A circadian rhythm-related \textit{MTNR1B} genetic variant (rs10830963) modulates glucose metabolism and insulin resistance after body weight loss secondary to biliopancreatic diversion surgery

La variante genética \textit{MTNR1B} (rs10830963), relacionada con el ritmo circadiano, modula el metabolismo de la glucosa y la resistencia a la insulina después de la pérdida de peso corporal secundaria a una cirugía de derivación biliopancreática

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Abstract

**Objective:** the rs10830963 SNP of the \textit{MTNR1B} gene may be related with biochemical changes after weight loss induced by caloric restriction. We investigated the role of this SNP on biochemical parameters after biliopancreatic diversion (BPD) surgery in morbid obese subjects.

**Patients and methods:** one hundred and fifty-four patients with morbid obesity, without diabetes mellitus type 2, were enrolled. Their biochemical and anthropometric parameters were recorded before the procedure and after one, two, and three years of follow-up. All subjects were genotyped (rs10830963) at baseline.

**Results:** the decrease in fasting insulin levels seen after the first year (delta: -3.9 ± 1.2 mIU/L vs. -1.8 ± 1.1 mIU/L; \(p = 0.03\)), the second year (delta: -5.0 ± 0.3 mIU/L vs. -2.3 ± 0.2 mIU/L; \(p = 0.01\)) and the third year (delta: -5.1 ± 1.9 mIU/L vs. -2.8 ± 1.1 mIU/L; \(p = 0.02\)) was higher in non-G-allele carriers than in G-allele carriers. Additionally, the improvement of HOMA-IR levels at year one (delta: -0.7 ± 0.2 mIU/L vs. -0.2 ± 0.2 mIU/L; \(p = 0.03\)), year two (delta: -1.0 ± 0.3 mIU/L vs. -0.5 ± 0.2 mIU/L; \(p = 0.01\)) and year three (delta: -1.2 ± 0.3 mIU/L vs. -0.4 ± 0.2 mIU/L; \(p = 0.03\)) was also higher in non-G-allele carriers than in G-allele carriers. Finally, basal glucose levels after the first year (delta: -10.1 ± 2.4 mg/dL vs. -3.6 ± 1.8 mg/dL; \(p = 0.02\)), the second year (delta: -16.0 ± 2.3 mg/dL vs. -8.4 ± 2.2 mg/dL; \(p = 0.01\)) and the third year (delta: -17.4 ± 3.1 mg/dL vs. -8.8 ± 2.9 mg/dL; \(p = 0.03\)) were higher in non-G-allele carriers than in G-allele carriers, too. Improvements seen in comorbidities were similar in both genotype groups.

**Conclusion:** our study showed an association of the rs10830963 \textit{MTNR1B} polymorphism after massive weight loss with lower glucose response, insulin resistance, and fasting insulin levels in G-allele carriers.

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Ethical approval: all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (HVUVA committee 3/2017) and with the 1964 Helsinki Declaration and later amendments or comparable ethical standards.

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Informed consent: an informed consent was obtained from all participants included in the study.

Author’s contribution: Daniel de Luís designed the study and performed the statistical analysis. Olatz Izaola performed the anthropometric evaluation and control of dietary intake. David Pacheco carried out the technical surgery procedures. David Primo performed the biochemical evaluations and genotypes. All the above authors contributed to the data analysis and reviewed the study critically for important intellectual content. They approved the version to be published and are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

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INTRODUCTION

The prevalence of obesity is alarming, and is considered a public health problem worldwide (1). Bariatric surgery, including bilipancreatic diversion, gastric bypass, sleeve gastrectomy, and son on, has emerged as the most effective strategy to treat obesity and its associated comorbidities (2). Non-surgical treatments may fail to provide substantial and long-term weight loss in morbid obesity cases (3,4). Among all the surgical procedures for the treatment of morbid obesity, BPD is considered a good procedure due to its low morbidity and mortality rate, and its efficacy (5).

The response of individuals with obesity, regardless of the form of treatment, is heterogeneous, indicating that the genetic background of obese subjects, besides environment, is involved in the success of weight loss therapy (6,7). The role of genetics in the modulation of weight loss is related to genes involved in different pathways such as thermogenesis regulation, appetite and satiety circuits, adipogenesis, energy expenditure, and cardiovascular risk factors.

The action of melatonin is mediated by two receptors: melatonin receptor 1 (MT1, encoded by MTNR1A) and melatonin receptor 2 (MT2, encoded by MTNR1B). MTNR1B is the ubiquitous receptor. A lot of evidence shows that particularly melatonin rhythm plays crucial roles in a variety of metabolic functions as an antioxidant, appetite controller, anti-inflammatory chronobiotic, and possibly epigenetic regulator (8). One of the common SNPs (single nucleotide polymorphisms) in the melatonin receptor type 1B (MTNR1B) gene is rs10830963. It has been related to impaired melatonin rhythm and signaling (8). The investigations studying the effect of this polymorphism in response to weight loss strategies are scarce, and there is no study in the literature that evaluates the effect of this SNP on biochemical modifications after bariatric surgery. Gorji et al. (9) reported that the rs10830963 SNP could be related with weight loss as induced by caloric restriction. The same investigators (10) have detected a relationship of this SNP with lipid response after a 2-year, weight-loss diet. In another study a significant interaction was detected between this SNP (11) and a dietary intervention with a hypocaloric, Mediterranean style-based diet on body weight loss and insulin resistance, too.

Therefore, we hypothesized that the MTNR1B genotype rs10830963 might influence metabolic changes after bilipancreatic diversion surgery in morbidly obese subjects during 3 years of follow-up.

MATERIALS AND METHODS

SUBJECTS AND SURGICAL PROCEDURE

We conducted a prospective study approved by the local Ethics Committee (HURH-Committee-3/2016). Individuals with an age range of 35-55 years who underwent bariatric surgery (bilipancreatic diversion) were included and signed a written informed consent. All the procedures performed in this study were in accordance with the Declaration of Helsinki.

A total of 154 Caucasian participants (112 females and 42 males) with grade-III obesity (body mass index ≥ 40) and without diabetes mellitus were enrolled in the study (Table I). The BPD without duodenal switch technique consisted of the set-up of a 175-cm alimentary limb and 70-cm common limb with the addition of partial gastrectomy and closure of the duodenal stump, transection of the small bowel half way from Treitz’s angle to the ileocecal valve, followed by a Roux-en-Y gastroenterostomy on the distal bowel loop and an end-to-side enterolocecestomy of the proximal bowel loop on the ileum, 50-75 cm before the ileocecal valve. After the first month past the surgical procedure, all study participants received the same diet based on the consumption of 1100-1400 calories; non-protein calories were distributed among fats (30 %, divided into 10 % saturated, 15 % monounsaturated, and 5 % polyunsaturated), and carbohydrates (70 %). Protein consumption was 1.5 g per kg of ideal weight (BMI, 22 kg/m²). All patients took two Multicentrum® tablets daily.
**Table I. Preoperative characteristics of the study participants**

|                          | Baseline | 1 year | 2 years | 3 years |
|--------------------------|----------|--------|---------|---------|
|                         | n = 154  | n = 153| n = 150 | n = 150 |
| Morbid obese             | 136      | 147    | 147     | 150     |
| Super obese              | 18       | 6      | 3       | 0       |
| Gender (female/male)     | 112/42   | 111/42 | 109/41  | 109/41  |
| Age (years)              | 47.1 ± 8.0 | 47.5 ± 4.8 | 48.0 ± 6.2 | 48.9 ± 5.9 |
| BMI (kg/m²)              | 49.8 ± 5.0 | 38.1 ± 4.0 | 34.7 ± 3.2 | 33.0 ± 4.1 |

Morbid: BMI > 40 kg/m² and < 50 kg/m². Superobese: > 50 kg/m². ANOVA test and Bonferroni’s post-hoc test.

**PROCEDURE**

Blood pressure, body weight, waist circumference, serum lipid levels (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides), insulin, fasting glucose, homeostasis model assessment of insulin resistance (HOMA-IR), and associated morbidities (percentage of study participants with hypertension or hyperlipidemia) were obtained at the last visit prior to surgery (baseline) and at each later postoperative visits after 1, 2 and 3 years following the operation. Percent excess weight loss (EWL%) was calculated in each visit. Exclusion criteria included severe liver or chronic renal disease, malignancies, coagulopathy, gastrointestinal tract diseases, and drug therapy for diabetes mellitus. Inclusion criteria included body mass index (BMI) ≥ 40 kg/m² and a history of failed weight loss on hypocaloric diets prior to surgery. The genotype of the MTNR1B gene (rs10830963) was evaluated in all subjects.

**ANTHROPOMETRIC PARAMETERS AND BLOOD PRESSURE**

Body weight was determined to an accuracy of 10 g using a manual scale (Seca, Birmingham, United Kingdom). Height was measured with the patient in an upright position to the nearest centimeter using a stadiometer (Seca Birmingham, United Kingdom). BMI was calculated using the formula: (weight [kg] / height x height [m²]); morbid obese patients were classified at 40–49.9 kg/m², and superobese obese subjects at above 50 kg/m². Waist circumferences (WC) were measured with a flexible, non-stretchable measuring tape (Type SECA, SECA, Birmingham, UK). Percent excess weight loss (EWL%) was calculated using the formula: (preoperative weight – current body weight x 100 / preoperative weight – ideal weight). Ideal weight was calculated with an ideal BMI of 22 kg/m². A bioimpedanciometry (BIA) was performed on all subjects after a fast of at least 8 hours; subjects were warned that they could not exercise or drink alcohol within 48 hours prior to the test. An alternating current of 0.8 mA at 50 kHz, produced by a calibrated signal generator (EFG, Akern, Firenze, Italy) (12), was used and applied to the skin by adhesive electrodes placed on the back of the hand and right foot. The parameter analyzed with BIA was total fat mass (kg). Blood pressure was measured twice after a 10-minute rest with a sphygmomanometer (Omron, Los Angeles, California, USA) and averaged.

The presence of comorbidities was defined as hypertriglyceridemia (triglycerides > 150 mg/dL), hypertension (systolic and diastolic blood pressures higher than 130 and 85 mmHg, respectively), low HDL cholesterol (< 40 mg/dL and 50 mg/dL for men and women, respectively). All patients with hypertension or hypertriglyceridemia were taking drugs at baseline (13).

**BIOCHEMICAL PARAMETERS AND GENOTYPING**

Blood samples were taken after an overnight fast of 10 hours. After centrifugation, the serum were stored at -40 °C. Plasma glucose levels were determined by using an automated glucose-oxidase method (glucose analyzer 2, Beckman Instruments, Fullerton, California, USA). Plasma insulin levels were measured by enzymatic colorimetric means (Insulin, WAKO Pure-Chemical Industries, Osaka, Osaka, Japan) (14), and the homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using values with the following formula: (glucose x insulin) / 22.5 (15). Serum total cholesterol, HDL-cholesterol, and triglyceride concentrations were determined by enzymatic colorimetric assays (Roche Diagnostics, Mannheim, Waden Winterberg, Germany). LDL-cholesterol was determined using Friedewald’s formula (16).

Genomic DNA was extracted from 5 mL of whole blood using a commercially available DNA blood kit (QIAamp®, Madison, WI, USA) following the manufacturer’s instructions. The genotyping (rs10830963) was performed using customized assays with the TaqMan® OpenArray™ Genotyping platform (Thermofisher, Pittsburg, Pens., USA). Samples were loaded using the AccuFill system, and amplification was performed on the QuantStudio 12K Flex Real-Time qPCR instrument (Thermofisher, Pittsburg, Pens., USA). A total volume of 5 μL with 2.5 μL of TaqMan OpenArray Master Mix (Applied Biosystems, Foster City, CA, USA) and 2.5 μL of human DNA sample was loaded and amplified on arrays following the manufacturer’s instructions. Genotype calling and sample clustering for OpenArray assays were performed in a TaqMan Genotyper (LifeTechnologies, Carlsbad, CA, USA).
STATISTICAL ANALYSIS

The IBM SPSS Statistics, version 23.0 (IL, USA), was used for all statistical analyses. The power analysis suggested that at least 150 subjects with an expected weight loss of 30 %, with a type-I error of 0.05 and a type-II error of 0.10 (power = 0.9). All analyses were performed under a dominant genetic model with the rs10830963 G-allele as risk allele (GG + GC vs. CC).

Data are reported as mean ± standard deviation. The normal distribution of variables was studied with the Kolmogorov-Smirnov test. Non-parametric variables were analyzed with the Mann-Whitney test and Wilcoxon’s test. The parametric test was analyzed with an ANOVA test and Bonferroni’s post-hoc test. Qualitative variables and the presence of comorbidities were analyzed with the chi-squared test, with Yates’ correction as necessary, and Fisher’s test as necessary. The statistical analysis to evaluate the time per group interaction was a univariate ANCOVA, adjusting by baseline weight, age, and gender. A p-value under 0.05 was considered statistically significant.

RESULTS

A total of 154 obese subjects were included, and 150 of these completed the follow-up at 3 years. With the above-mentioned power analysis, the sample was reached. Of the whole group, 112 subjects were female (72.7 %) and 42 were male (27.3 %). The allelic frequency carrying the C and G allele was found to be 75.3 % and 24.7 % respectively. The Hardy-Weinberg equilibrium was calculated with a statistical test (chi-squared), and the variant of the MTNR1B gene was in Hardy-Weinberg equilibrium (p = 0.31). The genotypic frequency was found to be 54.5 % (84 study participants) for the CC genotype, 41.6 % (64 study participants) for the CG genotype, and 3.9 % (6 study participants) for the GG genotype. The patients were divided into two groups: G-allele carriers (GG + GC, 45.5 %) and non-G-allele carriers (CC, 54.5 %). The gender distribution was similar for the different genotypes: CC, 25.0 % (n = 21) for males and 75.0 % (n = 63) for females, CG, 27.2 % (n = 18) for males and 71.9 % (n = 46) for females, and GG, 33.3 % (n = 2) for males and 66.6 % (n = 4) for females (p = 0.19). The average age was similar in all genotype groups (CC: 47.3 ± 7.2 years vs. CG + GG: 46.9 ± 9.2 years: p = 0.36), too. Table I shows the characteristics of all the participants included in the study. As expected, during the three-year follow-up period there was a gradual loss of study participants to follow-up.

Table II shows the anthropometric and blood pressure of subjects before and after their BPD. Weight loss was similar in both genotype groups (p = 0.21). All anthropometric parameters (body weight, waist circumference, and fat mass by BIA) showed a statistically significant reduction after surgery at 1, 2, and 3 years. Systolic and diastolic blood pressures improved in both groups, too. No preoperative differences in anthropometric parameters and blood pressure were observed between genotypes. Improvements in these variables were similar for both genotypes, and all parameters were significantly different from baseline values. In G-allele and non-G-allele carriers the percentage of excess of weight loss (EWL%) showed a significant improvement at all times during follow-up.

Table III shows the changes in all biochemical parameters. No significant preoperative differences in glucose, HOMA-IR, insulin, total cholesterol, HDL-cholesterol, LDL cholesterol, and triglyceride levels were detected between genotypes. Fasting glucose, insulin, HOMA-IR, total cholesterol, LDL-cholesterol, and triglyceride levels decreased in both genotype groups during follow-up. Although the improvements seen in glucose, insulin, and HOMA-IR were significant in both genotypes, this change was significantly higher amonse non-G-allele carriers, and as soon as 12 months after surgery was this improvement noticeable. This analysis has been adjusted for age, gender, and weight. These improvements were detected later, at 24 months, in G-allele-carriers. Thus, the decrease in fasting insulin levels after the first year (delta: -3.9 ± 1.2 mIU/L vs.

| Characteristic | rs10830963 (CC, n = 84) | rs10830963 (CG or GG, n = 70) |
|----------------|--------------------------|-----------------------------|
| **0 time**     |                          |                             |
| BMI            | 47.2 ± 5.1               | 47.0 ± 4.0                  |
| Weight (kg)    | 120.5 ± 23.6             | 121.8 ± 19.2                |
| Fat mass (kg)  | 47.0 ± 10.4              | 46.7 ± 8.1                  |
| WC (cm)        | 118.1 ± 9.1              | 120.3 ± 6.1                 |
| EWL%           | -                        | -                           |
| SBP (mmHg)     | 148.0 ± 7.1              | 149.1 ± 9.1                 |
| DBP (mmHg)     | 89.1 ± 4.1               | 90.1 ± 3.1                  |

DBP: diastolic blood pressure; SBP: systolic blood pressure; WC: waist circumference; EWl: percent excess weight loss; *p < 0.05 in each genotype group with baseline values, analyzed with Bonferroni’s test. There are no statistical differences in anthropometric and metabolic characteristics between the two genotype groups.

ANOVA, by baseline levels of weight, age, and gender.
A CIRCADIAN RHYTHM-RELATED MTNR1B GENETIC VARIANT (RS10830963) MODULATES GLUCOSE METABOLISM AND INSULIN RESISTANCE AFTER BODY WEIGHT LOSS SECONDARY TO BILIOPANCREATIC DIVERSION SURGERY

-1.8 ± 1.1 mIU/L; \( p = 0.03 \), the second year (delta: -5.0 ± 0.3 mIU/L vs. -2.3 ± 0.2 mIU/L; \( p = 0.01 \)), and the third year (delta: -5.1 ± 1.9 mIU/L vs. -2.8 ± 1.1 mIU/L; \( p = 0.02 \)) were higher in non-G-allele carriers than in G-allele carriers. On the other hand, the improvement seen in HOMA-IR levels at year one (delta: -0.7 ± 0.2 mIU/L vs. -0.2 ± 0.2 mIU/L; \( p = 0.03 \)), year two (delta: -1.0 ± 0.3 mIU/L vs. -0.5 ± 0.2 mIU/L; \( p = 0.01 \)) and at year three (delta: -1.2 ± 0.3 mIU/L vs. -0.4 ± 0.2 mIU/L; \( p = 0.03 \)) were also higher in non-G-allele carriers than in G-allele carriers. Finally, baseline glucose levels after the first year (delta: -10.1 ± 2.4 mg/dL vs. -3.6 ± 1.8 mg/dL; \( p = 0.02 \)), the second year (delta: -16.0 ± 2.3 mg/dL vs. -8.4 ± 2.2 mg/dL; \( p = 0.01 \)) and the third year (delta: -17.4 ± 3.1 mg/dL vs. -8.8 ± 2.9 mg/dL; \( p = 0.03 \)) were higher in non-G-allele carriers than in G-allele carriers. As a result of this slower decrease in the values of HOMA-IR, glucose, and insulin, these parameters were higher at year 1, 2, and 3 in G-allele carriers. Finally, HDL-cholesterol levels increased in both genotype groups during follow-up to the same extent.

Table IV shows the improvement seen in comorbidities (percentage of hypertriglyceridemia, hypertension, and low-HDL levels). These improvements (frequencies) were similar in both genotype groups and represented patients with normal values of these parameters and discontinuation of drug therapy.

| Table III. Evolution of biochemical parameters during follow-up (mean ± SD) |
|------------------|------------------|
| **rs10830963**   | **rs10830963**   |
| **CC (n = 84)**  | **CG or GG (n = 70)** |
| **0 time**       | **At 1 year**    | **At 2 years** | **At 3 years** |
| **Glucose (mg/dL)** | 105.2 ± 7.0  | 95.1 ± 6.0*  | 89.2 ± 8.1*  | 87.8 ± 4.9*  | 104.5 ± 9.1 | 100.9 ± 6.1* | 96.1 ± 7.2* | 95.7 ± 5.4* |
| **Total ch. (mg/dL)** | 199.8 ± 31.1 | 135.4 ± 14.1* | 129.2 ± 11.1* | 126.7 ± 9.0* | 201.9 ± 29.1 | 136.2 ± 21.4* | 130.9 ± 12.0* | 126.0 ± 13.1* |
| **LDL-ch. (mg/dL)** | 124.2 ± 10.1 | 71.5 ± 8.2* | 61.7 ± 8.0* | 62.0 ± 7.1* | 120.1 ± 21.3 | 78.0 ± 10.1* | 63.9 ± 8.0* | 60.3 ± 9.2* |
| **HDL-ch. (mg/dL)** | 49.5 ± 8.1 | 50.2 ± 7.2* | 51.7 ± 9.0* | 52.0 ± 8.1* | 50.7 ± 10.2 | 51.9 ± 7.9* | 52.0 ± 6.9* | 53.1 ± 6.2* |
| **TG (mg/dL)** | 168.9 ± 20.1 | 130.1 ± 18.4* | 110.1 ± 12.6* | 91.2 ± 12.1* | 170.1 ± 19.8 | 127.2 ± 19.0* | 111.8 ± 20.8* | 90.8 ± 13.1* |
| **Insulin (mIU/L)** | 18.1 ± 4.1 | 14.2 ± 5.1* | 13.1 ± 4.3* | 13.0 ± 5.0* | 17.9 ± 6.1 | 16.1 ± 4.1* | 15.2 ± 3.9* | 15.1 ± 6.9* |
| **HOMA-IR** | 4.2 ± 2.0 | 3.5 ± 1.5* | 3.2 ± 1.2* | 3.0 ± 0.9* | 4.1 ± 2.1 | 3.9 ± 1.9* | 3.6 ± 2.1* | 3.5 ± 2.3* |

LDL: low density lipoprotein; HDL: high density lipoprotein; Ch: cholesterol; TG: triglycerides; HOMA-IR: homeostasis model assessment; \( *p < 0.05 \), in each group with baseline values, analyzed with Bonferroni’s test; \( †p < 0.05 \) between genotypes ANCOVA, adjusting by baseline levels of weight, age, and gender.

| Table IV. Percentage of comorbidities during follow-up (mean ± SD) |
|------------------|
| **Baseline** | **1 year** | **2 years** | **3 years** |
| **n = 154** | **n = 153** | **n = 150** | **n = 150** |
| **Low levels of HDL-cholesterol** |
| CC | 32.7 % | 17.8 %* | 17.2 %* | 11.1 %* |
| CG + GG | 37.1 % | 18.8 %* | 17.3 %* | 10.1 %* |
| **High levels of hyperTG** |
| CC | 38 % | 19 %* | 19.7 %* | 9.8 %* |
| CG + GG | 40 % | 21.7 %* | 20.2 %* | 8.7 %* |
| **Hypertension** |
| CC | 19 % | 10.7 %* | 7.4 %* | 3.7 %* |
| CG + GG | 17.1 % | 11.5 %* | 5.8 %* | 2.9 %* |

\( *p < 0.05 \), in each group with baseline values; HyperTG: hypertriglyceridemia (triglycerides > 150 mg/dL). Hypertension: systolic and diastolic blood pressures higher than 130 and 85 mmHg, respectively; low HDL-cholesterol: < 40 mg/dL and 50 mg/dL for men and women, respectively). The percentage values presented are for the sample at each time point. No statistical differences were detected in each variable between genotype groups. The Chi-squared test was used.
DISCUSSION

In the present study we evaluated the contribution of variant rs10830963 of the MTNR1B gene on biochemical changes in a sample of non-diabetic morbid obese subjects undergoing biliopancreatic diversion surgery. Our data show a less powerful effect of G-allele of the rs10830963 variant in modulating glucose levels, insulin levels, and HOMA-IR levels after extensive weight loss during 3 years of follow-up.

As far as we know, this is the first time that an analysis of this polymorphism in a cohort of morbid obese subjects is carried out after bariatric surgery and over 3 years. The G-allele frequency of the MTNR1B polymorphism in our study (0.24) was equal to previous data in the literature (17,18). The similarity of our allelic frequency with the data reported in these previous studies of non-morbid obese subjects, and the absence of differences in baseline anthropometric and biochemical data, suggest that this SNP in the melatonin pathway does not have a direct impact on adiposity parameters.

Moreover, there are few interventional studies evaluating the relationship between weight loss and this SNP located in MTNR1B (9-11). In one study (9), Goñi et al. showed that the G-allele of the rs10830963 SNP could be related with weight loss as induced by caloric restriction. In a further study (10), the same group showed a relationship of this SNP with lipid response after 2 years on a weight-loss diet. Additionally, a negative relation was detected between G-allele (11) and weight loss secondary to a dietary intervention with a hypocaloric, Mediterranean style-based diet on insulin levels and insulin resistance, too. And the same effect of the minor allele (G) was observed with other different hypocaloric diets (19). Our present findings in the analysis of adiposity parameters suggested that the MTNR1B variant (rs10830963) may not affect total body weight and fat mass response, a result that seems logical when taking into account the extensive weight loss generated by the BPD surgery and the minor effect that an isolated SNP may have.

The second main finding of our study is that G-allele carriers showed less improvement in glucose, insulin, and HOMA-IR levels after weight loss than non-G-allele carriers, as well as a slower improvement of these parameters in comparison with non-G-allele carriers, as these were not observed until after 24 months of follow-up. The pathophysiological mechanisms by which the rs10830963 SNP of the MTNR1B gene affects these metabolic parameters remains unknown. It could be hypothesized that the effect of the MTNR1B SNP on the dynamics of melatonin expression might influence glucose metabolism. The effect of feeding on the rhythmic mRNA expression of clock or circadian rhythmic balance genes has also been shown in animals (20,21). Moreover, Grotenfeld et al. (22) have reported the relationship of this SNP with glucose levels. More specifically, they reported that, among females at high risk for gestational diabetes mellitus, G-allele carriers did not seem to benefit from lifestyle interventions. In other studies, the risk G-allele has been related to decreased insulin sensitivity (23) and decreased insulin secretion in response to glucose (24), too. Experimental studies have demonstrated that the G-allele of rs10830963 that leads to increased glucose levels was associated with reduced pancreatic cell function (HOMA-B) (25), and that G-allele carriers had a reduced suppression of hepatic glucose production during a hyperinsulinemic-euglycemic clamp, indicating the presence of hepatic insulin resistance (26). These differences in the response of glucidic metabolism parameters between genotypes we have not detected in improvement of comorbidities, determined as the percentage of study participants with hypertension or dyslipidemia.

All these previously mentioned metabolic results may be related to alterations in circulating melatonin levels. For example, G-allele carriers have a later melatonin offset and a longer duration of elevated melatonin levels (27). Perhaps, this disruption of the melatonin rhythm among carriers of the risk allele could produce an increase in food intake and metabolism. As a different hypothesis, it is possible that the G-allele may be involved in the regulation of MTNR1B gene expression or other gene expression that might influence the role of melatonin on glucose metabolism. These metabolic pathways are very complex and recently, Irvy et al. (28) have shown that the use of melatonin premedication improves the quality of recovery (sensation of pain and sleep) after bariatric surgery. Clear relationships of this polymorphism with the presence of polycystic ovary, risk of gestational diabetes, and type-2 diabetes mellitus have also been discovered (29-31).

Our study has limitations. Firstly, we did not measure serum melatonin levels in the study population, which prevented the potential analysis of the relationship between serum melatonin levels and the SNP. Secondly, we only analyzed one SNP of the MTNR1B gene, so other SNPs in this or in various other gene loci could be related with our observations. Thirdly, many uncontrolled, non-genetic factors could modify the relationships in our design (smoking habit, exercise, hormone status, and so on), as also could epigenetic factors. Fourthly, the lack of a dietary assessment throughout the study in order to measure carbohydrate, fiber, and fat intakes. Finally, our group of study participants was a morbid obese adult sample without diabetes mellitus, so the data obtained may not be extrapolated to other populations.

CONCLUSION

Our study showed an association of the rs10830963 MTNR1B polymorphism after massive weight loss with lower glucose response, insulin resistance, and fasting insulin levels in G-allele carriers.

REFERENCES

1. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the global burden of disease study 2013. Lancet 2014;384:766-81. DOI: 10.1016/S0140-6736(14)60460-8
2. Jaunoo SS, Southall PJ. Bariatric surgery. Int J Surg 2010;8:86-9. DOI: 10.1016/j.ijsu.2009.12.003
3. Kushner RF. Weight loss strategies for treatment of obesity. Prog Cardiovasc Dis 2014;56:465-72. DOI: 10.1016/j.pcad.2013.09.005
A circadian rhythm-related MTNR1B genetic variant (rs10830963) modulates glucose metabolism and insulin resistance after body weight loss secondary to biliopancreatic diversion surgery

4. Abete I, Astrup A, Martinez JA, Thorsdottir I, Zulet MA. Obesity and the metabolic syndrome: role of different dietary macronutrient distribution patterns and specific nutritional components on weight loss and maintenance. Nutr Rev 2010;68:214-31. DOI: 10.1111/j.1753-4887.2010.00280.x

5. de Luis DA, Gonzalez M, Pacheco D, Martin T, Torroba MC, Guerlar L, et al. Effect of C358A missense polymorphism of the endocannabinoid degrading enzyme fatty acid hydrolase on weight loss and cardiovascular risk factors 1 year after biliopancreatic diversion surgery. Surgery of Obesity and Related Disease 2010;6:516-20. DOI: 10.1016/j.soard.2010.01.005

6. de Luis D, Garcia Calvo S, Primo D, Izaoa O, Pacheco D. Polymorphism rs3123554 in the cannabinoid receptor type 2 (CB2R) gene is associated to metabolic changes after biliopancreatic diversion surgery. Endocrinol Diabetes Nutr 2019;66:157-63. DOI: 10.1016/j.endinu.2018.09.008

7. de Luis DA, Primo D, Izaoa O, Aller R. Adiponectin Gene Variant rs266729 Interacts with Different Macronutrient Distribution of Two Different Hypocaloric Diets. Lifestyle Genom 2020;13:20-7. DOI: 10.1159/000503863

8. Tuomi T, Nagorny CLF, Singh P, Bennett H, Yu Q, Alenkov I, et al. Increased melatonin signaling is a risk factor for type 2 diabetes. Cell Metab 2016;23:1067-77. DOI: 10.1016/j.cmet.2016.04.009

9. Gori L, Cuervo M, Milligro FI, Martinez JA. Gene-Gene Interplay and Gene-Diet Interactions Involving the MTNR1B rs10830963 Variant with Body Weight Loss. J Nutrigenet Nutrigenomics 2014;7:232-42. DOI: 10.1159/000380951

10. Gori L, Sun D, Heianza Y, Wang T, Huang T, Cuervo M, et al. Macronutrient-specific effect of the MTNR1B genotype on lipid levels in response to 2 year weight-loss diets. J Lipid Res 2018;59:155-61. DOI: 10.1194/jlr.P078634

11. de Luis DA, Izaoa O, Primo D, Aller R. Association of the rs10830963 polymorphism in melanin receptor type 1B (MTNR1B) with metabolic response after weight loss secondary to a hypocaloric diet based in Mediterranean style Clin Nutr 2018;37:1563-8. DOI: 10.1017/cnu.2017.08.015

12. Lukacik H, Johnson PE. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr 1985;41:810-7. DOI: 10.1093/ajcn/41.4.810

13. Expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Executive summary of the Third Report of the National Cholesterol Education Program (NCEP). JAMA 2001;285:2486-97. DOI: 10.1001/jama.285.19.2486

14. Duart MJ, Arroyo CO, Moreno JL. Validation of an insulin model for the reactions in RA. Clin Chem Lab Med 2002;40:1161-7. DOI: 10.1515/cclcm.2002.293

15. Mathews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-4. DOI: 10.1007/BF02880883

16. Friedenwald WT, Levy RJ, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preoperative ultracentrifuge. Clin Chem 1972;18:499-502. DOI: 10.1093/clinchem/18.6.499

17. Kelliny C, Ekelund U, Andersen LB, Brage S, Loos RJ, Wareham NJ, et al. Common genetic determinants of glucose homeostasis in healthy children: the European Youth Heart Study. Diabetes 2009;58:2939-45. DOI: 10.2337/db09-0374

18. Andersson EA, Holst B, Sparso T, Grupu N, Banasik K, Holmkvist J, et al. MTNR1B G24E variant associates with BMI and fasting plasma glucose in the general population in studies of 22,142 Europeans. Diabetes 2010;59:1539-48. DOI: 10.2337/db09-1757

19. De Luis DA, Izaoa O, Primo D, Aller R. Dietary-fat effect of the rs10830963 polymorphism in MTNR1B on insulin resistance in response to 3 months weight-loss diets. Endocrinol Diabetes Nutr 2020;67:43-52. DOI: 10.1016/j.endinu.2019.02.007

20. Yanagihara H, Ando H, Hayashi Y, Obi Y, Fujimura A. High-fat feeding exerts minimal effects on rhythmic mRNA expression of clock genes in mouse peripheral tissues. Chronobiol Int 2006;23:905-14. DOI: 10.1080/07420500600827103

21. Cano P, Jimenez-Ortega V, Larrad A, Reyes Toso CF, Cardinali DP, Esquillino AI. Effect of a high-fat diet on 24-h pattern of circulating levels of prolactin, luteinizing hormone, testosterone, corticosterone, thyroid-stimulating hormone and glucose, and pineal melatonin content in rats. Endocrine 2008;33:118-25.

22. Grotefent NE, Wansenius NS, Röö K, Laiuvi K, Stach-Lempinen B, Ortho-Melanter M, et al. Interaction between rs10830963 polymorphism in MTNR1B and lifestyle intervention on occurrence of gestational diabetes. Diabetologia 2016;59:1655-8.

23. Jonsson A, Ladenvall C, Ahlwa T. TS. Effects of common genetic variants associated with type 2 diabetes and glycemic traits on alpha- and beta-cell function and insulin action in humans. Diabetes 2013;62:2978-83.

24. Lyssenko V, Nagorny CL, Erdos MR, Wierup N, Jonsson A, Spiegel P, et al. Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. Nat Genet 2009;41:82-8.

25. Song JY, Wang HJ, Ma J, Xu ZY, Hinne A, Hebebrand J, et al. Association of the rs10830963 polymorphism in MTNR1B with fasting glucose levels in Chinese children and adolescents. Obes Facts 2011;4:197-203.

26. Sparso T, Bonnefond A, Andersson E, Bouallia- Najj N, Holmkvist J, Wegner L, et al. G-allele of intrinsic rs10830963 in MTNR1B confers increased risk of impaired fasting glycaemia and type 2 diabetes through an impaired glucose-stimulated insulin release: Studies involving 19,605 Europeans. Diabetes 2009;58:1450-6.

27. Lane JM, Chang AM, Bjonnes AC. Impact of common diabetes risk variant in MTNR1B on sleep, circadian, and melatonin physiology. Diabetes 2016;65:1741-51.

28. Iyv M, Golstein D, Welly W, Berkenstadt H. Melatonin premedication improves quality of recovery following bariatric surgery - a double blind placebo controlled prospective study. Surg Obes Relat Dis 2017;13:502-6.

29. Zhang X, Xie L, Zhong M, Yang B, Yang Q, Yang H, et al. The association between melanin receptor 1B gene polymorphisms and type 2 diabetes mellitus (2TDM) in Chinese populations: a meta-analysis. Ann Palliat Med 2020;9(3):957-66. DOI: 10.21037/apm-20-691

30. Yi S, Xu J, Shi H, Li W, Li Q, Sun X. Association between melanin receptor gene polymorphisms and polycystic ovarian syndrome: a systematic review and meta-analysis [published online ahead of print, 2020 May 28]. Biosci Rep 2020;BSR20200824. DOI: 10.1042/BSR20200824

31. Correction: A functional polymorphism rs10830963 in melatonin receptor 1B associated with the risk of gestational diabetes mellitus. Biosci Rep 2020;40(2):BSR20190744_COR. DOI: 10.1042/BSR20190744_COR