysis for correlation between glucose and aMT6s changes by genotype groups was conducted. Deviation from Hardy–Weinberg equilibrium was evaluated using a chi-squared test with one degree of freedom, and none of the 160 SNPs deviated from Hardy–Weinberg equilibrium. All genetic association analyses were conducted using PLINK.[24]

SNPs that achieved significance in the atenolol monotherapy group were confirmed in the cohort of Caucasians in PEAR who received atenolol add-on therapy. In the atenolol add-on therapy cohort, glucose change was defined as the glucose level immediately after atenolol treatment minus the level immediately prior to starting atenolol treatment. Glucose change outliers, significance of glucose change, and pharmacogenetic associations were assessed under the same statistical methodology described above. SNPs with the same direction of effect and a P-value less than the Bonferroni-corrected significance level under a one-sided hypothesis were considered statistically significant.

Table 1 Baseline characteristics of atenolol-treated Caucasians in PEAR

Characteristics	Atenolol monotherapy ($n = 232$)	Atenolol add-on therapy ($n = 207$)
Age (years)	49.6 ± 9.5	50.1 ± 9.5
Female, *n* (%)	109 (47.0)	82 (40.0)
BMI (kg/m2)	30.3 ± 5.6	30.3 ± 4.9
Fasting glucose (mg/dl)	92.0 ± 11.6	93.0 ± 11.9
Fasting insulin (uU/ml)	7.1 (4.9 – 10.7)	7.0 (4.7 – 10.4)
Systolic blood pressure (mmHg)	151.2 ± 12.4	152.2 ± 12.2
Diastolic blood pressure (mmHg)	97.9 ± 5.7	97.9 ± 5.9
Estimated glomerular filtration rate (mL/min)	93.4 ± 17.6	92.8 ± 19.3

Continuous characteristics are presented as mean and SD or median and interquartile range (IQR) when appropriate, and categorical characteristics are presented as frequency and percentages.
BMI, body mass index.

RESULTS

Baseline characteristics

The baseline characteristics of the 232 Caucasians who received atenolol monotherapy included in this study are summarized in **Table 1**. Participants were hypertensive based on the inclusion criteria, with mean systolic/diastolic blood pressures of 151.2 ± 12.4/97.9 ± 5.7 mmHg, and 47% were female. The average baseline BMI was 30.3 ± 5.6 kg/m^2, and the baseline median fasting glucose level was 89.5 mg/dl (IQR 85.0-97.0 mg/dl). Baseline characteristics of the 207 Caucasians who received atenolol add-on therapy are also included in **Table 1**.

Glucose and aMT6s changes during atenolol monotherapy

Table 2 summarizes glucose and aMT6s levels before and after atenolol treatment. Following ~9 weeks of atenolol monotherapy, the mean fasting glucose was 94.6 ± 10.2 mg/dl, which resulted in a mean 1.8 ± 10.1 mg/dl significant increase ($P = 0.017$, **Figure 2a**). High interindividual variability of baseline urinary aMT6s levels was observed with a median 10.6 ng/mg (IQR 5.7–18.5 ng/mg). Treatment with atenolol was associated with a significant reduction in aMT6s level (mean –4.5 ± 10.1 ng/mg, $P < 0.0001$, **Figure 2b**).

Although we observed an overall increase in fasting glucose levels and a decrease in aMT6s levels after atenolol monotherapy, there was no correlation between the glucose and aMT6s changes (Pearson's $r = 0.05$, unadjusted $P = 0.47$, **Figure 3**). After adjusting for clinical covariates the correlation improved but remained nonsignificant (Pearson's partial $r = 0.12$, $P = 0.10$). The additional analyses confirmed

Table 2 Glucose and 6-sulfatoxymelatonin (aMT6s) measurements of atenolol-treated PEAR Caucasians

	Atenolol monotherapy ($n = 194$)[a]			Atenolol add-on therapy ($n = 207$)		
	Baseline	**Posttreatment**	**Change**	**Pretreatment**	**Posttreatment**	**Change**
Fasting glucose (mg/dl)						
Mean ± SD	92.8 ± 11.4	94.6 ± 10.2	1.8 ± 10.1*	94.5 ± 12.9	97.0 ± 12.3	2.4 ± 11.6**
Median (25th, 75th percentile)	90.5 (85.5, 98.5)	94.0 (87.5, 100.5)	2.8 (–3.5, 7.0)	93.0 (87.0, 100.0)	96.5 (87.5, 104.5)	2.5 (–3.5, 8.5)
Normalized urine aMT6s (ng/mg)						
Mean ± SD	13.6 ± 11.5	9.1 ± 10.8	–4.5 ± 10.1***			
Median (25th, 75th percentile)	10.6 (5.7, 18.5)	6.1 (2.7, 10.9)	–2.8 (–9.0, 0.3)		Not Measured	
Urine creatinine (mg/dl)						
Median (25th, 75th percentile)	115.3 (61.8, 174.5)	129.4 (69.7, 191.7)	7.7 (–35.6, 53.6)			

[a]Reporting data from individuals included in the analysis investigating correlation between glucose change and aMT6s change.
*$P = 0.017$
**$P = 0.0029$
***$P < 0.0001$
aMT6s: 6-sulfatoxymelatonin

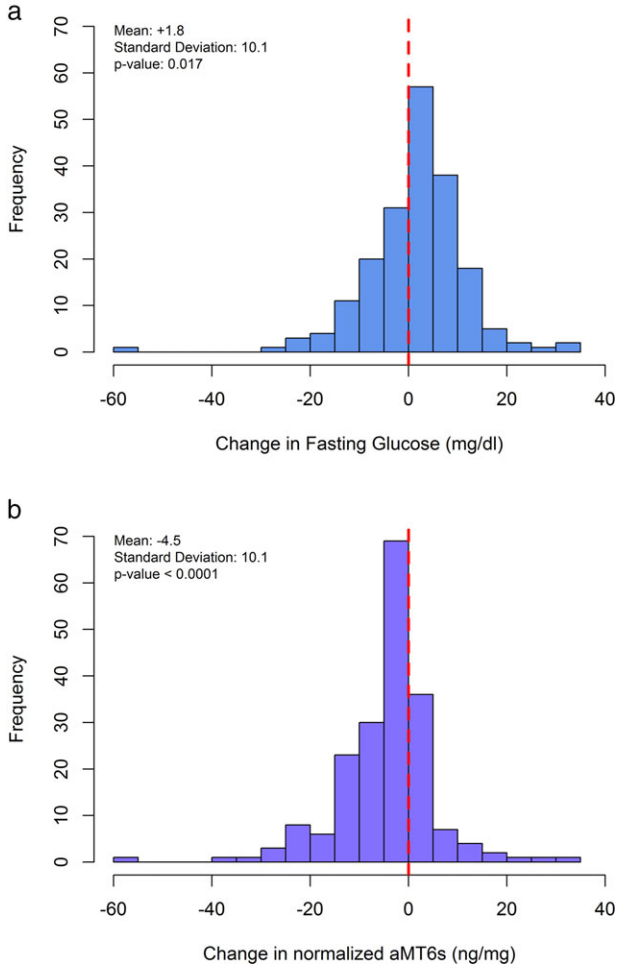

Figure 2 Glucose **(a)** and normalized aMT6s **(b)** changes after atenolol monotherapy in PEAR Caucasians ($n = 194$).

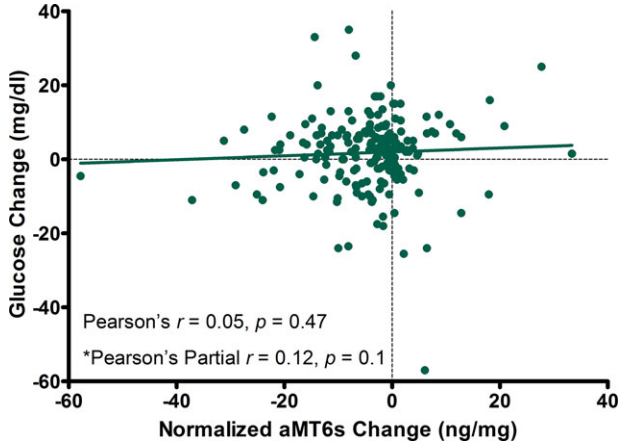

Figure 3 Correlation between change in normalized aMT6s and change in fasting glucose levels after atenolol monotherapy in PEAR Caucasians ($n = 194$). *Clinical covariates adjusted: age, gender, baseline glucose, baseline insulin, and BMI.

that there was no evidence of correlation between aMT6s levels and atenolol-related glucose change (log-transformed glucose and aMT6s ratios (Pearson's $r = 0.06$, unadjusted $P = 0.42$; Pearson's partial $r = 0.05$, $P = 0.50$); aMT6s tertiles and glucose change (trend $P = 0.94$); baseline log-transformed normalized aMT6s level and glucose change (Pearson's $r = -0.04$, $P = 0.58$; Pearson's partial $r = -0.08$, $P = 0.31$).

Pharmacogenetic effects of melatonin candidate genes and glucose change

Among PEAR Caucasians who received atenolol monotherapy, *PRKCB* rs11649514 was significantly associated with glucose change after adjusting for PC and clinical variables, including age, gender, BMI, and baseline levels of glucose and insulin (additive model $P = 9.9\times10^{-5}$, dominant model $P = 1\times10^{-4}$). The A allele of rs11649514 had an MAF of 0.10 among the atenolol monotherapy group, with two individuals carrying the A/A genotype and 43 individuals carrying the A/C genotype (**Supplemental Table 1**). A differential glucose response to atenolol by *PRKCB* rs11649514 genotype was observed ($P = 1\times10^{-4}$) despite no difference in the

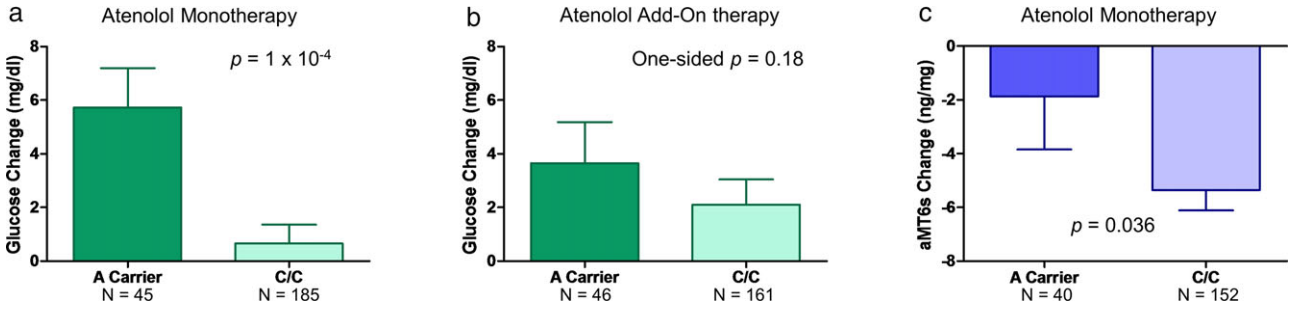

Figure 4 Atenolol-related fasting glucose and aMT6s changes by *PRKCB* rs11649514 genotype in PEAR Caucasians under a dominate genetic model. **(a)** Glucose change in atenolol monotherapy group. **(b)** Glucose change in atenolol add-on therapy group. **(c)** aMT6s change in atenolol monotherapy group (adjusted for age, gender, PC, and baseline aMT6s level). The adjusted mean and SE are presented.

baseline glucose level between genotype groups ($P = 0.52$, **Supplemental Figure 1**). Under a dominant genetic model, the rs11649514 A allele carriers had an average 5.7 ± 1.5 mg/dl increase in glucose compared with the homozygous C/C carriers, who had a 0.7 ± 0.7 mg/dl increase, which demonstrates an ~5 mg/dl greater increase in glucose between the two genotype groups (**Figure 4a**). In addition, within the same *PRKCB* region of the chromosome 16p12.2 locus, rs195993 ($P = 0.026$), rs17811655 ($P = 0.034$), rs198185 ($P = 0.036$), and rs7202459 ($P = 0.046$) were nominally associated with glucose change ($P < 0.05$) (**Supplemental Table 2**) independently of rs11649514 ($r^2 < 0.1$). The rs11649514 genotype independently contributed to 4% of the total variance in glucose response to atenolol monotherapy among all the variance explained by the model (44.1%), which was the third highest among all other variables behind baseline glucose (24%) and baseline insulin (13%). Furthermore, *PRKCB* rs11649514 was associated with a change in the aMT6s level ($P = 0.036$, **Figure 4c**) despite no difference in the baseline aMT6s level between genotype groups ($P = 0.29$). The *PRKCB* rs11649514 A allele carriers had an average 1.87 ± 1.97 ng/mg decrease in the aMT6s level compared with the homozygous C/C carriers, who had a 5.35 ± 0.76 ng/mg decrease. Subgroup analysis demonstrated no correlations between glucose and aMT6s changes by rs11649514 genotype groups (**Supplemental Table 3**).

PEAR Caucasians who received atenolol add-on therapy had very similar characteristics as the atenolol monotherapy group (**Table 1**). When receiving atenolol as an add-on therapy to HCTZ for ~9 weeks, a mean 2.4 ± 11.6 mg/dl ($P = 0.0029$) increase in glucose was observed (**Table 2**). No individuals were excluded due to outlier values for glucose change. Similar to the monotherapy group, rs11649514 had an MAF of 0.12 in the atenolol add-on group, and two individuals were observed carrying the A/A genotype and 44 carrying the A/C genotype. In the atenolol add-on therapy group, the A allele carriers had a consistent direction of pharmacogenetic effect as the monotherapy group, although not statistically significant. The A allele carriers had an average 3.7 ± 1.5 mg/dl increase in glucose and the homozygous C/C carriers had a 2.1 ± 0.9 mg/dl increase in glucose (one-sided $P = 0.18$, **Figure 4b**).

DISCUSSION

Through integrating genetic, metabolite, and clinical information from PEAR study participants, the results described herein demonstrate that in Caucasians with uncomplicated hypertension, ~9 weeks of atenolol monotherapy at clinically recommended doses was associated with significantly increased fasting glucose and decreased aMT6s levels. However, there was no correlation between glucose and aMT6s responses. Among the 16 melatonin pathway candidate genes investigated, *PRKCB* was associated with a change in fasting glucose after atenolol monotherapy, with one SNP (rs11649514) reaching statistical significance and four additional SNPs reaching nominal significance. To our knowledge, this is the first study investigating the relationship between the melatonin pathway and atenolol-related glucose dysregulation. Moreover, the prospective nature of PEAR provides the advantage of a clearly defined phenotype and more complete data collection, which is essential for genetic studies, and allowed the possibility to derive a causal relationship.

We observed increased fasting glucose and decreased aMT6s levels after ~9 weeks of atenolol monotherapy at commonly prescribed doses. The observed glucose increase was consistent with previous studies that indicate 6–12 months of atenolol treatment increases fasting glucose in mild to moderate hypertensives.[30,31] Previous studies noted a reduction in nocturnal melatonin release with atenolol treatment and a high variability in melatonin levels among healthy individuals.[16,18,19] Our findings in aMT6s response to atenolol treatment are in line with these observations, and we observed a high variability in endogenous aMT6s levels, even after exclusion of known night workers. Additionally, we observed interindividual variability in both the glucose and aMT6s responses to atenolol, which support a possible pharmacogenetic effect.

In our study, the atenolol-related decrease in endogenous aMT6s level was not correlated with the observed glucose change. While this observation is contrary to our hypothesis, there are several possible explanations for this. First, evidence from cell and animal studies suggest that the melatonin pathway plays an important role in regulating glucose metabolism,[3,32] and that melatonin supplementation

in DM-prone rodents ameliorates impaired glucose homeostasis and insulin resistance.[33–35] However, caution should be taken when extrapolating results from nocturnal animals to humans, given the difference in diurnal pattern. Second, although our understanding of atenolol-related glucose dysregulation is not yet complete, potential mechanisms for β-blocker-related hyperglycemia, including reduced insulin-stimulated glucose uptake due to vasoconstriction, impairment of insulin release, weight gain, and increased hepatic glucose output have been proposed.[36,37] It is possible that the observed aMT6s change might not play a major part in affecting glucose metabolism relative to other mechanisms.

Third, previous observational studies in humans suggest that a lower endogenous aMT6s concentration, measured from the first morning urine at baseline, is associated with greater risk for both insulin resistance and type 2 DM.[4,5] In these studies, individuals were divided into categories based on a single measurement of normalized aMT6s level. Individuals characterized in the lowest aMT6s level category had more than twofold increased risk for insulin resistance or developing type 2 DM compared with individuals in the highest category.[4,5] However, these were observational studies, and thus could not conclude a causal relationship between endogenous aMT6s level and risk for glucose dysregulation. Lastly, given the high interindividual variability of melatonin level, a mean 4.5 ng/mg decrease in aMT6s level may be within the normal physiological variability of melatonin, thus limiting the impact on glucose homeostasis. In the observational studies described above, the median difference in aMT6s levels between each of the aMT6s level categories was at least 10 ng/mg, and statistical significance for increased risk for type 2 DM or insulin resistance was observed between individuals in the highest category and those in the lowest category.[4,5]

We observed a potential pharmacogenetic association between *PRKCB* and atenolol-related glucose change. *PRKCB* encodes two alternative spliced major isoforms of protein kinase C (PKC) beta, PKCβ1 and PKCβ2.[38] PKC is involved in melatonin downstream signaling, including the melatonin-insulin regulation pathway.[27,28,32] Melatonin receptor signaling cascades have been suggested to play a crucial role in determining an individual's risk for glucose dysregulation.[9–11,39,40] PKCβ also has a well-characterized role in regulating insulin signaling and insulin resistance,[38,41] and a promoter polymorphism (rs2575390) has been associated with insulin resistance in Caucasians, potentially via reduced promoter activity.[42] SNP rs11649514 is located in the 12th intron of *PRKCB*, and is not in linkage disequilibrium with rs2575390. According to the HaploReg database,[43] rs11649514 is not in high linkage disequilibrium with other variants in Caucasians in the 1000 Genomes database, is located in a conserved region, and is associated with chromatin regulation and six altered regulatory motifs. The functional consequences of rs11649514, or whether it is in linkage disequilibrium with other functional SNPs, need further investigation. Nevertheless, we observed a significant differential glucose response to atenolol by rs11649514 genotype among individuals who received atenolol monotherapy. In individuals who received atenolol added to HCTZ therapy, this differential glucose response by genotype was directionally consistent with the monotherapy group but with a smaller effect size, and did not achieve statistical significance. Given that HCTZ is well documented to impair glucose regulation and increase the risk for DM,[12–14] potential confounding of the pharmacogenetic-mediated glucose effect by HCTZ treatment may exist in individuals receiving combination therapy.

This study has several limitations. We only included Caucasian individuals, as previous findings of the association between glucose dysregulation and *MTNR1B* or low melatonin concentration were originally discovered in Caucasian populations. Generalization of our findings to other race groups is not possible. We only focused on melatonin candidate genes, and did not include genetic variations with established association with fasting glucose level, which was a separate effort and has been published previously.[29] While consistent evidence from long-term clinical trials demonstrated that atenolol-based treatment strategies are associated with increased risk for developing DM,[12,13] we observed only moderate increases in glucose level after short-term atenolol therapy. Exposure to atenolol for only 9 weeks may not have elicited the full adverse glucose effect, and may have led to an underestimation of the potential glucose change. Although the glucose increase we observed is moderate, data from individuals with glucoses within the normal range ($<$100 mg/dl) demonstrated that each mg/dl increase in fasting glucose is associated with a 6% increased risk for DM, independent of other risk factors.[44] Moreover, fasting glucose level has been independently associated with a progressively increased risk for cardiovascular disease before reaching the cutoff for DM diagnosis.[45–47] PKCβ is involved in a wide range of pathways, and our genetic finding may not be specific to the melatonin pathway. Nevertheless, *PRKCB* rs11649514 genotype was not associated with baseline levels of either glucose or aMT6s level, but was associated with their changes during atenolol treatment. These interesting observations could suggest that rs11649514 has atenolol response-specific associations, and provide further support on the relevance of our finding to the melatonin pathway. PEAR participants were not instructed to provide a first morning urine sample, and the study design did not specify a specific timepoint for urine sample collection, which would have provided a more consistent estimation of endogenous aMT6s levels among participants before and after atenolol treatment. However, based on available urine sampling time documentation, $\sim$95% of the PEAR samples were collected during a morning study visit, as a consequence of requiring PEAR participants to fast overnight. Additionally, stringent exclusion criteria were implemented to eliminate potential confounding of the diurnal pattern of melatonin secretion, as described above.

β-blockers as a class consistently account for more than 160 million prescriptions per year in the US, and are used in approximately one in every three elderly Americans.[48,49] Treatment with atenolol, one of the most commonly prescribed β-blockers worldwide, may represent an environmental risk factor that deteriorates glycemic control in individuals who are genetically predisposed to DM or hyperglycemia. Increased glucose could serve as an intermediate phenotype for DM since individuals with higher

fasting glucose have significantly increased risk for developing DM, even within the normal-glycemic range.[44,50] A better understanding of potential mechanisms and genetic predictors of atenolol-related glucose dysregulation could open up the potential for individualized risk assessment, and streamlined use of diabetogenic medications in high-risk individuals.

In conclusion, we conducted the first study investigating the association between the melatonin pathway and atenolol-related glucose dysregulation, through incorporating genetic, melatonin metabolite, and clinical information from atenolol-treated Caucasians with uncomplicated hypertension. We confirmed that atenolol treatment at clinically recommended doses increases glucose levels and decreases melatonin metabolite levels. We add to the available literature that the decrease in melatonin metabolite level is not correlated with the post-atenolol glucose dysregulation. Additionally, *PRKCB,* a gene involved in the melatonin–insulin regulatory pathway, may play a role in mediating atenolol-related hyperglycemia. The role of PKCβ in the melatonin signaling cascade in relation to risk for glucose dysregulation in the presence or absence of atenolol treatment warrants further investigation. Validated genetic predictors of atenolol-related glucose dysregulation could be utilized for individualized risk assessment and a tailored, personalized drug therapy management approach, which could result in the avoidance of diabetogenic medications like β-blockers in individuals at high risk for DM.

Acknowledgments. PEAR was supported by the National Institute of Health Pharmacogenetics Research Network grant U01-GM074492 and the National Center for Advancing Translational Sciences under award number UL1 TR000064 (University of Florida); UL1 TR000454 (Emory University); and UL1 TR000135 (Mayo Clinic). PEAR efforts at the Mayo Clinic were also supported by funds from the Mayo Foundation. We also acknowledge and thank the contribution of the study participants, clinical and research staff, and study physicians.

Author Contributions. S-W.C. wrote the article; R.C-D., S-W.C., Y.G., C.W.M., T.L., A.L.B., J.G.G., A.B.C., S.T.T., and J.A.J. designed the research; S-W.C., Y.G., C.W.M., T.L., and N.N.K. performed the research; S-W.C. analyzed the data; R.C-D., and J.A.J. contributed new reagents/analytical tools.

Conflict of Interest/Disclosure. Y.G., T.Y.L., A.L.B., J.G.G., S.T.T., J.A.J., and R.M.C. had support from the National Institutes of Health, National Center for Advancing Translational Sciences, and Mayo Foundation for the submitted work. All authors declare no financial or other relationships with any organizations that might have a conflict of interest with the submitted work in the previous 3 years.

1. Axelrod, J. The pineal gland: a neurochemical transducer. *Science* **184**, 1341–1348 (1974).
2. Little, K.Y., Kirkman, J.A. & Duncan, G.E. Beta-adrenergic receptor subtypes in human pineal gland. *J. Pineal Res.* **20**, 15–20 (1996).
3. Peschke, E., Bahr, I. & Muhlbauer, E. Melatonin and pancreatic islets: interrelationships between melatonin, insulin and glucagon. *Int. J. Mol. Sci.* **14**, 6981–7015 (2013).
4. McMullan, C.J., Schernhammer, E.S., Rimm, E.B., Hu, F.B. & Forman, J.P. Melatonin secretion and the incidence of type 2 diabetes. *JAMA* **309**, 1388–1396 (2013).
5. McMullan, C.J., Curhan, G.C., Schernhammer, E.S. & Forman, J.P. Association of nocturnal melatonin secretion with insulin resistance in nondiabetic young women. *Am. J. Epidemiol.* **178**, 231–238 (2013).
6. Kawakami, N., Takatsuka, N. & Shimizu, H. Sleep disturbance and onset of type 2 diabetes. *Diabetes Care* **27**, 282–3 (2004).
7. Scheer, F.A., Hilton, M.F., Mantzoros, C.S. & Shea, S.A. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc. Natl. Acad. Sci. USA.* **106**, 4453–4458 (2009).
8. Leproult, R., Holmback, U. & Van Cauter, E. Circadian misalignment augments markers of insulin resistance and inflammation, independently of sleep loss. *Diabetes* **63**, 1860–1869 (2014).
9. Bouatia-Naji, N. *et al.* A variant near MTNR1B is associated with increased fasting plasma glucose levels and type 2 diabetes risk. *Nat. Genet.* **41**, 89–94 (2009).
10. Prokopenko, I. *et al.* Variants in MTNR1B influence fasting glucose levels. *Nat. Genet.* **41**, 77–81 (2009).
11. Lyssenko, V. *et al.* Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. *Nat. Genet.* **41**, 82–88 (2009).
12. Gupta, A.K. *et al.* Determinants of new-onset diabetes among 19,257 hypertensive patients randomized in the Anglo-Scandinavian Cardiac Outcomes Trial–Blood Pressure Lowering Arm and the relative influence of antihypertensive medication. *Diabetes Care* **31**, 982–988 (2008).
13. Cooper-Dehoff, R. *et al.* Predictors of development of diabetes mellitus in patients with coronary artery disease taking antihypertensive medications (findings from the INternational VErapamil SR-Trandolapril STudy [INVEST]). *Am. J. Cardiol.* **98**, 890–4 (2006).
14. Elliott, W.J. & Meyer, P.M. Incident diabetes in clinical trials of antihypertensive drugs: a network meta-analysis. *Lancet* **369**, 201–207 (2007).
15. Vaughan, G.M. *et al.* Nocturnal elevation of plasma melatonin and urinary 5-hydroxyindoleacetic acid in young men: attempts at modification by brief changes in environmental lighting and sleep and by autonomic drugs. *J. Clin. Endocrinol. Metab.* **42**, 752–764 (1976).
16. Stoschitzky, K. *et al.* Influence of beta-blockers on melatonin release. *Eur. J. Clin. Pharmacol.* **55**, 111–115 (1999).
17. Brismar, K., Hylander, B., Eliasson, K., Rossner, S. & Wetterberg, L. Melatonin secretion related to side-effects of beta-blockers from the central nervous system. *Acta Med. Scand.* **223**, 525–530 (1988).
18. Cowen, P.J., Fraser, S., Sammons, R. & Green, A.R. Atenolol reduces plasma melatonin concentration in man. *Br. J. Clin. Pharmacol.* **15**, 579–581 (1983).
19. Nathan, P.J., Maguire, K.P., Burrows, G.D. & Norman, T.R. The effect of atenolol, a beta1-adrenergic antagonist, on nocturnal plasma melatonin secretion: evidence for a dose-response relationship in humans. *J. Pineal Res.* **23**, 131–135 (1997).
20. Johnson, J.A. *et al.* Pharmacogenomics of antihypertensive drugs: rationale and design of the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study. *Am. Heart J.* **157**, 442–449 (2009).
21. Graham, C., Cook, M.R., Kavet, R., Sastre, A. & Smith, D.K. Prediction of nocturnal plasma melatonin from morning urinary measures. *J. Pineal Res.* **24**, 230–238 (1998).
22. Nowak, R., McMillen, I.C., Redman, J. & Short, R.V. The correlation between serum and salivary melatonin concentrations and urinary 6-hydroxymelatonin sulphate excretion rates: two non-invasive techniques for monitoring human circadian rhythmicity. *Clin. Endocrinol. (Oxf.).* **27**, 445–452 (1987).
23. BÜHLMANN. BÜHLMANN 6-Sulfatoxymelatonin ELISA. https://www.alpco.com/pdfs/01/01-EK-M6S.pdf:; 2011.
24. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
25. Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A. & Reich, D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* **38**, 904–909 (2006).
26. Reiter, R.J. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocr. Rev.* **12**, 151–80 (1991).
27. Kanehisa, M. & Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30 (2000).
28. Kanehisa, M. *et al.* Data, information, knowledge and principle: back to metabolism in KEGG. *Nucleic Acids Res.* **42**, D199-205 (2014).
29. Gong, Y. *et al.* PROX1 gene variant is associated with fasting glucose change after antihypertensive treatment. *Pharmacotherapy* **34**, 123–130 (2014).
30. Aberg, H., Morlin, C. & Lithell, H. Different long-term metabolic effects of enalapril and atenolol in patients with mild hypertension. *EGTA Group. J. Hum. Hypertens.* **9**, 149–153 (1995).
31. Helgeland, A., Strommen, R., Hagelund, C.H. & Tretli, S. Enalapril, atenolol, and hydrochlorothiazide in mild to moderate hypertension. A comparative multicentre study in general practice in Norway. *Lancet* **1**, 872–875 (1986).
32. Peschke, E. Melatonin, endocrine pancreas and diabetes. *J. Pineal Res.* **44**, 26–40 (2008).
33. Nishida, S., Segawa, T., Murai, I. & Nakagawa, S. Long-term melatonin administration reduces hyperinsulinemia and improves the altered fatty-acid compositions in type 2 diabetic rats via the restoration of Delta-5 desaturase activity. *J. Pineal Res.* **32**, 26–33 (2002).
34. Sartori, C. *et al.* Melatonin improves glucose homeostasis and endothelial vascular function in high-fat diet-fed insulin-resistant mice. *Endocrinology* **150**, 5311–5317 (2009).

35. Zanuto, R. *et al.* Melatonin improves insulin sensitivity independently of weight loss in old obese rats. *J. Pineal Res.* **55**, 156–165 (2013).
36. Sarafidis, P.A. & Bakris, G.L. Antihypertensive treatment with beta-blockers and the spectrum of glycaemic control. *QJM.* **99**, 431–436 (2006).
37. Barzilay, J.I., Davis, B.R. & Whelton, P.K. The glycemic effects of antihypertensive medications. *Curr. Hypertens. Rep.* **16**, 410 (2014).
38. Kawakami, T., Kawakami, Y. & Kitaura, J. Protein kinase C beta (PKC beta): normal functions and diseases. *J. Biochem.* **132**, 677–682 (2002).
39. Bonnefond, A. *et al.* Rare MTNR1B variants impairing melatonin receptor 1B function contribute to type 2 diabetes. *Nat. Genet.* **44**, 297–301 (2012).
40. Peschke, E. *et al.* Melatonin and type 2 diabetes - a possible link? *J. Pineal Res.* **42**, 350–358 (2007).
41. Tabit, C.E. *et al.* Protein kinase C-beta contributes to impaired endothelial insulin signaling in humans with diabetes mellitus. *Circulation* **127**, 86–95 (2013).
42. Osterhoff, M.A. *et al.* Identification of a functional protein kinase Cbeta promoter polymorphism in humans related to insulin resistance. *Mol. Genet. Metab.* **93**, 210–215 (2008).
43. Ward, L.D. & Kellis, M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res.* **40**, D930–D934 (2012).
44. Nichols, G.A., Hillier, T.A. & Brown, J.B. Normal fasting plasma glucose and risk of type 2 diabetes diagnosis. *Am. J. Med.* **121**, 519–524 (2008).
45. Jansson, S.P., Svardsudd, K. & Andersson, D.K. Effects of fasting blood glucose levels and blood pressure and treatment of diabetes and hypertension on the incidence of cardiovascular disease: a study of 740 patients with incident Type 2 diabetes with up to 30 years' follow-up. *Diabet. Med.* **31**, 1055–1063 (2014).
46. Sung, J., Song, Y.M., Ebrahim, S. & Lawlor, D.A. Fasting blood glucose and the risk of stroke and myocardial infarction. *Circulation* **119**, 812–819 (2009).
47. Stacey, R.B., Leaverton, P.E., Schocken, D.D., Peregoy, J.A. & Bertoni, A.G. Prediabetes and the association with unrecognized myocardial infarction in the multi-ethnic study of atherosclerosis. *Am. Heart J.* **170,** 923–928 (2015).
48. The Use of Medicines in the United States: Review of 2011: IMS Institute for Healthcare Informatics 2012.
49. Health, United States, 2013: With Special Feature on Prescription Drugs: National Center for Health Statistics 2014.
50. Tirosh, A. *et al.* Normal fasting plasma glucose levels and type 2 diabetes in young men. *N. Engl. J. Med.* **353**, 1454–1462 (2005).

Supplementary information accompanies this paper on the *Clinical and Translational Science* website. (http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1752-8062)