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***CLOCK* 3111T/C genetic variant influences the daily rhythm of autonomic nervous function: relevance to body weight control**

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Abstract

Background/Objectives—Humans carrying the genetic risk variant *C* at the circadian *CLOCK* (Circadian Locomotor Output Cycles Kaput) 3111T/C have been shown to have more difficulties to achieve desired weight loss than TT carriers. We tested the hypothesis that the daily rhythm of autonomic nervous function differs in *CLOCK* 3111C carriers, leading to reduced effectiveness in weight control.

Subjects/Methods—We recruited 40 overweight/obese Caucasian women (BMI>25), 20 carrying *CLOCK* 3111C (CC and TC) and 20 non-carriers with matched age and BMI who participated in a dietary obesity treatment program of up to 30 weeks. Following the treatment, ambulatory electrocardiography was continuously monitored for up to 3.5 days when subjects underwent their normal daily activities. To assess autonomic function, heart rate variability analysis (HRV) was performed hourly to obtain mean (mean RR) and standard deviation of normal-to-normal heartbeat intervals (SDNN), and two parasympathetic measures, namely, proportion of differences between adjacent NN intervals that are >50 msec (pNN50), and high-frequency (HF: 0.15–0.4 Hz) power.

Results—In the TT carriers, all tested HRV indices showed significant daily rhythms (all *P* values <0.0001) with lower mean RR, SDNN, pNN50, and HF during the daytime as compared to the nighttime. The amplitudes of these rhythms except for SDNN were reduced significantly in the *C* carriers (mean RR: ~19.7%, *P*=0.001; pNN50: 58.1%, *P*=0.001; and HF: 41.1%, *P*=0.001). In addition, subjects with less weight loss during the treatment program had smaller amplitudes in the

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rhythms of mean RR ($P < 0.0001$), pNN50 ($P = 0.007$) and HF ($P = 0.003$). Furthermore, the rhythmicity-weight-loss associations were much stronger in the C carriers as compared to the TT carriers (mean RR: $P = 0.028$, pNN50: $P = 0.0002$; HF: $P = 0.015$).

Conclusions—The daily rhythm of parasympathetic modulation may play a role in the influence of the *CLOCK* variation on body weight control.

Keywords

Circadian rhythm; obesity; autonomic nervous system; heart rate variability; genetics

Introduction

Humans carrying the genetic variant C at the circadian gene *CLOCK* (Circadian Locomotor Output Cycles Kaput) 3111T/C are more likely to be obese, exhibit greater difficulties in controlling body weight than non-carriers (TT) and have a worse prognosis in dietary weight loss treatments^{1–9}. Among the many clock genes and other metabolic genes examined in our ONTIME study (registered at clinicaltrials.gov as NCT02829619), the *CLOCK* 3111C Single-Nucleotide Polymorphism (SNP; rs1801260) is the only SNP that is independently associated with total weight loss and weight loss evolution in a dietary program, i.e., C carriers lost ~23% less during the same weight loss program than T carriers². In addition, the effect of this SNP on obesity or obesity-related-metabolic alterations has also been demonstrated and replicated in several populations with different environment and genetic background^{4–8,10}. Because the *CLOCK* 3111C SNP is present in 46% of the U.S. population¹, understanding the mechanisms underlying the obesity risk associated with this genetic variation is of great relevance to public health.

Many physiological processes and functions, including motor activity and autonomic control, display daily rhythms that are in synchrony to the day-night cycle¹¹. These rhythms are generated and/or orchestrated by the circadian system that is composed of a large number of cell-autonomous clocks in the brain and in peripheral organs and tissues¹¹. The molecular basis for the circadian clocks consists of a family of proteins that function together in a transcriptional–translational feedback loop with the *CLOCK* protein as a key transcription factor of this loop¹². A recent study showed that the *CLOCK* 3111C SNP resulted in higher expression of *CLOCK* and also increased the expression of *PER2*— a transcriptional target of *CLOCK*¹³, suggesting a functional role of the genetic variant in the circadian control and, thus, altered circadian/daily rhythms of behavior and physiology in human C carriers.

The circadian control is important for metabolism and body weight control^{14,15}. Perturbing the circadian control, as occurs in shift workers with sleep deprivation and/or irregular sleep-wake schedule, is associated with increased adiposity¹⁶ and increases the risk of obesity and diabetes^{17,18}. In terms of the underlying mechanisms, it has been proposed that autonomic nervous system (ANS) is essential for the pathway through which the circadian clocks regulate metabolism¹⁵. This proposed theory is based on two established links: (i) The autonomic nervous system (ANS) affects energy balance *via* its influences on eating behavior and energy storage/consumption¹⁹. It is generally believed that low sympathetic

activity and high parasympathetic activity reduce energy expenditure and predispose onset of obesity (MONA LISA Hypothesis: ‘most obesities known are low in sympathetic activity’)²⁰. The hypothesis is supported by many studies on the innervation of adipose tissue by autonomic nerves, showing that the sympathetic nervous system is catabolic while the parasympathetic nervous system is anabolic²¹. (ii) The central circadian clock at the hypothalamus (Suprachiasmatic nucleus: SCN) has specific neural projections to control the sympathetic/parasympathetic balance in ANS activity²². In humans ANS activity and its responses to behavior-related stress are modulated by the circadian system, displaying endogenous circadian variations^{23–26}.

Based on these findings, we hypothesize that humans carrying risk C allele have reduced daily ANS activity rhythm due to differences in the circadian control and that the reduction is more pronounced in those individuals with more difficulty achieving desired weight loss. To test the hypotheses, we studied 20 women carrying the risk allele *CLOCK* 3111C allele (CC and TC) and 20 women TT carriers who participated in and completed a dietary obesity treatment program of up to ~30 weeks. We collected continuous electrocardiography (ECG) recordings up to 3.5 days during their normal daily activity and obtained heartbeat intervals (Fig. 1). Using heart rate variability (HRV) analysis²⁷—an established noninvasive technique to assess ANS activity from heartbeat intervals, we examined the daily rhythm of autonomic function and its relation with weight loss during the obesity treatment.

Materials and Methods

Subjects

We recruited 40 overweight/obese Caucasian women (BMI>25) including 20 C risk allele carriers (including 3 CC carriers and 17 TC carriers) and 20 TT carriers (Table 1). The C carriers were selected from the database of the clinics of Murcia (Spain). From the previously established database, we arranged the list of the female carriers of the C allele (n=119) in ascending order according to their code numbers. We then randomly divided them in two groups (i.e., potential participants and non-participants) using a computer-executed software (<http://www.randomization.com>). Those women classified as participants were contacted by phone until 20 of them accepted to participate in the study. The TT carriers (n=20) were selected as controls to match for age, obesity parameters, energy intake and expenditure (Table 1) and menopausal status (32% were postmenopausal). Participants receiving thermogenic, lipogenic, or sleep drugs, or melatonin or those diagnosed with insomnia, cognitive disorders, diabetes mellitus, chronic renal failure, hepatic diseases or cancer were excluded from the study. The written informed consent was obtained before subjects were included in the study. Subjects attended outpatient obesity clinics in the city of Murcia, located in southeastern Spain. All procedures were in accordance with good clinical practice and patient data were approved by the Ethical Committee of the University of Murcia.

Procedure

All 40 subjects participated in a dietary obesity treatment program of up to ~30 weeks. The duration of the program varied depending on the weight-loss goal. Dietetic treatment was

based on the principles of the Mediterranean diet and the distribution of the macronutrient components adhered to the recommendations of the Spanish Society of Community Nutrition²⁸. Subjects attended a 60-minute group therapy session once per week. Certified nutritionists led the program sessions. Details of the weight loss program are described in the Supplementary Materials and at ClinicalTrials.gov (NCT02829619). After the treatment program, subjects underwent a field study of up to 4 days for the assessment of daily rhythm of cardiac activity (see below).

DNA isolation and clock genotyping

DNA was isolated from blood samples using standard procedures (Qiagen, Valencia, CA, USA). We performed genotyping of the *CLOCK* 3111T/C SNP using a TaqMan assay with allele-specific probes on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to the standardized laboratory protocols previously used for this genetic variant¹. DNA samples are deposited in the database of the Institute of Health Carlos III belonging to the Ministry of Economy and Competitiveness (Registration number: C0003626).

Obesity related parameters

Total body fat was measured by bioelectrical impedance, using TANITA TBF-300 (Tanita Corporation of America, Arlington Heights, IL, USA) equipment. Subjects were requested not to drink liquids during the 2 h before the measurement. Blood for biochemical determinations were collected in the morning after overnight fast. All the parameters were collected before the start of the weight loss program. Height and weight of each subject were obtained at the same time of the day in each clinical visit.

Ambulatory study and data acquisition

During a visit in the outpatient obesity clinics, research staff showed consented subjects the operation of a Holter monitor (MyECG E3-80, Mircostar Company, Taipei; Physiologuard, Taipei) and the standard procedure for data collection of 4-lead ECG (i.e., placing/removing electrodes and starting/stopping a recording). After the visit, subjects brought the device home, together with written instructions. During the ambulatory study phase of ~3.5 days, subjects continuously wore the electrodes and the device except when: (i) showering; and (ii) the battery of the device was being charged, which occurred only once on the second day of the study. ECG waveforms were continuously recorded at 1000 Hz, and data were saved directly to a memory card within the device. For all 40 participants, the ECG recordings were collected during the same period that was between three to five months after the dietary obesity treatment program. All the ECG data collected by MG and CB were first de-identified and then were provided to MT, HY, KH who performed the heart rate variability analysis and statistical analysis.

Heart rate variability analysis

R waves in the ECG were identified with a QRS wave detector based on amplitude and the first derivative of the ECG waveform²⁹. Identified R waves were visually scanned by a trained technician to ensure that only normal R waves were included for further analysis (see

Supplementary Materials for details). To assess autonomic nervous system activity, we performed heart rate variability analysis in both time and frequency domains according to published standards²⁷. In the time domain analysis, mean and SD of normal-to-normal (SDNN) beat intervals were calculated, along with the percentage of differences between adjacent normal-to-normal intervals that were >50 ms (pNN50) as a parasympathetic activity measure. In the frequency domain analysis, power spectra of RR intervals were calculated in non-overlapped 10-min windows by fast Fourier transform of 3.41-Hz cubic spline resampled data. The high frequency (HF) power at 0.15–0.40 Hz was calculated as another measure of parasympathetic activity. Data were re-sampled into hourly bins for subsequent analyses.

Statistical analysis

C carriers were pooled for analyses because T/C carriers had obesity related characteristics similar to those of C/C subjects⁷. Three statistical models were performed (Online Tables 1, 2 and 3). To assess daily rhythms of HRV variables and the effects of the genotype on the rhythms, we performed cosinor analyses using mixed models²⁵. In the first models, daily rhythms were estimated using a 24-h sinusoidal and a 12-h harmonic: $Y_i = \mu + a_1 f_1(t_i) + b_1 g_1(t_i) + a_2 f_2(t_i) + b_2 g_2(t_i) + e_i$, where Y_i is the i th data point of a HRV variable at time t_i , μ is the mean (MESOR), $f_1(t_i) = \cos(2\pi t_i/24)$ and $g_1(t_i) = \sin(2\pi t_i/24)$ represent the cosine and sine components of the fundamental 24-hr rhythm, and $f_2(t_i) = \cos(2\pi t_i/12)$ and $g_2(t_i) = \sin(2\pi t_i/12)$ describe the 12-hr harmonic. In the models, HRV variables are included as outcome measures (a log transform was applied on HF to ensure a normal distribution); the four sinusoidal functions, genotype-group (TC/CC and TT), and their interactions as fixed factors; and subject as a random effect on the MESOR/intercept. For those HRV variables with significant interactions between genotypes and the sinusoidal functions, we further performed a bootstrap analysis to determine whether the interactions were due to the group differences in amplitude, in acrophase or both (see Supplementary Materials). To explore the associations between HRV daily rhythms and weight loss, we used similar cosinor mixed models in which HRV indices are included as outcome variables, $f_1(t_i)$, $g_1(t_i)$, weight loss and their interactions as independent variables, and subject as a random effect (Online Table 2). In the third model (Online Table 3), the genotypic group and its interaction with $f_1(t_i)$, $g_1(t_i)$ was added to model 2 to assess the effects of genotype on the association of weight loss. Two subjects (one C carrier and one TT carrier) did not complete the weight-loss program and, thus, were excluded for the analysis related to weight loss. In all the models, the potential effect of use of medication (yes or no) was also accounted for. All the analyses were performed using JMP 11 pro (SAS Institute Inc, North Carolina). The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Results

Participant characteristics

The duration of the weight loss treatment was not significantly different between two groups of selected subjects (24 ± 21 weeks for C carriers and 22 ± 16 weeks for non-carriers; $P > 0.7$). The efficacy of treatment was based on mean values of weight loss. C carriers lost less

weight (~3.5kg) as compared to non-carriers despite that all the obesity related parameters were similar between the two groups at baseline (Table 1). Habitual total energy intake, physical activity values, and sleep duration were also similar between the two genotype-groups. The only group differences were the times of wake-up and breakfast, i.e., the C carriers woke up ~31 minutes later ($P<0.05$) and ate their breakfasts ~55 minutes later in the morning ($P<0.01$).

Daily rhythm in heart rate variability

All tested HRV indices showed significant daily variations (Fig. 2). Mean normal-to-normal heart beat interval (mean RR) was larger at ~0:00–8:00AM with an evident peak in the early morning hours between 3–6AM, and became smaller during the daytime and early evening hours (Fig. 2A). SDNN was lowest at around the bedtime (0:00–1:00AM), increased across the sleep period, and gradually decreased after wake-up and throughout the daytime and evening (Fig. 2B). Consistently, the parasympathetic activity indices including pNN50 and HF showed lower values during the daytime and higher values during the nighttime (Fig. 2C, 2D). Note that the log transform of HF (lnHF) was used to ensure a normal distribution. Cosinor mixed models confirmed the daily HRV variations and revealed significant 24-h sinusoidal components in all test HRV indices (all P values <0.0001). Additionally, all measures except for SDNN showed significant 12-h harmonics, indicating that daily HRV rhythms did not follow a simple sinusoidal form.

Reduced daily HRV rhythm in C carriers

For all tested HRV variables, there were significant group differences in the 24-h components ($P<0.05$ for the interaction term: group x 24-h components) (Fig. 2). Based on the bootstrap analysis (see Supplementary Materials), such group differences were characterized by reduced amplitudes of 24-h sinusoidal components in the risk carriers except for SDNN, i.e., reduction was ~19.7% for mean RR ($P=0.001$), 58.1% for pNN50 ($P=0.001$), and 41.1% for HF ($P=0.001$) (Fig. 3A; Table 2). In the 24-h SDNN component, the amplitude was similar between groups while the acrophase was significantly delayed about ~1.9 hours in the C carriers (Table 2). The group differences in these daily HRV rhythms were independent of the effects of medication.

The similar group differences were also observed in the overall fitted daily rhythms (i.e., combining the 24-h and 12-h components). The bootstrap analysis showed that the C carriers had smaller peak-to-trough amplitudes in mean RR (11.1% smaller) and parasympathetic activity rhythms (pNN50: 57.2% smaller, HF: 34.2% smaller) as compared to TT (Fig. 3B). The bootstrap analysis also revealed that the C carriers had smaller peak and trough values for mean RR; a smaller peak value but a similar trough value for pNN50; and a larger trough value but a similar peak value for HF (Figs. 3C and 3D). The seemingly discrepancy between the results of pNN50 and HF may be due to the different sensitivities of the two parasympathetic measures at different levels of vagal tone, i.e., HF may be not sensitive at relatively high levels of vagal tone (e.g., during sleep) due to a saturation effect³⁰ while pNN50 may be not sensitive at relatively low levels of vagal tone³¹ (see Supplementary Materials for details). In addition, the C carriers had an advanced peak (i.e., ~0.68 h earlier) and a delayed trough (i.e., ~4.04 h delayed) in the overall fitted rhythm of mean RR (Table

2). No significant group differences in the peak and trough locations were observed for the fitted rhythms of the parasympathetic indices (i.e., pNN50 and HF).

Interactive effects of *CLOCK* 3111 T/C SNP and daily parasympathetic activity rhythm on total weight loss

We further examined whether the amplitudes of 24-h HRV rhythm were associated with weight loss. When combining subjects in both groups, subjects who lost less weight had smaller amplitudes of 24-h sinusoidal components in the daily rhythms of mean RR ($P < 0.0001$), pNN50 ($P = 0.007$) and HF ($P = 0.003$). No such association was found for SDNN ($P > 0.3$). These associations between the 24-h HRV components and weight loss were different between two groups ($P = 0.028$ for mean RR; $P = 0.0002$ for pNN50; and $P = 0.015$ for HF) (Fig. 4). These rhythmicity-weight-loss associations were highly significant within the C carriers ($P = 0.0001$, $P < 0.0001$ and $P = 0.002$ for mean RR, pNN50, and HF, respectively) but became less or not significant within the TT carriers ($P = 0.0001$, $P > 0.7$ and $P = 0.014$ for mean RR, pNN50 and HF, respectively). For instance, the predicted amplitude of the 24-h component in the daily pNN50 (or lnHF) rhythm was $\sim 2.9\%$ (or 0.13) in the 3 C carriers with weight loss of 9.7 Kg and was reduced to $\sim 1.14\%$ (or 0.07) in the 5 C carriers with weight loss < 5.3 Kg; and the predicted amplitude of the 24-h component in the daily pNN50 (or lnHF) rhythm was similar between the 8 non-carriers with weight loss 9.7 Kg and the 5 non-carriers subject with weight loss < 5.3 Kg (Figs. 4H, 4I).

Discussion

This study shows for the first time that the 24-h profile of heart rate variability in women differs across *CLOCK* 3111T/C genetic variants. As compared to TT carriers, risk allele C carriers had a reduction of 34–57% in the daily rhythm amplitude of parasympathetic activity as estimated by pNN50 and HF power. The reduction was caused by reduced parasympathetic activity during the nighttime when vagal tone is supposed to be high (indicated by pNN50) and increased parasympathetic activity during the daytime when vagal tone is supposed to be low (as indicated by HF). Consistently, the daily rhythm amplitude of mean RR was also reduced with decreased values during the nighttime and increased values during the daytime. More interestingly, the amplitude of the parasympathetic rhythm was associated with the weight loss during the obesity treatment. It is accepted that the autonomic nervous system plays an important role in obesity and obesity-related metabolic alterations^{32,33}. The observed increase in parasympathetic activity during the daytime in C carriers is consistent with the MONA LISA hypothesis²⁰, suggesting reduced energy expenditure that can explain less weight loss as observed in these individuals.

One of the main problems in weight loss treatments is the dramatic inter-individual variability in response to the treatment³⁴. It is proposed that the elucidation of the genetic component in prognosis of weight management could assist in development of more effective and individually tailored therapeutic strategies³⁵. A previous study in 1495 overweight/obese subjects showed that carriers of the risk allele C had several behavioral and metabolic alterations that could be implicated in their weight loss difficulties such as shorter sleep duration, higher plasma ghrelin concentrations (the "hunger hormone"),

delayed breakfast time, evening preference and less compliance with a Mediterranean Diet pattern, as compared with TT homozygotes⁷. The current study suggests the alteration of autonomic function as a common mechanism underlying weight control difficulties and possibly other metabolic alterations in C carriers. Our results highlight the importance to examine the 24-h pattern of autonomic function together with the genetic background in order to predict the individual outcome of obesity treatment based on low-energy diets.

Though the current study is focused on the understanding of the difficulty to achieve desired weight loss in C carriers, our results may also have implications on risk for obesity in these individuals. Indeed, many studies showed the effect of this SNP on obesity or obesity-related-metabolic alterations^{4-8,10}. For instance, our previous study showed that C carriers weight 3 kg more (87 kg vs 84 kg) than T carriers after controlling for other factors such as age gender and clinical center¹. However, no significant associations of the SNP with BMI or waist circumference were reported in the recent studies from the GIANT consortium^{36,37}. The seemingly inconsistent results may be caused by the different threshold of the p value for a significance level used in the GWAS studies (i.e., due to more associations were tested in the GWAS studies). It is worth noting that weight loss and obesity are different outcomes such that different behaviors and genetic factors may be accounting for them. Currently there have been no GWAS studies investigating weight loss as a result of dietary treatments.

A concern on previous studies of the link between autonomic function and obesity is that timing of assessment is seldom considered. In chronobiology, it is established that autonomic function displays 24-h variations with higher sympathetic activity and lower parasympathetic activity during the daytime compared with nighttime³⁸. Due to the daily variations, a certain level of ANS activity can be physiological at one time point but might be pathophysiological at a different time point of the day³⁹. There is evidence that changes in these daily variations in autonomic function may be implicated in the risk for obesity-related pathologies⁴⁰. Here we found that daily parasympathetic activity variations are implicated in the response to an obesity treatment, especially in women carrying *CLOCK* 3111C.

The mechanisms underlying the effects of the genetic variant on daily variations of ANS activities are yet to be determined. One possibility is that daily rhythms in human physiology are produced by the daily behavioral cycles. Specifically, scheduled behaviors such as meal times, food intake, and physical activity levels may differ in C carriers during the daytime, leading to different ANS activities. We accounted for these potential behavioral effects in the current study by selecting the C carriers and non-carriers with matched physical activity levels as well as many other external factors including energy intake and sleep duration. The only group differences were that the C carriers woke up and ate their breakfasts later in the morning. These differences may explain the delayed peak of the overall heart rate variability (SDNN) but could not explain the reduced daily amplitudes of mean RR and parasympathetic indices in C carriers.

The observed reduction in parasympathetic activity in C carriers during the night might be associated with reduced sleep quality. This sleep hypothesis may explain the difficulty of

weight control in the C carriers because decreased sleep quality leads to increased hunger and appetite, decreased concentrations of the satiety hormone leptin, increased concentrations of the hunger hormone ghrelin when caloric intake is controlled, and increased weight gain when caloric intake is *ad libitum*³⁸. To explore this possibility, we examined previously collected Polysomnography-based sleep measures of the 40 participants, including arousals, obstructive apnea events, and desaturation events. We found no significant group differences (all P values >0.5)⁴. Note that these sleep measures were collected before the current study. Thus, simultaneous assessments of sleep and autonomic function are required to formally confirm or refute the link between autonomic function and sleep dynamics in risk C carriers.

Alternatively, daily rhythms of behavior and physiology can be contributed by the *central circadian clock* which prepares the body for the anticipated environmental and behavioral cycles¹¹. In mammals, the central circadian clock is the hypothalamic suprachiasmatic nucleus (SCN), which generates self-sustained oscillations with a period of ~24 h and controls the circadian rhythms of core physiological variables such as heart rate, body temperature and motor activity¹¹. A slight change in the circadian clock can have significant influences on behavior and physiology. For instance, the intrinsic period of the circadian clock is associated with chronotype (i.e., a longer period >24 h for evening-types and a shorter period <24h for morning-types). Future studies using more sophisticated circadian protocols are necessary to determine whether the intrinsic properties of the circadian clock may be different in C carriers, contributing to reduced parasympathetic activity directly or indirectly via its interaction with sleep-wake cycles.

Study limitations

Because daily activity and the circadian rhythm were not monitored/controlled simultaneously, we could not determine their separate contributions to the altered daily HRV rhythm of in C carriers. To better control the effects of activity and posture on autonomic nervous system activity, it is ideal to monitor activity and posture simultaneously with the ANS activity. In addition, the sample size in this study was relatively small. We did power calculation, and found that 40 subjects allowed the identification of the reported key effects with power > 80%, e.g., group differences in the amplitudes of daily HRV rhythms: >98% for both pNN50 and HF; the associations between the amplitudes and weight loss: >84% for pNN50 and HF. HRV allows only reliable assessment of parasympathetic activity. Whether daily sympathetic activity rhythm differs in C carriers are worth further investigating. Furthermore, the HRV assessment was performed only once after the weight-loss intervention such that the ability of the HRV daily rhythm to predict the effectiveness of achieving desired weight loss is to be determined. Thus, future studies with better design and large sample sizes are warranted to confirm the findings in this pilot study.

Conclusions

The daily rhythm of vagal modulation is reduced in women carrying *CLOCK* 3111C as compared to non-carriers. The reduction negatively correlates with the effectiveness of achieving desired weight loss in these C carriers. These findings suggest that altered daily

ANS activity may underlie the effect of genetic variant of *CLOCK* 3111T/C on body weight control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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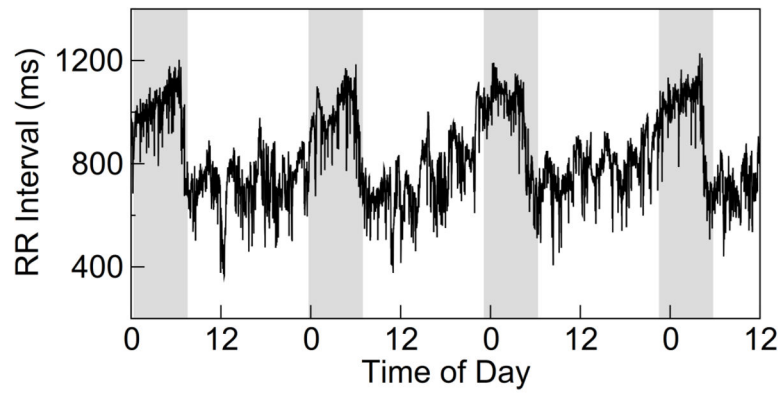


Fig. 1. A representative heartbeat recording of a TT carrier. The grey shaded areas indicate subject's habitual sleep episodes.

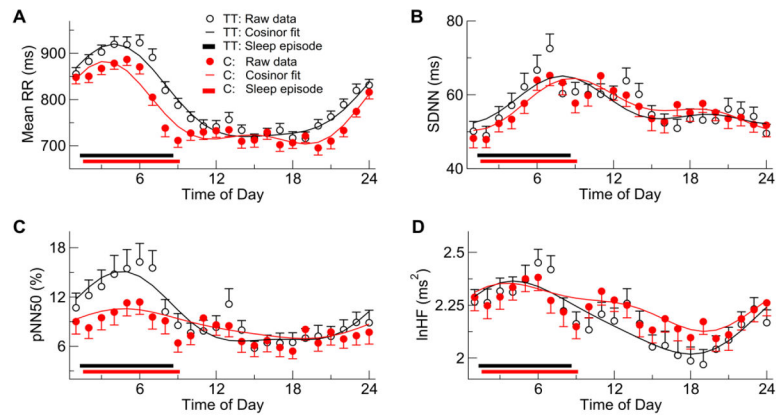


Fig. 2. Daily pattern of heart rate variability (HRV). Shown are (means \pm SE) group averages of mean RR, SDNN (a marker for overall HRV), pNN50 (a time-domain marker of cardiac vagal modulation), and HF (a frequency-domain marker of cardiac vagal modulation). The log transform of HF (lnHF) was used to HF to ensure a normal distribution. Solid curves are the fits based on cosinor mixed models.

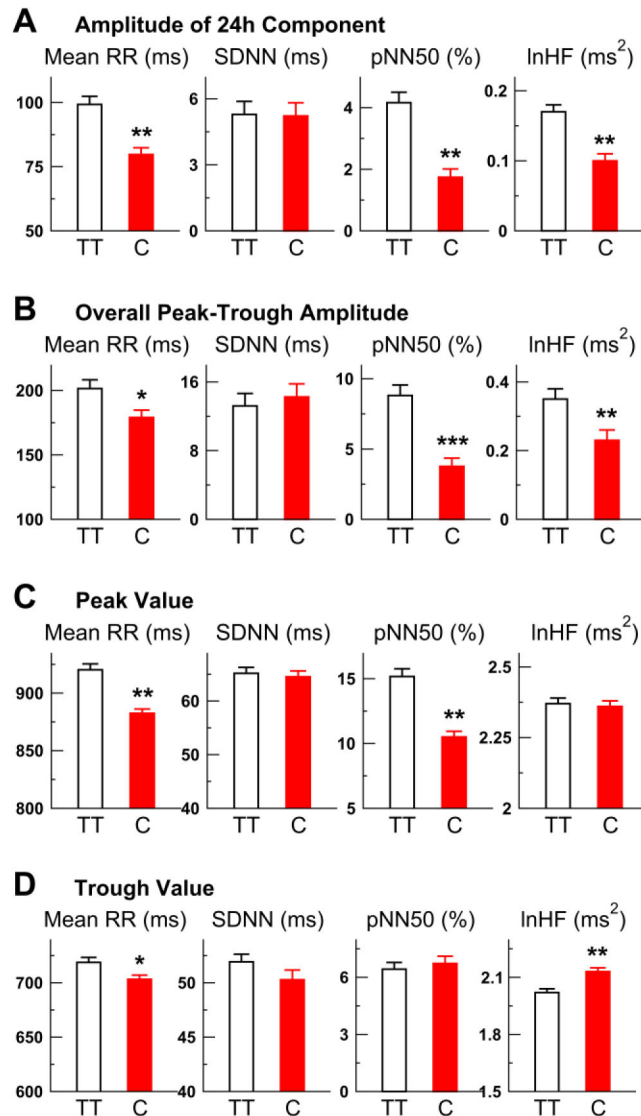
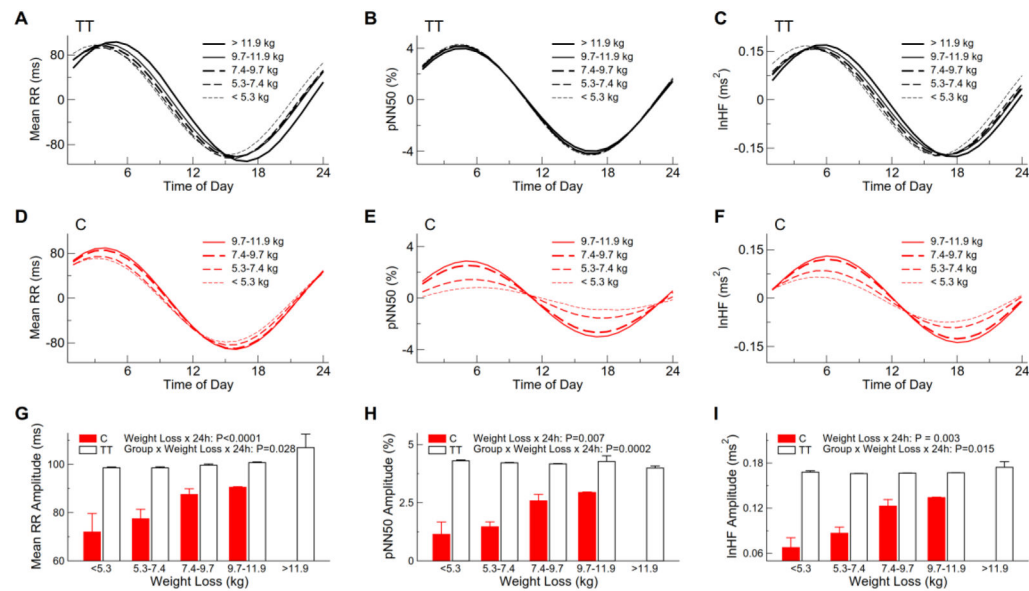


Fig. 3. Effect of the genetic variant *CLOCK* 3111T/C on the amplitude of daily heart rate variability rhythm. The rhythms of HRV indices (mean RR, SDNN, pNN50, HF) were estimated using 24-h sinusoids and 12-h harmonics. The log transform of HF (lnHF) was used to HF to ensure a normal distribution. (A–D) Group averages (SE) of the amplitudes/coefficients of the 24-h components (A), the peak-trough amplitudes of the overall fits (B), the peak values (C) and the trough values (D). Significant group differences are indicated by * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.0001$).

**Fig. 4.**

Effect of the genetic variant *CLOCK* 311T/C on the association between weight loss and daily heart rate variability rhythm. To demonstrate the weight-rhythm association, the 24-h components in the daily rhythms of heart rate variability indices (**A–F**) and the corresponding amplitudes (**G–I**) in five categories of weight loss (i.e., <5.3kg, 5.3–7.4 kg, 7.4–9.7 kg, 9.7–11.9kg, and >11.9 kg) were shown. Note that there were no C carriers in the category of >11.9 kg. Results were based on mixed models with weight loss (a continuous variable), group, coefficients of 24-h components, and their interactions (weight loss x 24-h coefficients) as the fixed effects and subject as a random effect.

Table 1

General characteristics of the study population. Data are presented as mean \pm SD. NS indicates $P > 0.1$. * Two subjects (one for each of C and TT carriers) did not complete the weight-loss program and, thus, were excluded for the analysis related to weight loss.

Obesity parameters	<i>CLOCK 311T/C</i>		
	Risk carriers(TC+CC)	Non-risk carriers(TT)	Group difference
	n=20 (17TC+3CC)	n=20	P value
	Mean \pm SD	Mean \pm SD	
Age (y)	42.0 \pm 9.7	46.5 \pm 11.04	NS
Height (m)	1.63 \pm 0.06	1.63 \pm 0.05	NS
Weight (kg)	74.87 \pm 8.83	76.32 \pm 12.00	NS
BMI(kg/m ²)	28.14 \pm 2.41	28.66 \pm 4.41	NS
Waist (cm)	89.10 \pm 9.55	91.60 \pm 10.80	NS
Hip (cm)	105.65 \pm 8.72	110.25 \pm 10.50	NS
Body fat (%)	35.03 \pm 3.72	34.81 \pm 6.26	NS
Glucose (mg/dl)	84.80 \pm 20.47	87.11 \pm 10.16	NS
Insulin (μ UI/mL)	10.27 \pm 13.99	6.03 \pm 3.16	NS
Total Tryclicerydes (mg/dl)	104.35 \pm 43.25	87.00 \pm 28.90	NS
Total cholesterol (mg/dl)	198.10 \pm 24.67	194.83 \pm 26.08	NS
Cholesterol HDL (mg/dl)	57.80 \pm 12.99	54.33 \pm 14.36	NS
Cholesterol LDL (mg/dl)	119.45 \pm 18.65	123.03 \pm 26.90	NS
Leptin (ng/mL)	21.14 \pm 9.65	20.34 \pm 9.32	NS
adiponectin (ng/mL)	74.60 \pm 26.62	78.70 \pm 25.24	NS
IL6 (μ g/mL)	24.79 \pm 5.90	24.81 \pm 6.18	NS
Total energy intake (kcal/day)	1980 \pm 767	1893 \pm 601	NS
Physical Activity Test (METs)	3614 \pm 2802	2842 \pm 2886	NS
Sleep duration (hours)	7.8 \pm 0.6	7.4 \pm 0.8	NS
Bedtime	0:36 \pm 55(min)	0:21 \pm 47(min)	NS
Wake-up Time	8:18 \pm 46(min)	7:47 \pm 38(min)	0.028
Breakfast Time	9:30 \pm 64(min)	8:35 \pm 48(min)	0.004
Lunch Time	14:51 \pm 29(min)	14:20 \pm 23(min)	NS
Dinner Time	21:32 \pm 32(min)	21:25 \pm 24(min)	NS
Intervention Duration (weeks)	24 \pm 21	22 \pm 16	NS
Total weight loss (Kg)	6.3 \pm 3.2	9.8 \pm 5.1	0.045*
Medication use (Total)	9	9	NS
Antihypertensives	2	2	NS
Lipid lowering drug	1	1	NS
Antidiabetics	0	1	NS
Antidepressives	3	4	NS
Anxiolytics	2	3	NS
Thyroid hormone	3	0	0.036
Anticonceptives	1	3	NS

Table 2

Parameter estimates of daily/circadian profiles of heart rate variability measures. Results were obtained using bootstrap. Amplitudes in 24-h and 12-h components are peak to mesor values.

	Group T (n=20) mean \pm SD	Group C (n=20) mean \pm SD	Group difference P-value
Mean RR (msec)			
<u>24-h component</u>			
Amplitude	99.24 \pm 3.15	79.73 \pm 2.62	0.001
Acrophase(h)	3.83 \pm 0.13	3.51 \pm 0.14	NS
<u>12-h harmonic</u>			
Amplitude	23.99 \pm 3.18	35.96 \pm 2.80	0.005
Acrophase(h)	4.13 \pm 0.27	3.19 \pm 0.14	0.003
<u>Overall</u>			
Peak-trough amplitude	201.50 \pm 6.78	179.07 \pm 5.71	0.012
Peak time(h)	3.98 \pm 0.17	3.30 \pm 0.13	0.002
Trough time(h)	14.62 \pm 1.14	18.68 \pm 1.67	0.045
Peak value	920.21 \pm 5.17	882.46 \pm 3.73	0.001
Trough value	718.70 \pm 4.61	703.19 \pm 3.87	0.011
SDNN (msec)			
<u>24-h component</u>			
Amplitude	5.29 \pm 0.59	5.22 \pm 0.6	NS
Acrophase(h)	8.33 \pm 0.42	10.32 \pm 0.44	0.001
<u>Overall</u>			
Peak-trough amplitude	13.20 \pm 1.46	14.25 \pm 1.52	NS
Peak time(h)	7.98 \pm 0.31	8.74 \pm 0.34	NS
Trough time(h)	22.84 \pm 3.05	24.95 \pm 0.37	NS
Peak value	65.18 \pm 1.10	64.53 \pm 1.09	NS
Trough value	51.93 \pm 0.70	50.28 \pm 0.89	NS
pNN50 (%)			
<u>24-h component</u>			
Amplitude	4.16 \pm 0.34	1.74 \pm 0.27	0.001
Acrophase(h)	4.5 \pm 0.26	5.52 \pm 0.6	NS
<u>12-h harmonic</u>			
Amplitude	1.36 \pm 0.31	0.43 \pm 0.21	0.014
Acrophase(h)	4.64 \pm 0.48	3.68 \pm 1.96	NS
<u>Overall</u>			
Peak-trough amplitude	8.81 \pm 0.75	3.77 \pm 0.58	0.0001
Peak time(h)	4.58 \pm 0.31	4.85 \pm 0.89	NS
Trough time(h)	15.99 \pm 2.70	18.56 \pm 1.59	NS
Peak value	15.17 \pm 0.60	10.50 \pm 0.44	0.001
Trough value	6.43 \pm 0.35	6.73 \pm 0.38	NS
HF			

	Group T (n=20) mean \pm SD	Group C (n=20) mean \pm SD	Group difference P-value
<u>24-h component</u>			
Amplitude	0.17 \pm 0.01	0.10 \pm 0.01	0.001
Acrophase(h)	4.93 \pm 0.26	5.83 \pm 0.50	NS
<u>12-h harmonic</u>			
Amplitude	0.03 \pm 0.01	0.04 \pm 0.01	NS
Acrophase(h)	2.34 \pm 1.14	1.95 \pm 0.64	NS
<u>Overall</u>			
Peak-trough amplitude	0.35 \pm 0.03	0.23 \pm 0.03	0.002
Peak time(h)	4.06 \pm 0.46	3.64 \pm 0.76	NS
Trough time(h)	18.00 \pm 0.57	19.08 \pm 0.50	NS
Peak value	2.37 \pm 0.02	2.36 \pm 0.02	NS
Trough value	2.02 \pm 0.02	2.13 \pm 0.02	0.001

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