METABOLISM CLINICAL AND EXPERIMENTAL 64 (2015) 1650–1657

Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Brief Report

Common type 2 diabetes risk variant in *MTNR1B* worsens the deleterious effect of melatonin on glucose tolerance in humans

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ARTICLE INFO

Article history:
Received 7 February 2015
Accepted 6 August 2015

Keywords:
MTNR1B
Type 2 diabetes
Glucose tolerance
Melatonin
Genetic variation

ABSTRACT

Aims. The common *MTNR1B* genetic variant rs10830963 is associated with an increased risk of type 2 diabetes (T2D). To date, no experimental study has tested the effect of the *MTNR1B* variant on glucose metabolism in humans during exposure of the melatonin receptors to their ligand. The aim of this study was to investigate whether this *MTNR1B* variant influenced the effect of melatonin (5 mg) on glucose tolerance assessed by an oral glucose tolerance test (OGTT; 75 g) at different times of the day (morning and evening) as compared to a placebo.

Methods. Seventeen normoglycemic women (24 ± 6 years; BMI 23.0 ± 3.3 kg/m^2) completed the study (11 carriers of the risk allele [CG] and 6 noncarriers [CC]).

Results. The effect of melatonin on glucose tolerance depended on the genotype. In the morning, the effect of melatonin (melatonin-placebo) on the glucose area under the curve (AUC) above baseline differed significantly ($P = 0.036$) between the carriers and noncarriers. This effect of melatonin in the carriers was six times as large as that in the noncarriers. The *MTNR1B* SNP explained over one-quarter (26%) of the inter-individual differences in the effect of melatonin on glucose AUC. However, in the evening, the effect of melatonin on glucose AUC of the carriers and noncarriers did not differ significantly ($P > 0.05$).

Conclusions. *MTNR1B* rs10830963 risk variant worsens the effect of melatonin on glucose tolerance, suggesting the importance of genotyping and personalized recommendations, especially in people consuming food when melatonin levels are elevated. Large-scale

Abbreviations: AUC, area under the curve; CIR, corrected insulin response; C_{120}, concentration 120 min after the start of the OGTT; DI, disposition index; GWAS, genome-wide-association studies; ISI, Insulin Sensitivity Index; MAF, minor allele frequency; MT2, melatonin receptor type 2 (Mel1b); OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; TF, time fasting.

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http://dx.doi.org/10.1016/j.metabol.2015.08.003

studies in vulnerable populations are necessary to translate these results into real-world, clinically relevant recommendations.

1. Introduction

The discovery of *MTNR1B* as a novel type 2 diabetes (T2D) risk gene sparked great interest in the role of melatonin in glucose metabolism [1–5]. This gene encodes the melatonin type 2 receptor Mel1b (MT2), a G protein-coupled receptor that mediates effects of melatonin and that is expressed in various tissues including pancreatic β-cells.

Genome-wide-association studies (GWAS) have found an association between *MTNR1B* rs10830963 and increased fasting plasma glucose levels and T2D risk [1,2,6]. Follow-up studies confirmed this association with T2D risk [7–9]. However, although the genetic association of rs10830963 with T2D risk is now established, the functional impact of this *MTNR1B* variant on glucose control is poorly characterized, because – to date – measures of glucose control, insulin secretion and insulin sensitivity have been performed during the daytime, when endogenous melatonin levels are very low and thus without the ligand of the receptor available to induce its effect.

Nevertheless, Mel1b is expressed in β-cells implying that *MTNR1B* rs10830963 might affect pancreatic glucose sensing and/or insulin release [1,6]. Furthermore, Lyssenko et al. showed increased *MTNR1B* expression in pancreatic islets of carriers of the rs10830963 risk genotype compared to non-carriers and impaired insulin responses to oral and intravenous glucose, reporting a negative correlation between *MTNR1B* mRNA levels and insulin secretion [1]. Indeed, association of the rs10830963 risk allele with impaired insulin secretion is well-established and an inverse association with insulin sensitivity has also been reported [10,11]. Importantly, among more than 60 variants associated with T2D and/or glycemic traits, the common *MTNR1B* risk variant rs10830963 had the most significant adverse influence on the disposition index, a product of both insulin secretion and insulin sensitivity, as estimated by an oral glucose tolerance test (OGTT) [12].

Despite that GWAS have provided robust evidence for an association of the common *MTNR1B* risk variant with glycemic traits, the magnitude of its effect is small. Notably, all the metabolic assessments in these GWAS were performed during daytime hours when endogenous melatonin concentrations were very low or absent [13]. In addition, only a few functional studies have examined the impact of manipulating melatonin concentration on glycemic measures [14,15], and very few such studies have included humans. Functional studies in animal models are restricted mostly to nocturnal rodents, from which data cannot be directly translated to (diurnal) humans [16]. While in humans melatonin is produced when we are sleeping and fasting, in nocturnal rodents melatonin is produced when they are awake and eating, and as such, the biological effects of melatonin may be very different [13]. To date no experimental study has been performed in humans in order to test the effect of the *MTNR1B* variant on glucose metabolism during exposure of the melatonin receptors to their ligand.

A new approach to investigate the role of melatonin and the *MTNR1B* diabetes risk variant in glucose metabolism could be to test the effect of exogenous melatonin administration during the daytime on glucose metabolism between carriers and non-carriers of the *MTNR1B* risk variant. The aim of the current study was to assess whether *MTNR1B* rs10830963 influences the magnitude of effect of exogenous melatonin administration on glucose tolerance at different times of the day (morning and evening), as compared to placebo administration.

2. Research Design and Methods

2.1. Subjects

This study consisted of members of the female rugby team at the University of Murcia, Spain who fulfilled both inclusion and exclusion criteria and for whom blood for DNA extraction was available (n = 17 from a total of n = 21). Data from all the participants were collected within one month (April) to minimize any influence of seasonal effects. The population was a homogenous set of very healthy, young women. The exclusion criteria included endocrine, renal, hepatic, or psychiatric disorders; impaired glucose tolerance based on standard criteria; prescription drugs or other pharmacological treatment, except oral contraceptives; major weight fluctuations ±3 kg in the past 3 years; recent shift work (within the last 2 years); travel across more than one time zone (within the last 1 year); and sleep disorders.

Seventeen nondiabetic, nonobese, young women of European ancestry (mean ± SD; 24 ± 6 years; BMI: 23.0 ± 3.3 kg/m^2) completed the study (Table 1). Only two of the participants were smokers. All the participants had normal fasting glucose and insulin levels and normal glucose tolerance (<7.8 mmol/L, 2 h after 75 g OGTT; Table 1). There were no significant differences in the anthropometric, biochemical (including morning melatonin saliva concentrations), chronotype (morningness–eveningness scores), or habitual sleep characteristics of the carriers and noncarriers of the risk allele. The participants gave written informed consent, and the study was approved by the local Ethics Committee of the "Virgen de la Arrixaca" University Hospital in Murcia, Spain.

2.2. OGTT

To determine the effects of melatonin administration on glucose tolerance, each participant underwent four OGTTs after a 10 h fast on four nonconsecutive days, two after a placebo and two after melatonin (5 mg) administration, as previously described [15]. The placebo was administered once in the morning (9 A.M.) and once in the evening (9 P.M.). The same was true for melatonin administration. For each of the four OGTTs, an oral glucose load of 75 g was given 15 min after the administration of the placebo or melatonin. During each OGTT, blood samples were obtained immediately prior to the administration of the melatonin/placebo and 30, 60, 90,