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Effect of oral melatonin treatment on insulin resistance and diurnal blood pressure variability in night shift workers. A double-blind, randomized, placebo-controlled study

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ABSTRACT

Background: Night shift work is associated with sleep disturbances, obesity, and cardiometabolic diseases. Disruption of the circadian clock system has been suggested to be an independent cause of type 2 diabetes and cardiovascular disease in shift workers. We aimed to improve alignment of circadian timing with social and environmental factors with administration of melatonin.

Methods: In a randomized, placebo-controlled, prospective study, we analysed the effects of 2 mg of sustained-release melatonin versus placebo on glucose tolerance, insulin resistance indices, sleep quality, circadian profiles of plasma melatonin and cortisol, and diurnal blood pressure profiles in 24 rotating night shift workers during 12 weeks of treatment, followed by 12 weeks of wash-out. In a novel design, the time of melatonin administration (at night or in the morning) depended upon the shift schedule. We also compared the baseline profiles of the night shift (NS) workers with 12 healthy non-night shift (NNS)-working controls.

Results: We found significantly impaired indices of insulin resistance at baseline in NS versus NNS ($p < 0.05$), but no differences in oral glucose tolerance tests nor in the diurnal profiles of melatonin, cortisol, or blood pressure. Twelve weeks of melatonin treatment did not significantly improve insulin resistance, nor did it significantly affect diurnal blood pressure or melatonin and cortisol profiles. Melatonin administration, however, caused a significant improvement in sleep quality which was significantly impaired in NS versus NNS at baseline ($p < 0.001$).

Conclusions: Rotating night shift work causes mild-to-moderate impairment of sleep quality and insulin resistance. Melatonin treatment at bedtime improves sleep quality, but does not significantly affect insulin resistance in rotating night shift workers after 12 weeks of administration.

1. Introduction

Impaired glucose tolerance is a prodromal stage of type 2 diabetes mellitus, a cardiometabolic disease affecting millions of individuals during their work life with a sharply increasing incidence [1]. Besides obesity and lack of physical activity, two major known risk factors for the development of type 2 diabetes [2], an increasing prevalence of night shift work was identified as another factor driving the incidence of this disease [3,4]. Several prospective studies have shown that night shift workers have an increased risk of type 2 diabetes. A 5-fold increase in onset of type 2 diabetes/obesity was found in a prospective study that compared 402 night shift workers with 336 daytime-only workers during a median follow-up of four years [5]. In another study, the incidence of metabolic syndrome was 77 % higher in 309 rotating night shift workers as compared to 1220 non-night shift workers during 6.6 years of follow-up [6].

One important and independent pathogenic factor driving impaired glucose tolerance is disruption of the circadian timing system, as shown

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by the diabetes / obesity phenotype of CLOCK gene knockout mice [7]. We have previously reported that circadian-adapted light therapy improves diurnal blood pressure control in night shift workers [8], whilst exercise intervention generated discrepant results depending on the ability of the exercise program to enhance physical exercise capability [9,10].

The central circadian clock in the suprachiasmatic nuclei (SCN) are strongly influenced by daylight, which in turn drives the circadian rhythm in melatonin. Melatonin is the major humoral mediator of a broad range of cellular functions including glucose homeostasis and diurnal blood pressure regulation [11,12]. Administration of melatonin at bedtime enhances the endogenous rise in melatonin levels at the onset of darkness [13]. In two seminal studies, it was shown that disruption of the endogenous diurnal melatonin rhythm is associated with ischemic stroke [14,15]. Whilst it is well known that melatonin supplementation supports sleep and to adjust circadian rhythms [16,17]. In addition, several recent studies have shown that melatonin administration may improve glucose tolerance and cardiovascular function in patients with type 2 diabetes mellitus [18].

The EuRhythDia consortium was formed to investigate the prospect of improving circadian blood pressure control and reducing the risk of diabetes mellitus / metabolic syndrome through adjusting the internal clock in individuals in whom circadian rhythms are disrupted by night shift work. We therefore performed a prospective, randomized, placebo-controlled trial with sustained-release melatonin to assess its effects on markers of glucose homeostasis and insulin resistance as well as on 24 h blood pressure profiles in long-term rotating night shift workers.

2. Study participants and methods

2.1. Study participants

This randomized, double-blind, placebo-controlled prospective clinical trial was performed with 24 long-term rotating night shift workers; 12 non-night shift workers underwent baseline examination as a control group. Night shift workers had worked a minimum of three night shifts per month for at least six months before the start of the study. We excluded individuals with any severe somatic or psychic disease or signs of obstructive sleep apnea, individuals who had taken any melatonin supplements within four weeks before study start, those whose caffeine intake was above 750 mg/day, and those who had been on a transmeridian flight (3 time zones or more) within four weeks before the start of the study. Non-night shift workers were included if they were healthy, had never undertaken any night shift work; all other criteria for night shift workers were also applied to them. All study participants received detailed information and gave written informed consent to participate before their inclusion into the study. The study protocol adhered to the principles of the Declaration of Helsinki. This clinical study was approved by the National Competent Authority and registered at EudraCT (No. 2012–005254–30), and it was approved by the Ethics Committee of the Chamber of Physicians of Hamburg (decision no. PVN4419).

2.2. Study protocol

All study participants underwent medical history, full clinical examination, blood sampling for routine clinical chemistry, and oral glucose tolerance testing (OGTT) at baseline, and a 24-hour blood pressure monitoring. The next day, study participants returned the 24-hour blood pressure monitors and remained on a study ward for 26 h, during which time blood samples were drawn every three hours for the determination of plasma melatonin and cortisol concentrations. The validated German version of the Horne-Östberg questionnaire [19] and the Pittsburgh Sleep Quality Index (PSQI [20]) were used to assess morningness – eveningness and sleep quantity and quality, respectively, in the study participants at each study visit. PSQI scores $\leq$5 were used to define good sleep; scores >5 were used to define poor sleep. We utilized validated German translations of the Horne-Östberg questionnaire (D-MEQ) and the PSQI [21,22].

Whilst non-night shift workers only underwent the baseline examination, night shift workers were then randomized to receive sustained-release melatonin (2 mg, oral) or matching placebo at bedtime for 12 weeks. Thereafter, the same set of clinical investigations was performed (12 weeks, post-intervention). Then, the intervention ceased and the study participants were invited to a final examination after an additional 12 weeks without administration of study drug (24 weeks, wash-out). All study participants were tested in a dedicated phase 1 clinical trial unit. As our focus was to assess the persisting effects of night shift work, the night shift workers were studied on a non-night shift day within 3–7 days after the end of a night shift.

The primary objective of this trial was to assess the efficacy of timed administration of sustained-release melatonin over 12 weeks of treatment to improve diurnal control of glucose metabolism and blood pressure in rotating night shift workers. The study was powered to detect a 10 % improvement in the area under the curve for glucose during OGTT, which was considered clinically relevant.

2.3. Study medication

Study medication was prepared by the pharmacy of the University Medical Center Hamburg-Eppendorf. It consisted of either 2 mg melatonin in a sustained-release preparation (Circadin®, RAD Neurim Pharmaceuticals, Tel Aviv, Israel) or matching, optically identical placebo tablets which were also supplied by Neurim Pharmaceuticals. This melatonin preparation is an approved drug in Germany.

Study participants were asked to take one tablet of melatonin or placebo between 0.5 and 1 h before bedtime every day, except on days of one single or two subsequent night shifts, when medication was to be paused. This meant that study medication was taken in the evening on days off work, in the morning on days after each of three or more subsequent night shifts, or not at all on days after one single or two subsequent night shifts.

Compliance with study medication was assessed by analyzing patient diaries and by pill counts when the medication containers were returned to the study center. The individual number of tablets taken was calculated as the difference in number of tablets dispensed at the beginning and returned after the intervention; it was expressed as percent of the number of tablets that should have been ingested according to each participant's shift schedule as noted in the diary. Compliance was assumed if 70 – 100 % conformity with the study protocol was reached.

2.4. Biochemical analyses

Serum glucose and glycated hemoglobin (HbA_{1c}) concentrations as well as routine laboratory values were measured by standard clinical chemistry methods in the local laboratory. Serum insulin was measured by enzyme-linked immunoassay (Mercodia, Uppsala, Sweden). Plasma melatonin and cortisol concentrations were measured in 3-hourly lithium heparin plasma samples by specific radioimmunoassays with reagents obtained from Stockgrand Ltd. (University of Surrey) as described previously [23].

2.5. Assessment of insulin resistance

The homeostasis model assessment of insulin resistance (HOMA-IR) index, a widely used parameter to estimate the extent of insulin resistance in an individual based on fasting insulin and fasting glucose concentrations, was calculated according to the formula HOMA-IR = fasting insulin [mU/l] * fasting glucose [mg/dl] / 405 [24]. The quantitative insulin sensitivity check index (QUICKI), which was first introduced in 2000 by Katz and co-workers [25], was calculated from fasting glucose and insulin concentrations by the formula QUICKI = 1 / (log

(fasting insulin) + log(fasting glucose)).

2.6. 24-hour blood pressure measurements

24 h blood pressure measurements were performed with the BPLab® ambulatory blood pressure monitoring system (OOO Petr Telegin, Nizhny Novgorod, Russia). Measurements were taken every 15 min during daytime (07:00 – 21:00) and every 30 min during nighttime. 24 h blood pressure recordings were completed by 11 out of 12 non-night shift workers.

Dipper status was calculated according to published recommendations [26] from the difference between mean systolic blood pressure levels during individual wake-times and sleep-times. Dipper status was categorized into two groupings, i.e. dipper ($\geq$ 10 % decline in mean BP from wake-time to sleep-time) and non-dipper ($<$ 10 % decline in mean BP).

2.7. Statistical analyses

Variables were tested for normal distribution using the Kolmogorov–Smirnov test. Differences between groups were tested for significance using a two-tailed t test for normally distributed variables or the nonparametric Mann–Whitney U test for non-normally distributed variables. Dipper status, chronotypes, and insulin resistance index scores were assessed using the chi-square test. P for trend is given for comparisons of more than two groups; Fisher's exact test was used for calculating statistical significances for the comparison of categorical variables from two groups. Repeated measurement analysis of variance (ANOVA) was used for time courses of parameters measured at baseline, at 12 and at 24 weeks. Data are presented as median with 25th and 75th percentiles or as mean with standard error of the mean, as appropriate and indicated. All statistical analyses were performed using SPSS (version 25; IBM Corp., Armonk, NY, USA) and GraphPad Prism (version 6.01, GraphPad Software, San Diego, CA, USA). For all tests, P less than 0.05 was considered significant.

3. Results

3.1. Baseline characteristics of the study groups

Thirty-four rotating night shift workers were screened. Seven individuals did not consent to study participation because they did not agree to participate in the overnight stay at the study centre. Therefore, 27 night shift workers were included in the study. Of these, two study participants randomized to the melatonin group and one individual randomized to the placebo group dropped out for personal reasons (withdrawal of consent because of hourly blood sampling during baseline visit, N = 1; inability to meet study schedules, N = 1, termination of night shift work, N = 1). The primary end-point after 12 weeks of intervention could thus be assessed for 12 individuals per group. During

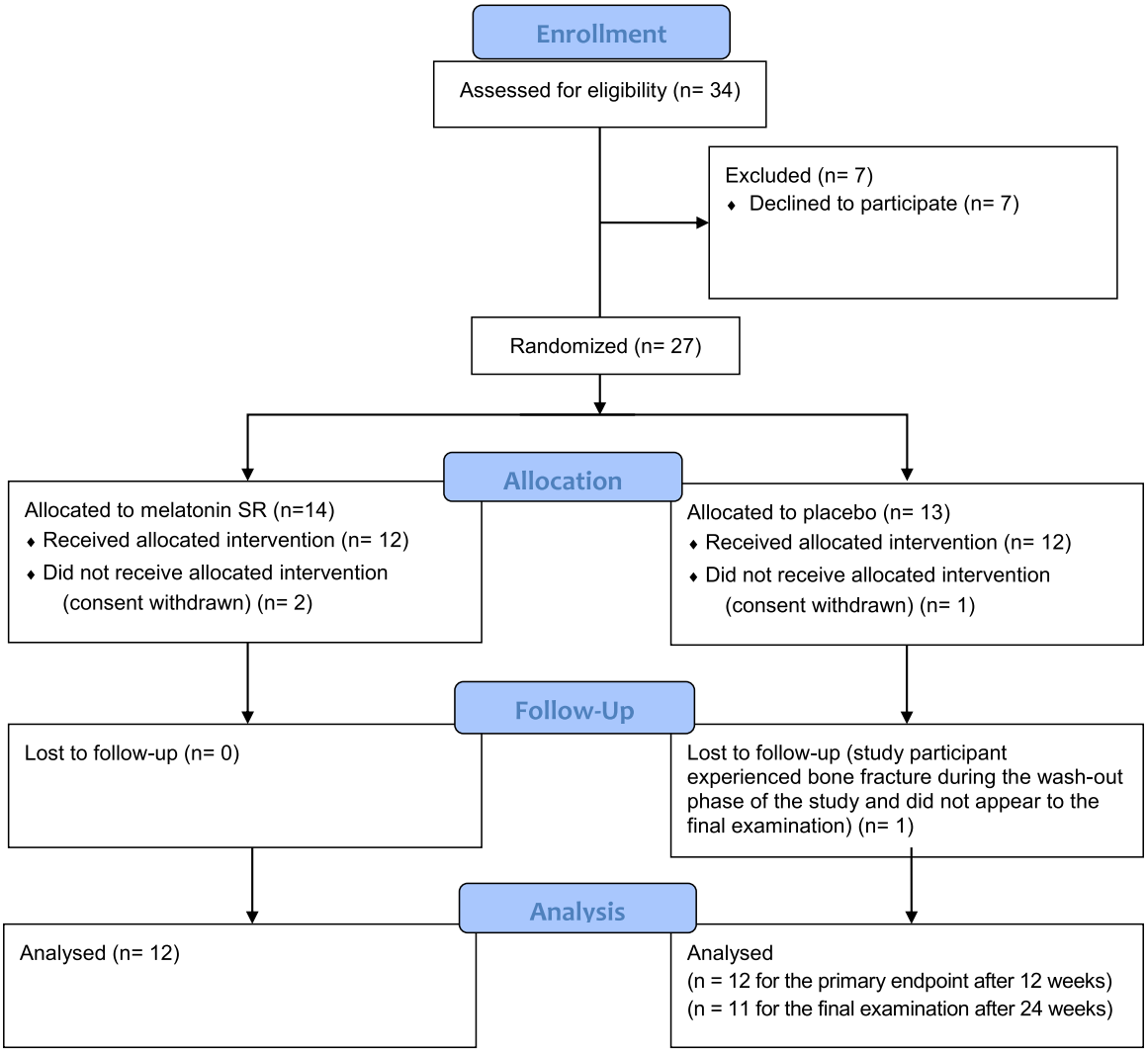

Fig. 1. CONSORT study flow diagram.

the subsequent wash-out phase, one more study participant dropped out (tibial plateau fracture during sports); this study participant (placebo group) was not replaced. Fig. 1 shows the CONSORT study flow diagram.

The night shift workers were recruited from various professional fields (nurses, police officers, fire workers, dock workers) with no significant difference between the melatonin and placebo groups. The mean duration of their night shift history was 11.9 ± 10.6 years; the mean number of night shifts per month was 8.7 ± 4.7 with no significant differences between both groups (Table 1). At baseline, there was no significant difference in anthropometric and cardiometabolic parameters, neither between night shift and non-night shift workers nor between the night shift workers randomized to the melatonin and placebo groups, respectively (Table 1).

Based on the Horne-Östberg Morningness-Eveningness Questionnaire, the majority of study participants were intermediate types (5 non-night shift workers, 41.7 %; 17 night shift workers, 70.8 %)). Four participants in each of the groups were evening types, and two non-night shift workers and three night shift workers were morning types. There were no significant differences between the two groups. Within the night shift workers, morning, evening, and intermediate chronotypes were equally distributed (melatonin, 2 / 2 / 8 participants, placebo, 1 / 2 / 9 participants; p = n.s.).

During the 12 weeks' intervention phase, the study participants performed 24.0 ± 3.3 (melatonin group) and 19.6 ± 3.4 night shifts (placebo group; p = n.s.).

3.2. Effects of melatonin treatment on glucose tolerance and insulin resistance

At baseline, the areas under the concentration-time curve for serum glucose and serum insulin during OGTT were not significantly different between night shift workers and non-night shift workers. During 12 weeks of double-blind administration of study medication, melatonin did not significantly affect serum glucose or serum insulin concentrations as compared to baseline or compared to placebo (Fig. 2).

The night shift workers were significantly more insulin-resistant than the non-night shift workers. The HOMA index was significantly higher and the QUICKI index was significantly lower in night shift workers at baseline (Fig. 3a and b). However, melatonin treatment did not significantly affect either of these two indices of insulin resistance. Neither the area under the curve for glucose nor for insulin was significantly different between night shift workers and controls, nor was there any significant effect of melatonin treatment on these parameters (Fig. 3c and d).

Table 1
Baseline characteristics of the study population.

	Night shift	Non-night shift		Night shift		
	n = 24	n = 12	p	Melatonin (n = 12)	Placebo (n = 12)	p
Anthropometrics						
Sex (m/f)	13/11	6/6	ns	4/8	9/3	ns
Age (years)	36.6 ± 11.5	36.9 ± 11.2	ns	38.3 ± 11.6	34.8 ± 11.5	ns
BMI (kg/m^2)	26.9 ± 6.4	24.0 ± 2.5	ns	26.1 ± 5.1	27.8 ± 7.7	ns
Waist/Hip ratio	0.9 ± 0.1	0.8 ± 0.1	ns	0.8 ± 0.1	0.9 ± 0.1	ns
Work History						
No. of nights shifts / month	8.7 ± 4.7	n.a.		9.3 ± 4.5	8.2 ± 5.0	ns
Night shifts since (years)	11.9 ± 10.6	n.a.		12.5 ± 9.7	11.4 ± 11.8	ns
Cardiometabolic parameters						
Systolic blood pressure (mm Hg)	119.7 ± 10.9	120.3 ± 12.0	ns	119.5 ± 8.6	119.9 ± 13.3	ns
Diastolic blood pressure (mm Hg)	72.9 ± 8.5	73.0 ± 6.1	ns	71.5 ± 8.0	74.3 ± 9.1	ns
Heart rate (1/min)	63.9 ± 12.7	67.0 ± 13.1	ns	68.3 ± 12.6	59.4 ± 11.6	ns
Fasting glucose (mg/dL)	87.5 ± 7.4	85.7 ± 6.5	ns	87.1 ± 6.3	87.8 ± 8.5	ns
HbA1c (%)	5.1 ± 0.3	5.0 ± 0.3	ns	5.1 ± 0.3	5.1 ± 0.3	ns

Abbreviations: BMI, body mass index; HbA1c, glycated hemoglobin. Data are given as mean ± standard deviation.

3.3. Effects of melatonin treatment on physiological diurnal rhythms

The diurnal rhythms of plasma melatonin (Fig. 4a and c) and plasma cortisol (Fig. 4b and d) were not significantly different between night shift workers and non-night shift workers. As expected, melatonin treatment caused a significant prolongation of the nightly elevation in plasma melatonin, which was reversed after wash-out (week 24) (Fig. 4a).

At baseline, the 24-hour blood pressure profile was not significantly different between night shift workers and non-night shift workers. In both groups, systolic blood pressure levels tended to be slightly lower during the second investigation, i.e., after 12 weeks of intervention; however, by comparison with placebo, melatonin treatment did not significantly affect the diurnal variation in systolic or diastolic blood pressure (Fig. 5). There were seven vs. eight dippers among non-night shift workers and night shift workers, respectively. Four non-night shift workers were non-dippers, and one was a reverse dipper, as compared to 13 non-dippers and three reverse dippers among night shift workers. Melatonin treatment did not significantly affect the proportion of dippers versus non-dippers compared to placebo (Table 2).

3.4. Effects of melatonin treatment on sleep quality

At baseline, sleep quality was highly significantly worse in night shift workers than in non-night shift workers, with only five night shift workers considering their sleep quality as good (21 %) and 17 individuals having poor sleep quality (71 %), contrasted by nine non-night shift workers with good sleep quality (75 %) and only two having poor sleep quality (17 %). Melatonin treatment significantly shifted this proportion towards better sleep quality ($p < 0.001$); after 12 weeks of medication intake, 50 % of the melatonin-treated night shift workers indicated good sleep quality, whilst the proportion of good vs. poor sleepers remained almost unchanged in the placebo group (Table 3).

3.5. Compliance with medication and safety of melatonin treatment

Compliance with the pre-determined medication schedule was similar in both groups (melatonin group, 90.0 ± 2.0 %; placebo group, 93.5 ± 1.9 %; p = n.s.).

The safety of melatonin intake was regarded as good. There was a total of 74 adverse events (AEs) that affected 10 out of 12 individuals in the melatonin group and 5 out of 12 individuals in the placebo group. 35 AEs occurred during the wash-out period; none of the AEs was considered to be related to study medication. One serious adverse event occurred during the wash-out phase in a participant allocated to placebo; this event was not considered related to study medication (accident during action sports). Table 4 gives a complete overview of adverse events during this trial.

4. Discussion

The present study has three major findings: Firstly, rotating night shift workers have impaired glucose tolerance compared to non-night shift workers at baseline. Secondly, sustained-release melatonin treatment for twelve weeks improved sleep quality in rotating night shift

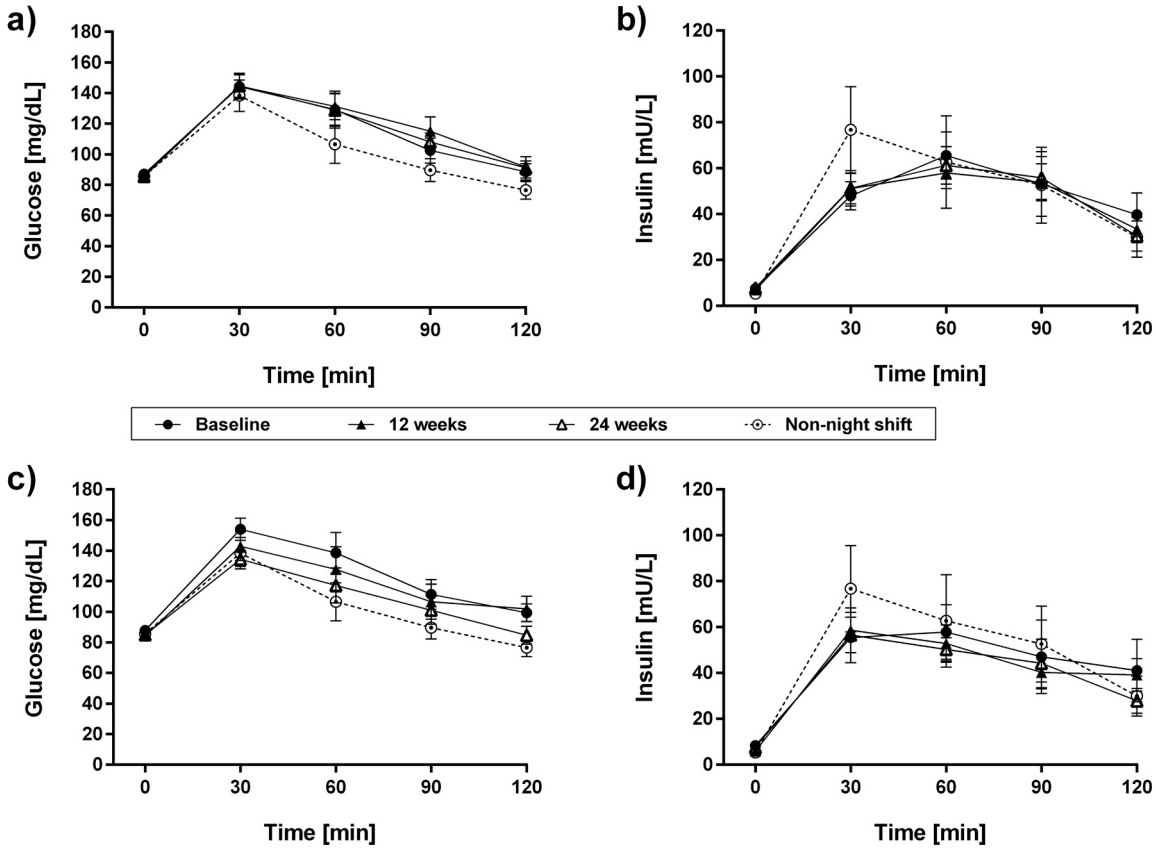

Fig. 2. Time course of serum glucose and insulin concentrations during oral glucose tolerance testing in night shift workers at baseline, after 12 weeks, and after 24 weeks as compared to non-night shift workers. Night shift workers were randomly allocated to melatonin (**a** and **b**) or placebo (**c** and **d**) between baseline and week 12; week 24 was a final examination after 12 weeks of wash-out.

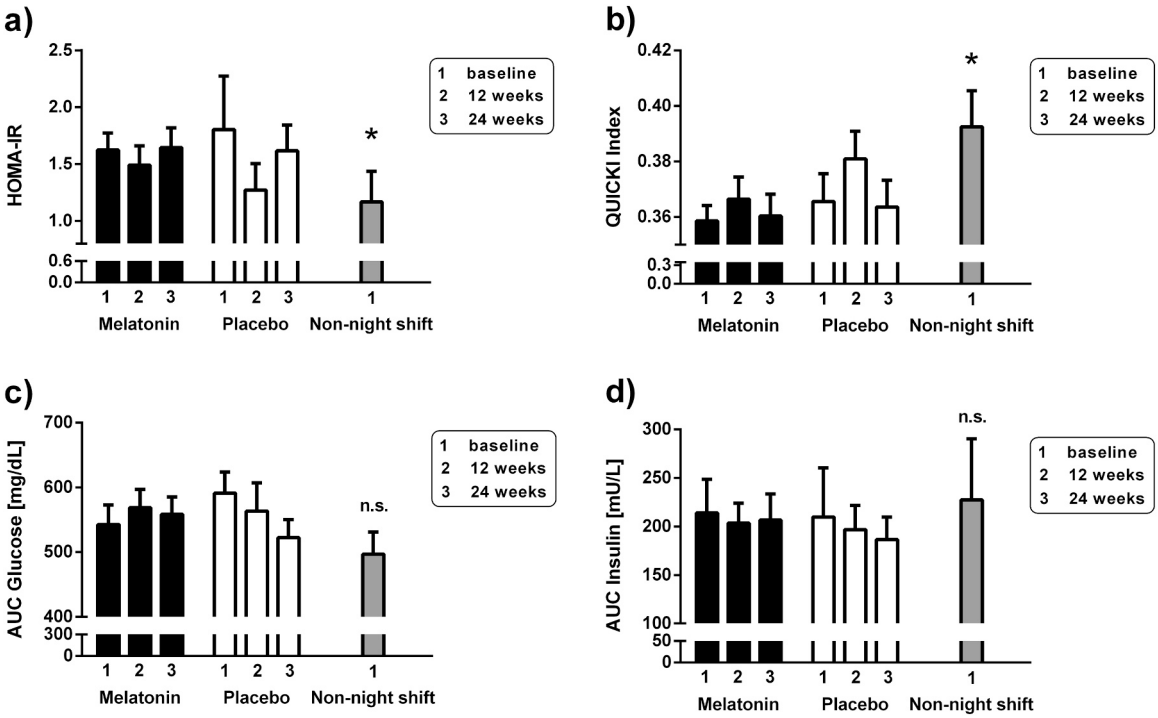

Fig. 3. Insulin resistance indices (**a**: HOMA-IR, **b**: QUICKI) and area under the curve (AUC) for glucose (**c**) and insulin (**d**) during oral glucose tolerance testing at baseline, 12 weeks, and 24 weeks in night shift workers as compared to non-night shift workers (baseline only). Data are presented as mean ± SD. *$p < 0.05$ versus baseline in night shift workers.

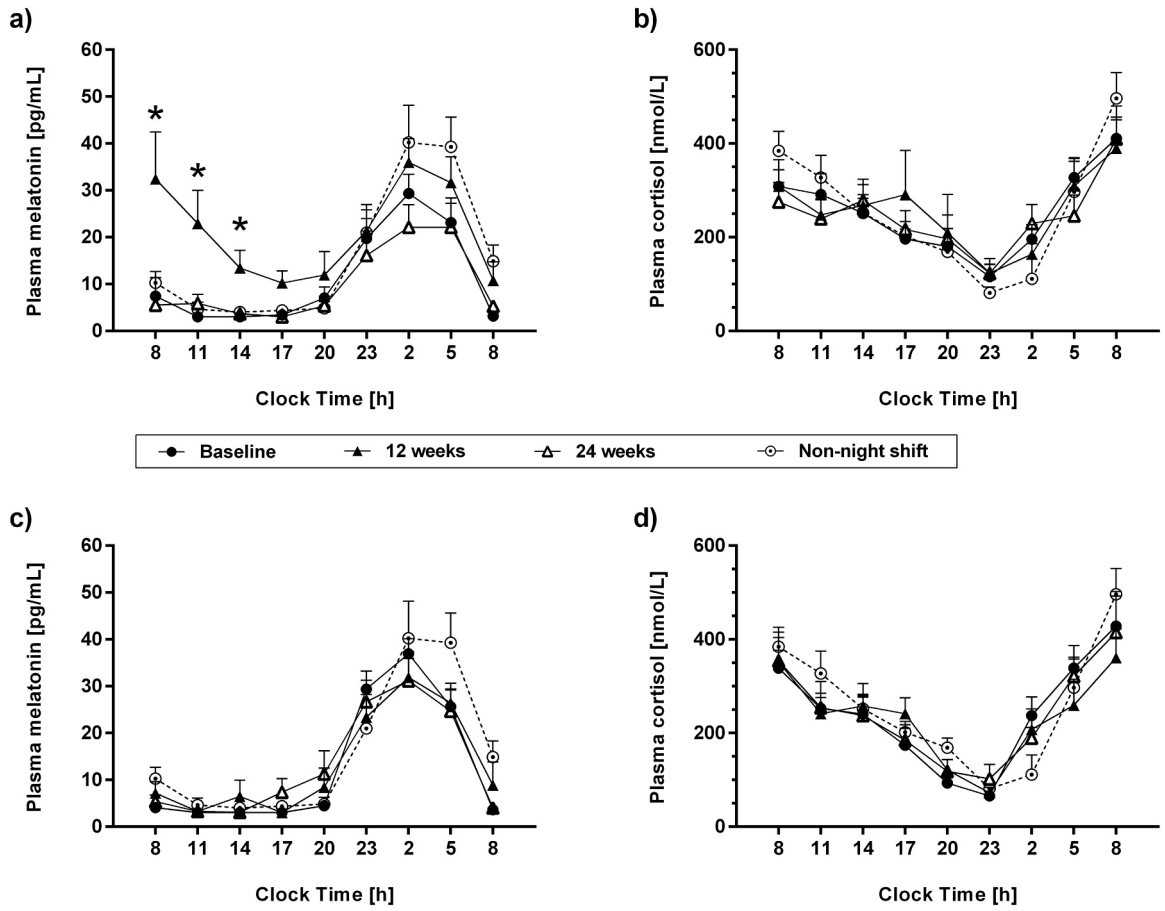

Fig. 4. Plasma melatonin and cortisol concentrations in night shift workers at baseline, after 12 weeks, and after 24 weeks as compared to non-night shift workers. Night shift workers were randomly allocated to melatonin (**a** and **b**) or placebo (**c** and **d**) between baseline and week 12; week 24 was a final examination after 12 weeks of wash-out. *$p < 0.05$ versus baseline.

workers, but it did not affect diurnal rhythms of melatonin and cortisol. Thirdly, melatonin treatment does not improve glucose homeostasis, indices of insulin resistance, or diurnal blood pressure control in rotating night shift workers.

Rotating night shift workers in this study were significantly more insulin-resistant than non-night shift workers at baseline and throughout the study, as judged by a significantly higher HOMA index and a significantly lower QUICKI index. Both of these indices are based upon fasting glucose and insulin concentrations, but they use different algorithms. However, this level of insulin resistance was insufficient to affect the time course of glucose and insulin in oral glucose tolerance testing.

Previous studies have suggested a relationship between night shift work, disruption of circadian rhythms, and glucose tolerance, diabetes mellitus, and metabolic syndrome. In a cross-sectional analysis of 111 active and 98 former rotating night shift workers with 69 daytime workers, we recently found significantly elevated glycated haemoglobin (HbA1c) and diastolic blood pressure levels in active and former night shift workers [27]. Similar findings were reported from two prospective studies [5,6]. In the first study mentioned, the difference in HbA1c levels was numerically small (below 4 % difference compared to daytime workers), but significant, and no significant difference was found in fasting glucose or insulin levels, although insulin tended to be somewhat higher in rotating night shift workers [27]. No metabolic parameters have been reported in the epidemiological long-term survey by de Bacquer and colleagues [6]. However, they reported that accumulated years of shift work gradually increased the risk of developing metabolic syndrome. The prospective study by Pietroiusti and colleagues comprised a comparatively high number of nurses performing a high frequency of night shifts (n = 402) or no night shifts at all (n = 336); they found a major difference in the incidence of metabolic syndrome during 4 years of follow-up. However, metabolic blood parameters were not reported in this study either [5].

We analysed several measures for physiological diurnal rhythms, i.e., the 24 h plasma concentration profiles of melatonin and cortisol, two well-known hormones with prominent circadian rhythms driven by the central circadian clock in the suprachiasmatic nuclei [28,29], and blood pressure, which is characterized by a physiological nighttime drop [12,30]. We did not find any significant differences in these markers of circadian alignment in the night shift workers included in this study. This observation is in line with previous studies in both simulated [31,32] and real-world [33,34] shift work have shown that the rhythms of these circadian markers are resistant to phase shifting, indicating that the endogenous SCN rhythm is slow to adapt to shift work schedules [35]. Nonetheless, in the study by Rizza and colleagues [27], we observed significant differences in clock gene expression in circulating peripheral blood mononuclear cells between active night shift workers and daytime workers. In a previous study, we reported the effects of timed light therapy on blood pressure in rotating night shift workers [8]. 12 weeks of light therapy caused a significant reduction of systolic blood pressure at nighttime in that study, which was associated with a significant restoration in the proportion of dippers. Non-dipper status is associated with an increased risk of cardiovascular disease [36]; it is more common in night shift workers than in daytime workers [8,37]. However, we did not find any changes in HbA1c or fasting glucose levels in that study, and the blood pressure-reducing effects of light therapy were associated with reduced renal excretion of catecholamine

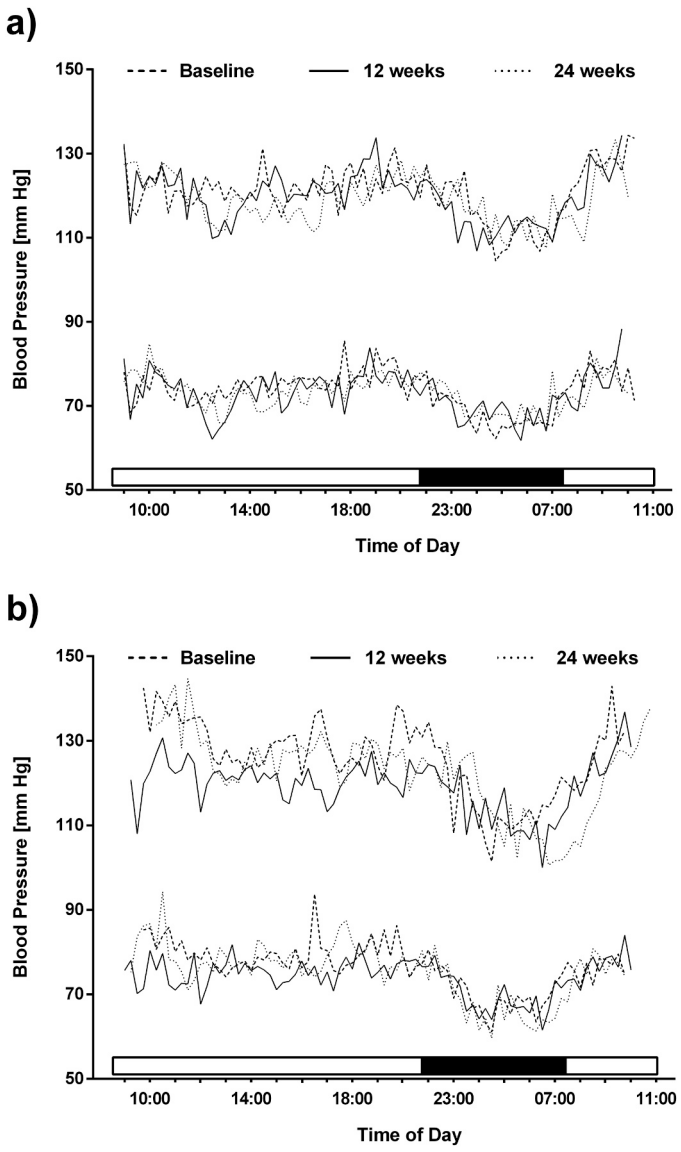

Fig. 5. 24-hour blood pressure profiles comparing systolic and diastolic arterial blood pressure between non-night shift workers with night shift workers allocated to the melatonin (**a**) and placebo groups (**b**) at baseline, at weeks 12 and 24. The graphs display means without indicators of variability for better visibility of the 24-hour profiles.

Table 2
Effects of melatonin versus placebo on dipper status of 24 h blood pressure.

	Non-night shift	Night shift	P	Melatonin	Placebo	p
Baseline	7 / 4 / 1	8 / 13 / 3	n.s.	5 / 6 / 1	3 / 7 / 2	n.s.
12 weeks	n.d.	5 / 17 / 2	n.d.	3 / 9 / 0	2 / 8 / 2	n.s.
24 weeks	n.d.	8 / 15 / 0	n.d.	3 / 8 / 1	5 / 6 / 0	n.s.

Data shown are numbers of dippers / non-dippers / reverse dippers. Abbreviations: n.d., not determined. There were no significant differences between any of the groups.

metabolites, whilst we did not observe changes in diurnal melatonin or cortisol profiles by treatment.

We therefore believe that the frequency and duration of night shift work, e.g., rotating night shift work versus continuous night shift work,

Table 3
Effects of melatonin versus placebo on sleep quality.

	Non-night shift	Night shift	P	Melatonin	Placebo	p
Baseline	9 / 2 / 1	5 / 17 / 2	0.005	2 / 9 / 1	3 / 8 / 1	n.s.
12 weeks	n.d.	10 / 13 / 1	n.d.	6 / 6 / 0*	4 / 7 / 1	n.s.
24 weeks	n.d.	10 / 13 / 0	n.d.	6 / 6 / 0*	4 / 7 / 0	n.s.

Data shown are numbers of individuals having good sleep / poor sleep / no answer. Sleep quality was assessed by the Pittsburgh Sleep Quality Index (PSQI) [20]. A PSQI score below or equal to 5 was considered as "good sleep", a score above 5 was considered as "poor sleep". 12 weeks indicated the investigation after 12 weeks of intervention; 24 weeks indicates the investigation 12 weeks after the end of the intervention (i.e., wash-out). Abbreviations: n.d., not determined.

* $p < 0.05$ for trend over time within the treatment group.

Table 4
Adverse events during the clinical trial.

MedDRA term	Melatonin		Placebo		P
System organ class	**Intervention Phase**	**Wash-out Phase**	**Intervention Phase**	**Wash-out Phase**	
Cardiac disorders	5	-	-	-	n.s.
Gastrointestinal disorders	4	5	-	2	n.s.
Infections and infestations	5	3	-	3	n.s.
Injury, poisoning, and procedural complications	-	-	1	-	n.s.
Musculoskeletal and connective tissue disorders	5	3	-	-	n.s.
Nervous system disorders	11	9	8	8	n.s.
Vascular disorders	-	2	-	-	n.s.
Total	30	22	9	13	n.s.

Data display the absolute number of study participants experiencing each adverse event. P values denote statistically significant differences between melatonin and placebo groups. Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities; n.s., not statistically significant.

number of days off between changes in shifts, and number of night shifts per month, may play important roles in defining the influence of night shift work on the degree of disruption of circadian rhythms and on associated physiological parameters.

Single nucleotide polymorphisms (SNP) in the melatonin receptor 1B gene have been shown to confer a genetic risk of type 2 diabetes; specifically, carriers of the G allele of the MTNR1B SNP rs10830963 have an increased risk of type 2 diabetes [38]. This genetic effect is mediated, at least in part, by an extended duration of the nighttime elevation of serum melatonin into the morning hours [39]. Whilst we have not assessed genotypes in our study population, the prolonged elevation of melatonin into the morning hours as reported for carriers of the MTNR1B G allele may suggest that the extended elevation of melatonin levels during and after treatment in our study (as depicted in Fig. 4a) may have counter-acted any beneficial effect of melatonin via normalization of diurnal rhythms. However, this speculation needs experimental validation in future studies.

Melatonin has a prominent role in mediating many, but not all, of the physiological effects influenced by the central circadian clock [40]. Beyond its well-described effects on sleep and jet lag [16,17], evidence has accumulated recently to suggest that exogenous melatonin may

exert favorable effects on glucose metabolism and, thereby, be a novel treatment option for type 2 diabetes. A study in rats suggested that melatonin administration improved insulin resistance, hyperlipidemia, and antioxidant capacity [41]. Various molecular mechanisms of melatonin have been described that may contribute to these effects, e.g., stimulation of insulin secretion by pancreatic β cells [42] and its antioxidant activity [43]. A recent meta-analysis of 15 studies in humans revealed positive effects of melatonin supplementation on fasting blood glucose, insulin resistance, and HbA1c levels [18]. However, this meta-analysis also showed that doses of 10 mg/day and above produced greater benefit than smaller doses, and intervention times of 10 weeks or more are needed to demonstrate significant effects of melatonin supplementation. Importantly, many of the controlled trials included in this meta-analysis had been performed in patients with additional medical conditions like long-term antipsychotics use, Parkinson's disease, end-stage renal disease, or chronic liver disease [18], shedding some doubt on the utility of melatonin as a treatment in classical type 2 diabetes.

Despite these controversial data from interventional trials with exogenous melatonin, epidemiological evidence suggests that low endogenous melatonin secretion is associated with the development of type 2 diabetes in man [44,45], and diabetic rats and human patients with type 2 diabetes show decreased diurnal melatonin serum levels [46]. In addition, common genetic variants in the melatonin receptor 2 gene (MTNR1B) have been linked to type 2 diabetes risk in genome-wide association studies [47]. Based on these data and the repeatedly confirmed effect of exogenous melatonin to improve alignment of the central clock with external environmental stimuli [16,17,48], we used a different, novel treatment approach in this study.

By adjusting the timing of melatonin administration to each participant's shift schedule, i.e., to evening bedtime on days off work and morning bedtime after each of three or more shifts, respectively, and pausing melatonin intake on days after one single or two subsequent night shifts, we aimed to strengthen the alignment of the participant's circadian rhythms to individual shift schedules while taking into consideration the slow adaptation of SCN-driven rhythms to changes in shift schedules. With this design we aimed to strengthen the circadian phase-setting properties of exogenous melatonin over its additional, e.g., antioxidant effects, and assessed its effects on multiple parameters of glucose tolerance and diurnal patterns of melatonin, cortisol, and blood pressure. Using a dose of 2 mg of sustained-release melatonin in a pharmaceutical formulation that has received marketing approval in Europe for the treatment of primary insomnia with poor sleep quality in people above the age of 55 years, our goal was to avoid effects of melatonin which occur at high concentrations, e.g., the antioxidant actions. We found a significant elevation of melatonin plasma concentration lasting well into the early afternoon hours as well as improvements in sleep parameters, but no detectable influence on glucose metabolism and diurnal blood pressure control. These data suggest that melatonin has only mild, subclinical effects on these cardiometabolic parameters, if any. Other molecular mechanisms unrelated to circadian rhythms may have contributed to the effects reported in previous studies.

Melatonin was well tolerated in our study. The side effects observed were related to those previously reported for this drug. Only one study participant dropped out of our study; unblinding revealed that he was assigned to placebo treatment, and had experienced an accident during action sports during the wash-out phase.

Our study has several limitations. Based on a small number of participants, minor effects of melatonin treatment may have remained undetected. However, our study was powered to detect clinically relevant changes in cardiometabolic parameters, and there were no observable trends in these parameters that may have missed statistical significance due to small sample size. Secondly, the rotating night shift workers had no marked disruptions of the circadian SCN clock at baseline. Therefore, we cannot exclude that melatonin treatment might help to improve cardiometabolic status in shift workers undertaking more intense rotating or permanent night shift work with a greater disruption of circadian rhythms. In addition, the study participants were pre-diabetic, whilst beneficial effects of melatonin on metabolic parameters have been shown in patients with established type 2 diabetes [18]. Finally, study duration was 12 weeks; a more prolonged intervention may be needed to uncover any long-term beneficial effects of melatonin on diabetes development.

In conclusion, our study shows that 12 weeks of sustained-release melatonin administration are well tolerated and help to improve sleep quality in rotating night shift workers. However, melatonin's effects at the dosing scheme applied here were not sufficient to cause clinically relevant changes in diurnal blood pressure profiles, glucose tolerance, or insulin resistance indices.

CRediT authorship contribution statement

Skene Debra J.: Data curation, Methodology, Writing – review & editing. **Kastner Mariola:** Data curation, Writing – review & editing. **Federici Massimo:** Conceptualization, Writing – review & editing. **Marx Nikolaus:** Conceptualization, Writing – review & editing. **Schwedhelm Edzard:** Data curation, Methodology, Writing – review & editing. **Middleton Benita:** Data curation, Writing – review & editing. **Laing Anika:** Investigation, Project administration, Writing – review & editing. **Hannemann Juliane:** Conceptualization, Formal analysis, Investigation, Project administration, Writing – original draft, Supervision. **Böger Rainer:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – original draft.

Declaration of Competing Interest

None.

Data Availability

Data will be made available on request.

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