



Associations of sleep quality, sleep apnea and autonomic function with insulin secretion and sensitivity: HSCAA study



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ARTICLE INFO

Article history:

Received 29 January 2020

Received in revised form

13 March 2020

Accepted 15 March 2020

Available online 19 March 2020

Keywords:

Pancreatic β -cell function

Insulin resistance

Actigraphy

Apnea/hypopnea index

Heart rate variability

Glucose intolerance

ABSTRACT

Rationale and purpose: Although sleep disorders are shown to be involved in occurrence of diabetes, impacts of several quantitative parameters related to sleep on insulin secretion and sensitivity is yet to be elucidated. We cross-sectionally examined relationships among quantitative sleep quality, sleep apnea, and autonomic function with insulin secretion and sensitivity in 399 patients without previous diagnosed diabetes who underwent 75-g oral glucose tolerance test (75gOGTT).

Method: Poor sleep quality (PSQ) was defined as an activity index ≥ 50 by actigraphy. Sleep apnea was measured by apnomonitor, while standard deviation of all normal-to-normal R-R intervals (SDNN) was measured by active tracer. Parameters of insulin secretion and sensitivity were measured by 75gOGTT.

Results: Patients with PSQ exhibited significantly lower insulinogenic index ($r = 0.155$, $p < 0.01$), a parameter of insulin secretion, with the association independent of other clinical factors including apnea and SDNN ($\beta = -0.156$, $p < 0.01$). In contrast, presence of sleep apnea ($r = -0.143$, $p < 0.05$) and the lower SDNN ($r = -0.150$, $p < 0.01$) were significantly and inversely associated with BIGTT-S, an insulin sensitivity parameter, with the association of SDNN with BIGTT-S remaining significant even after adjustments for PSQ and sleep apnea ($\beta = -0.111$, $p < 0.05$).

Conclusion: Poor sleep quality is an independent predictor of pancreatic β -cell function, which could be involved in occurrence of type 2 diabetes.

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1. Introduction

General metabolism, including adiposity and glucose homeostasis, appears to be regulated by integrated central brain functions, such as the hypothalamus, brainstem, and dopaminergic brain reward system [1–5], resulting in effects on feeding behavior, energy homeostasis, pancreatic insulin secretion, and cardiovascular function. Sleep duration and quality have been shown to be associated with the incidence and severity of diabetes [6–8], and sleep disorders or sleep disordered breathing [9–11], with/without autonomic nervous dysfunction reported as potential mechanisms, which may link central brain dysfunction, adiposity, and pancreatic β -cell dysfunction [3,12,13].

Although sleep has been shown to be associated with development of type 2 diabetes mellitus in population studies [14,15], reports regarding its effect on insulin secretion and insulin sensitivity is limited [16,17]. Easily examined symptoms of sleep disordered breathing have been found to be strongly associated with insulin resistance and incidence of type 2 diabetes in older adults [18]. However, those previous reports estimated sleep quality using self-reported questionnaires, while no known study has examined the impact of quantitatively measured sleep duration, quality, and apnea on insulin secretion and sensitivity.

Although autonomic nervous dysfunction is a common complication observed in patients with diabetes mellitus, it is generally present even in a pre-diabetes state [19–21], suggesting that impairment of autonomic function may progress or even contribute to development of diabetes. Heart rate variability (HRV) is a long-term time-domain measurement based on 24-h Holter records, more sensitive and reproducible than shorter term test methods [22], with that parameter proven useful for detecting

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diabetic autonomic neuropathy [23]. In the Atherosclerosis Risk In Communities study, decreased HRV was shown to be significantly associated with diabetes incidence [24], suggesting that autonomic dysfunction can be attributed to the pathogenesis of the disease. However, the impact of autonomic nervous dysfunction on insulin secretion and its sensitivity has not been clarified. Moreover, the mutual impacts of sleep duration, quality, and apnea, as well as autonomic nervous function on insulin secretion and sensitivity have yet to be elucidated.

The Hyogo Sleep Cardio-Autonomic Atherosclerosis (HSCAA) Study, begun in October 2010, was designed to analyze the mutual impacts of quantitative sleep duration, quality, and apnea, and also autonomic nervous function on metabolic and cardiovascular outcomes in patients with cardiovascular risk factors. In the present study, 399 patients who agreed to undergo a 75-g oral glucose tolerance test (75gOGTT) were examined for quantitative sleep duration, quality, and apnea, and autonomic nervous function in order to investigate their mutual associations with insulin secretion and sensitivity.

2. Methods

2.1. Study design and participants

All subjects agreed to participate in the study by providing written informed consent and the protocol was approved by the Ethics Committee of Hyogo College of Medicine (approval no. 2351). This cross-sectional study was conducted as part of the HSCAA Study, which was instituted to examine the impacts of sleep and autonomic imbalance on metabolic, renal, and cardiovascular events [25,26]. Patients with 1 or more cardiovascular risk factors, including obesity, smoking, presence of cardiovascular event history, hypertension, dyslipidemia, diabetes mellitus, and chronic kidney disease, and being treated at the Division of Diabetes, Endocrinology and Metabolism of Hyogo Medical College Hospital (Hyogo, Japan) were registered. Due to the concept and purpose of this cohort study, patients with regular use of hypnotics, or treated by Psychiatrist were not registered. Among 976 patients registered from October 2010 to December 2018, those with a formal diagnosis of malignant neoplasm, endocrine disease, kidney failure, or diabetes under treatment, or who refused an 75gOGTT test were excluded. Thus, 399 patients who underwent an examination for sleep quality (n = 344), sleep apnea (n = 332), and/or heart rate variability (n = 319) were analyzed in the present study (Fig. 1).

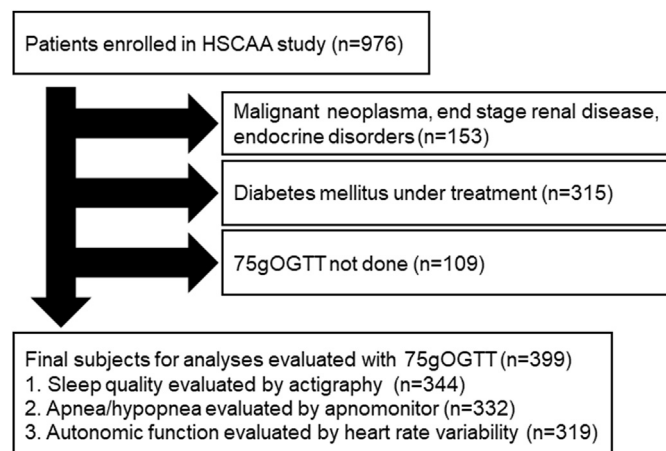


Fig. 1. Subject enrollment.

2.2. Anthropological and risk factors

We obtained the medical history of each subject, and measured height and body weight. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m²). Smoking and alcohol intake were based on self-reported habits. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or treatment for hypertension. Dyslipidemia was defined as the presence of low density lipoprotein cholesterol (≥ 140 mg/dl), high density lipoprotein cholesterol (≤ 40 mg/dl), an elevated triglyceride level (≥ 150 mg/dl), or treatment for dyslipidemia [27].

2.3. Biochemical parameters, 75gOGTT, and calculation

Blood samples were obtained in the morning after an overnight fast and then quickly centrifuged to obtain plasma. Whole blood was used for hemoglobin A1c, and EDTA-plasma for glucose (Glu) and insulin (IRI) determinations. Glucose was measured using a glucose oxidase method and insulin with radio-immunometric assay findings (Insulin RIA-BEAD II; Dinabot Co., Tokyo, Japan). For estimation of insulin secretion and resistance, the following indices were calculated; Homeostasis Model Assessment (HOMA)- $\beta = (20 \times \text{IRI } \mu\text{U/ml}) / (\text{Glu mmol/L} - 3.5)$ [28] and HOMA-IR = $(\text{Glu mmol/L} \times \text{IRI } \mu\text{U/ml}) / 22.5$ [29].

After a 12-h overnight fast, a standard 75gOGTT was performed to categorize glycemic abnormalities [normal glucose tolerance (NGT), impaired glucose tolerance (IGT), diabetes type], and to examine insulin secretion and sensitivity, with plasma samples for measuring glucose and insulin obtained at 0, 30, 60, 90, and 120 min after glucose loading. The subjects underwent this test within a week before or after the sleep test to examine association of insulin secretion and sensitivity with sleep parameters. The following indices were calculated in this study: Insulinogenic index = $(\text{IRI}_{30} \text{ pmol/L} - \text{IRI}_0 \text{ pmol/L}) / (\text{Glu}_{30} \text{ mmol/L} - \text{Glu}_0 \text{ mmol/L})$ [30], corrected insulin response (CIR) = $[(100 \times \text{IRI}_{30} \text{ pmol/L}) / (\text{Glu}_{30} \text{ mmol/L} \times (\text{Glu}_{30} \text{ mmol/L} - 3.89))]$ [31], Incremental area under the curve of insulin (IncaAUC insulin) = $[(\text{IRI}_{30} \text{ pmol/L} + \text{IRI}_0 \text{ pmol/L}) / 2] \times 30 + [(\text{IRI}_{120} \text{ pmol/L} + \text{IRI}_{30} \text{ pmol/L}) / 2] \times 90 - (120 \times \text{IRI}_0 \text{ pmol/L})$, BIGTT-AIR = $\exp[8.2 + (0.00178 \times \text{IRI}_0 \text{ pmol/L}) + (0.00168 \times \text{IRI}_{30} \text{ pmol/L}) - (0.000383 \times \text{IRI}_{120} \text{ pmol/L}) - (0.314 \times \text{Glu}_0 \text{ mmol/L}) - (0.109 \times \text{Glu}_{30} \text{ mmol/L}) + (0.0781 \times \text{Glu}_{120} \text{ mmol/L}) + (0.18 \times \text{gender}) + (0.032 \times \text{BMI})]$ as an index of rapid insulin response, BIGTT-S = $\exp[4.9 - (0.00402 \times \text{IRI}_0 \text{ pmol/L}) - (0.000556 \times \text{IRI}_{30} \text{ pmol/L}) - (0.00127 \times \text{IRI}_{120} \text{ pmol/L}) - (0.152 \times \text{Glu}_0 \text{ mmol/L}) - (0.00871 \times \text{Glu}_{30} \text{ mmol/L}) - (0.0373 \times \text{Glu}_{120} \text{ mmol/L}) - (0.145 \times \text{gender}) - (0.0376 \times \text{BMI})]$ [32], Matsuda index = $10,000 / [\sqrt{(\text{Glu}_0 \text{ mmol/L} \times \text{IRI}_0 \text{ pmol/L} \times \text{mean PG mmol/L} \times \text{mean IRI pmol/L})}]$ as an index of insulin sensitivity [33], QUICKI = $1 / (\log \text{IRI}_0 \text{ pmol/L} + \log \text{Glu}_0 \text{ mmol/L})$ [34], and Disposition index = Insulinogenic index \times QUICKI.

2.4. Assessments of sleep duration, quality, and apnea

Actigraphy has not traditionally been used in routine diagnosis of sleep disorders, but its technological advances as well as its validity, have made its use common [35]. Actigraphy can offer reliable results with an accuracy close to those with polysomnography, a gold standard for sleep assessment, for estimating sleep time, but also moderately useful for sleep stage estimation [36]. Since the use of polysomnography is physically difficult for many subjects, we used an actigraphy device (Ambulatory Monitoring, Inc., Ardley, New York, USA), which senses motion as acceleration, was placed on the wrist of the non-dominant arm and

used for quantitative analysis of sleep quality, as previously described [25,37]. Activity index as a parameter of sleep quality was calculated as total body motion during sleep time, with a higher activity index value considered to be related to lower sleep quality. Poor sleep quality (PSQ) was defined as an activity index value equal to or greater than 50, as previously described [37,38]. Physical activity (mean activity counts per minute of body motion during awake and sleep time), sleep/awake physical activity ratio (parameter for diurnal rhythm), total sleep time (total minutes as sleep), %sleep (percentage of sleeping time of total time lying in bed), sleep efficiency (percentage of time scored as sleep), and time awake after sleep onset (number of minutes noted as awake during sleep time) were also analyzed. In this study, actigraphy findings suggest any subjects showed any signs of night work, or recent travel across multiple time zones. Apnomonitor (SAS- 21001, Teijin, Tokyo, Japan) results combined with measurements of percutaneous oxygen saturation were used to determine apnea-hypopnea index (AHI), as previously reported [39].

2.5. Assessment of autonomic nervous function

To analyze cardiac autonomic nervous activity, heart rate variability (HRV) was measured in a noninvasive manner with an Active Tracer device (AC-301A®, Arm Electronics, Tokyo, Japan) and a MemCalc Chiram 3 system, version 2.0 (Suwa Trust, Tokyo, Japan), as previously described [25,26,39,40]. According to the recommendations for clinical use of HRV [22], the standard deviation of the NN(RR) interval (SDNN) was calculated.

2.6. Statistical analysis

The patients were divided into the poor sleep quality (PSQ) and control groups using an activity index cut-off value of 50, and sleep apnea and control (without sleep apnea) groups using an AHI cut-off value of 5. For autonomic nervous function, the subjects were divided into low and high SDNN based on the median SDNN value. To compare variables including insulin secretion and sensitivity between the groups, a non-repeated t-test and an analysis of variance (ANOVA) with Tukey-Kramer's test (continuous variables with normal distribution), Mann-Whitney's test (continuous variables with skewed distribution), and a chi-squared test (categorical variables) were utilized, as appropriate. Pearson's correlation coefficient and multiple linear regression analyses were performed to explore independent relationships among variables. For the analyses, indices of insulin secretion and sensitivity were natural logarithm-transformed (ln) to normalize skewed distribution. To analyze factors independently associated with insulinogenic index and BIGTT-S, multivariate linear regression analyses including age, gender, body mass index, current smoking, alcohol, hypertension, dyslipidemia, and HbA1c as covariates in all Models. PSQ (Model 1), sleep apnea (Model 2), low SDNN (Model 3), PSQ and sleep apnea (Model 4), PSQ and low SDNN (Model 5), sleep apnea and low SDNN (Model 6), and all PSQ, sleep apnea and low SDNN (Model 7) were included as additional covariates. All statistical analyses were performed using the Statistical Package for the Social Sciences software platform (PASW Statistics, version 18.0). All reported *p* values are 2-tailed and were considered to be statistically significant at <0.05.

3. Results

3.1. Basal/demographic characteristics of patients with PSQ, sleep apnea, and low SDNN

Comparisons of baseline/demographic characteristics among subjects categorized by sleep quality, sleep apnea, and HRV are

shown in Table 1. Those with PSQ exhibited significantly older age, male prevalence, and higher BMI, and a greater portion showed hypertension comorbidity. Rather surprisingly, AHI and SDNN values were not significantly different among the groups. Subjects with sleep apnea exhibited significantly higher BMI, HbA1c, and HOMA-IR, whereas activity index and SDNN were not significantly different among the groups. Those with lower SDNN showed significantly older age and higher BMI, HbA1c, and HOMA-IR values. Again, activity index and AHI did not show significant differences among the groups. Subjects with PSQ, sleep apnea or low SDNN tended to exhibit higher rates of diabetes type by 75gOGTT (Table 1 and Supplemental Fig. S1).

3.2. PSQ associated with decreased insulin secretion, and sleep apnea and low SDNN with reduction in insulin sensitivity

Results of comparisons between the categories of parameters for insulin secretion (insulinogenic index, CIR, disposition index, BIGTT-AIR) and sensitivity (Inc AUC insulin, BIGTT-S, Matsuda index) calculated using 75gOGTT results are presented in Fig. 2. Insulinogenic index, CIR, and disposition index values for the PSQ subjects were significantly lower, whereas parameters for insulin sensitivity were not significantly different. To examine whether an activity index had a linear association with parameters of insulin secretion, we compared insulinogenic index, CIR, and disposition index using quadrants of activity indices (Q1 1.7–21.1, *n* = 86; Q2 21.1–29.8, *n* = 86; Q3 29.9–41.4, *n* = 86; Q4 41.6–90.9, *n* = 86). As shown in Supplemental Fig. S2, only the highest quadrant exhibited decreased parameters of insulin secretion, indicating an activity index value equal to or greater than 50 for PSQ as a reasonable threshold. Thus, a clear threshold representing poor sleep quality exists. We also examined whether other parameters shown in actigraphy findings were associated with insulin secretion and sensitivity. As indicated in Supplemental Table S1, wake after sleep onset, another representative parameter of PSQ, was significantly and inversely associated with parameters related to insulin secretion (insulinogenic index, CIR-1, disposition index, HOMA-β), but not with insulin sensitivity (IncAUC insulin, BIGTT-S, Matsuda index, HOMA-IR). Total sleep time, %sleep, and sleep efficiency were not significantly associated with parameters of insulin secretion or sensitivity, while physical activity ratio, potentially representing diurnal rhythm, also did not show a significant association with insulin secretion or sensitivity indices. Rather surprisingly and in marked contrast with PSQ, patients with sleep apnea exhibited lower BIGTT-S and lower Matsuda index, and higher IncAUC insulin values, suggesting a reduction in insulin sensitivity. Insulinogenic index, CIR-1, and disposition index values were not significantly different in patients with apnea as compared with the control group. Similar to apneic patients, those with low SDNN also exhibited significantly lower BIGTT-S and Matsuda index values, while there were no significant differences in regard to parameters of insulin secretion.

Sleep quality, sleep apnea, and autonomic function have been reported to have a mutual interaction [41], thus the associations of the individual components with indices of insulin secretion and sensitivity shown in the present study may be attributed to confounding effects by the others. Even though activity index showed only a weakly significant association with AHI and not with SDNN in the present cohort (Supplemental Fig. S3), we performed 2-way ANOVA to examine the interactions among PSQ, sleep apnea, and low SDNN in regard to insulin secretion and sensitivity. Of interest, subgroup analyses indicated that the reduced insulinogenic index and disposition index values in the PSQ group were more prominent in the group with sleep apnea than that without it (Fig. 3a). In contrast, when the subjects were categorized based on presence or

Table 1
Comparisons of clinical characteristics categorized by sleep quality, sleep apnea, and heart rate variability.

	Sleep quality (activity index)		Sleep apnea (AHI)		Heart rate variability (SDNN)	
	≥50	<50	>5	≤5	Low	High
Number	46	298	179	153	159	160
Age, years	64.6 ± 2.0**	57.9 ± 0.8	57.9 ± 1.0	60.0 ± 1.1	60.3 ± 1.0*	56.7 ± 1.1
Male gender, n (%)	36 (78.3)**	121 (40.5)	84 (46.9)	71 (46.1)	73 (45.9)	71 (44.4)
Body mass index, kg/m ²	24.8 ± 0.7*	24.1 ± 0.2	24.9 ± 0.3**	23.2 ± 0.3	24.5 ± 0.4*	23.7 ± 0.3
Current smoking, n (%)	18 (39.1)	57 (19.1)	42 (23.6)	31 (20.1)	91 (26.2)	73 (21.0)
Alcohol, n (%)	20 (43.5)	104 (34.9)	66 (37.1%)	57 (37.0%)	54 (34.2%)	60 (37.5%)
Hypertension, n (%)	31 (67.4)*	181 (60.5)	109 (60.9)	97 (63.0)	101 (63.5)	92 (57.5)
Dyslipidemia, n (%)	25 (54.3)	166 (55.5)	103 (57.5)	84 (54.5)	93 (58.5)	83 (51.9)
HbA1c, %	5.4 ± 0.0	5.3 ± 0.0	5.3 ± 0.0**	5.2 ± 0.0	5.3 ± 0.0*	5.2 ± 0.0
HOMA-IR	0.54 ± 0.07	0.56 ± 0.02	0.60 ± 0.03**	0.48 ± 0.02	0.62 ± 0.04*	0.49 ± 0.02
HOMA-β	527.4 ± 70.1	578.5 ± 24.6	589.6 ± 28.9	517.1 ± 31.9	570.5 ± 31.3	559.6 ± 34.4
OGTT						
NGT, n (%)	13 (28.3%)	116 (39.0%)	61 (34.1%)	61 (39.9%)	55 (38.9%)	69 (42.9%)
IGT, n (%)	19 (41.3%)	140 (46.8%)	82 (45.8%)	72 (46.9%)	71 (44.7%)	74 (46.4%)
DM Type, n (%)	14 (30.4)**	42 (14.2%)	36 (20.1%)	20 (13.2%)	33 (20.8%)*	17 (10.7%)
Activity index	63.4 ± 1.6**	27.3 ± 0.6	33.0 ± 1.3	31.8 ± 1.3	32.7 ± 1.3	31.3 ± 1.2
AHI	6.9 (2.4–14.0)	6.0 (1.8–13.6)	12.9 (8.3–20.6)**	1.7 (0.7–3.1)	5.0 (1.7–12.5)	6.8 (2.0–14.9)
SDNN, ms	103.8 (85.1–133.8)	116.9 (97.4–140.5)	118.6 (98.1–134.4)	113.2 (89.3–143.8)	95.1 (82.3–106.2)**	137.6 (125.2–161.2)

Data are presented as the mean ± standard error or median (25th–75th percentile) for continuous variables, and number (%) for dichotomous variables. P values are shown for comparisons of mean values for the groups (unrepeated t-test) and median (Mann-Whitney test), or percentages (chi-squared test). CVD, cardiovascular disease; NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus; AHI, apnea hypopnea index; SDNN, standard deviation of NN(RR) interval. **p* < 0.05, ***p* < 0.01 between groups.

absence of low SDNN, no clear trends showing an association of PSQ with reductions in insulin secretion indices were found (Fig. 3b), suggesting a potential interaction of sleep quality and autonomic nervous function. Notably, subjects with both sleep apnea and low SDNN showed significantly lower values for BIGTT-S as compared to the other groups (Fig. 3c). Furthermore, the Matsuda index for that group was also significantly lower as compared to subjects without sleep apnea and low SDNN.

3.3. PSQ inversely associated with insulin secretion and low SDNN with sensitivity index, independent of other clinical parameters and sleep apnea

We next examined other clinical factors associated with representative indices of insulin secretion and sensitivity, insulinogenic index, and BIGTT-S. As shown in Table 2, simple regression analysis results revealed BMI to be significantly positively correlated with insulinogenic index, whereas age, alcohol drinking habit, hypertension, HbA1c, and PSQ each showed a significantly inverse correlation with that index. Additionally, BIGTT-S was significantly and inversely associated with male gender, BMI, current smoking habit, hypertension, dyslipidemia, and HbA1c, as well as sleep apnea and low SDNN. Rather unexpectedly, age was found to be positively associated with BIGTT-S. As expected, both insulinogenic index and BIGTT-S were significantly decreased in accordance with worsening stage of glycemic abnormalities (NGT, IGT, diabetes type) (Supplemental Fig. S4).

To further examine whether the significant associations of PSQ, sleep apnea, and low SDNN with insulinogenic index and BIGTT-S were independent of potential clinical confounders, multiple linear regression analyses were performed with age, gender, BMI, smoking, alcohol, hypertension, dyslipidemia, and HbA1c used as covariates (Table 3). Those results showed that PSQ (Model 1) remained significantly and inversely associated with insulinogenic index, while low SDNN (Model 3) showed a significant inverse association with BIGTT-S. The significant association of sleep apnea with BIGTT-S was lost after adjustment with clinical confounders (Model 2). Furthermore, even after addition of sleep apnea (Model 4), low SDNN (Model 5), as well as both sleep apnea and low SDNN (Model 7) to Model 1, PSQ remained significantly and inversely

associated with insulinogenic index. Similarly, the significant inverse association of low SDNN with BIGTT-S was not confounded by PSQ (Model 5), sleep apnea (Model 6), or both (Model 7). When subjects with diabetes type (n = 56) were excluded from analyses, similar trends of associations of PSQ with insulinogenic index were observed with borderline significance (Model 1: *p* = 0.086, Model 4: *p* = 0.11, Model 5: *p* = 0.057, Model 7: *p* = 0.074) probably due to decrease in statistical power.

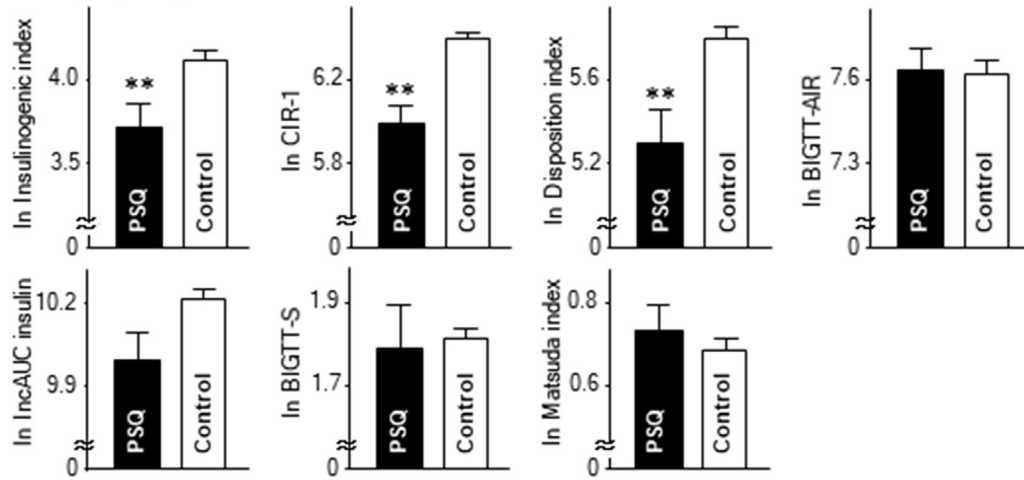
4. Discussion

Sleep has been shown to be associated with development of type 2 diabetes mellitus in population studies [14,15], though there are various factors related to sleep problems, including sleep duration, sleep efficiency, sleep quality, diurnal rhythm, sleep disordered breathing, and autonomic nervous dysfunction. Few studies have investigated the associations of sleep problems with insulin secretion and sensitivity [16,17], while no examinations of the impact of the mutual interaction of various aspects of sleep problems have been reported. This is the first known study to assess the impact of the mutual interactions of poor sleep quality, sleep apnea, and decreased autonomic function on insulin secretion and sensitivity in patients without diabetes under treatment.

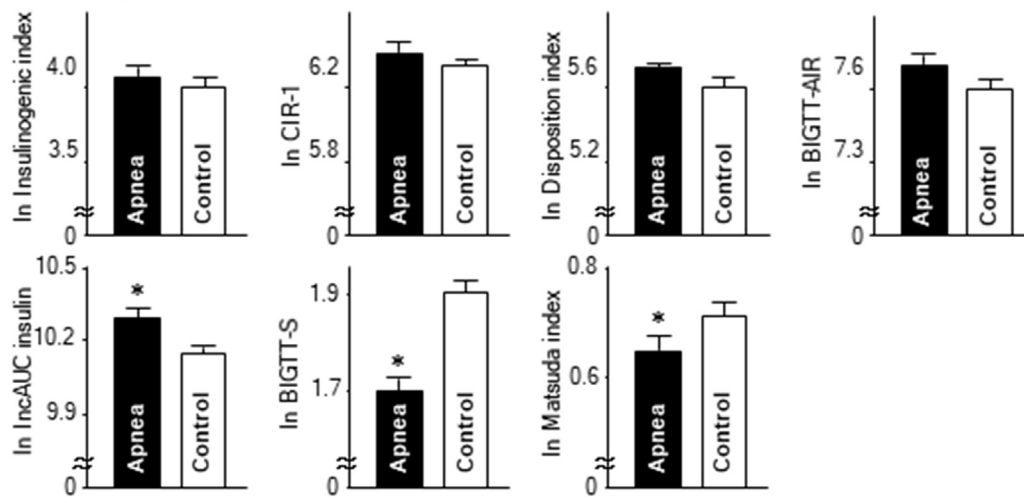
4.1. Sleep duration and quality

Previous studies that used a self-reported questionnaire found glycemic control deterioration in type 2 diabetes patients with short sleep time or poor sleep quality [6–8]. In addition, it has been reported that difficulty in falling asleep increased the risk of developing diabetes in non-diabetic populations [14,16], and that risk was also shown to be elevated in subjects with either shorter or longer sleep time [15]. In another study, van Dijk and colleagues noted that both short and long sleep durations, as determined by sleep questionnaire findings, were associated with lower insulin sensitivity, but not with insulin secretion [17]. However, no known previous study has objectively measured sleep duration and quality, and then examined their association with insulin secretion and sensitivity. Recently, So-Ngerm and colleagues conducted a clinical trial and objectively confirmed sleep extension shown by

a) Sleep quality



b) Sleep apnea



c) Heart rate variability

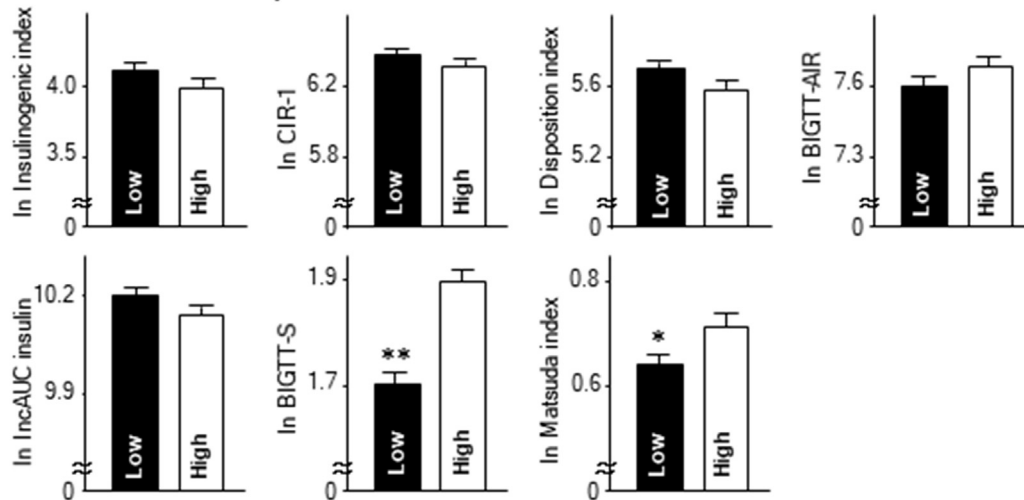
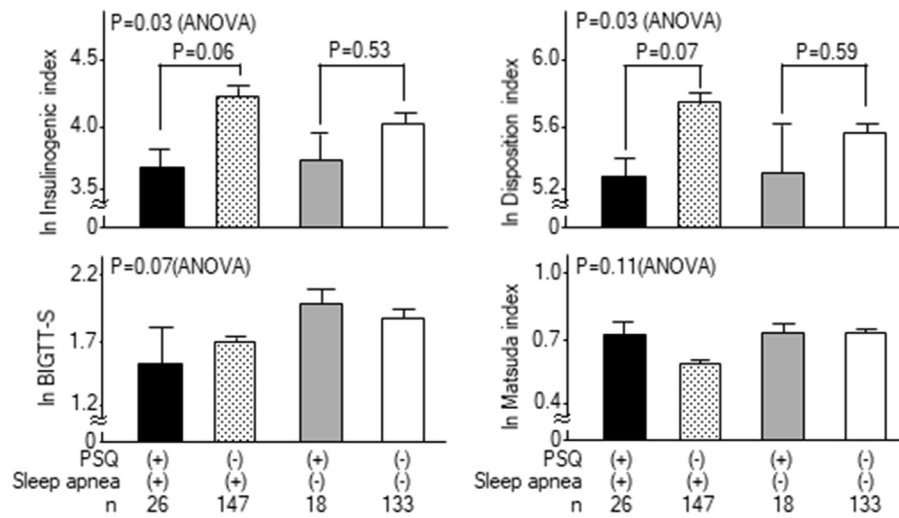
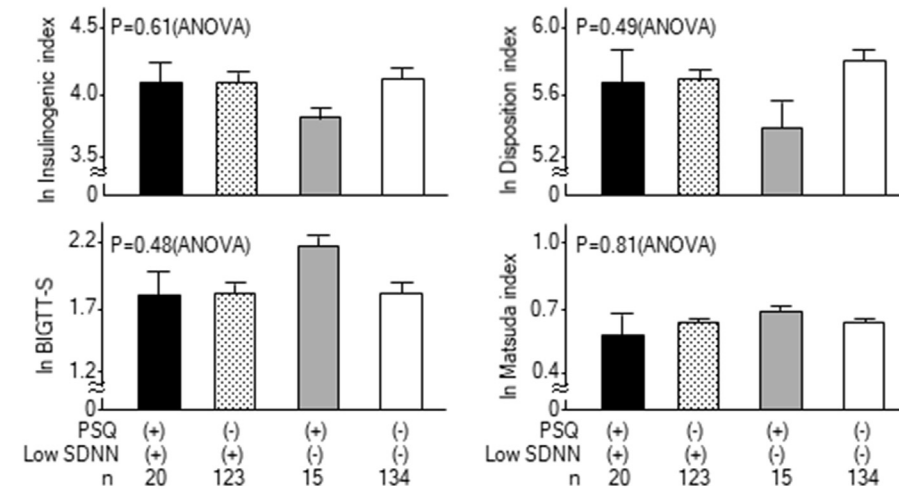


Fig. 2. Comparisons of indices of insulin secretion and sensitivity between control group and subjects with (a) poor sleep quality (PSQ), (b) sleep apnea, and (c) low and high heart rate variability (SDNN). Parameters of insulin secretion [insulinogenic index, corrected insulin response (CIR-1), disposition index, BIGTT-AIR] and insulin sensitivity (IncAUC insulin, BIGTT-S, Matsuda index) were calculated based on plasma glucose and insulin shown at 0, 30, 60, 90, and 120 min of a 75-g oral glucose tolerance test. These parameters were natural logarithm transformed to achieve a normal distribution. Each column shows the mean \pm standard error. * $p < 0.05$, ** $p < 0.01$; Student's t-test.

a) Poor sleep quality and apnea



b) Poor sleep quality and low SDNN



c) Sleep apnea and low SDNN

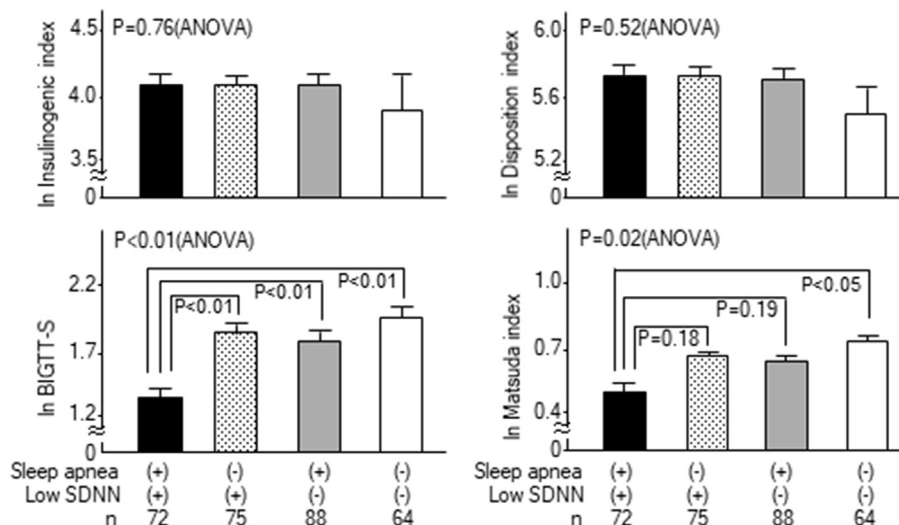


Fig. 3. Interactive effects of parameters [poor sleep quality (PSQ), sleep apnea, low SDNN] on insulin secretion (insulinogenic index, disposition index) and sensitivity (BIGTT-S, Matsuda index). Two-way ANOVA findings are shown. a) PSQ and sleep apnea, b) PSQ and low SDNN, c) sleep apnea and low SDNN. Indices of insulin secretion and sensitivity were natural logarithm-transformed to achieve a normal distribution. Each column shows the mean ± standard error. P values were calculated using Tukey-Kramer's test with ANOVA.

Table 2
Analyses of factors associated with insulinogenic index and BIGTT-S.

Variables	Insulinogenic Index	BIGTT-S
	r	r
Age	-0.207**	0.135**
Body mass index	0.210**	-0.609**
HbA1c	-0.169**	-0.193**
Gender		
male (n = 187)	4.01 ± 0.08	1.63 ± 0.06**
female (n = 212)	4.20 ± 0.06	1.99 ± 0.05
Current smoking		
yes (n = 88)	4.10 ± 0.09	1.62 ± 0.09*
no (n = 311)	4.11 ± 0.06	1.87 ± 0.05
Alcohol		
yes (n = 144)	3.91 ± 0.10*	1.84 ± 0.06
no (n = 255)	4.22 ± 0.05	1.80 ± 0.05
Hypertension		
yes (n = 243)	4.00 ± 0.06**	1.74 ± 0.06*
no (n = 156)	4.29 ± 0.08	1.95 ± 0.06
Dyslipidemia		
yes (n = 212)	4.12 ± 0.07	1.62 ± 0.05**
no (n = 187)	4.10 ± 0.07	2.07 ± 0.06

Pearson's coefficients of correlation analysis was performed for continuous variables (age, BMI, HbA1c), and Student's t-test for categorical variables. Data are presented as the mean ± standard error for t-test. PSQ, poor sleep quality; SDNN, standard deviation of NN(RR) interval. *p < 0.05, **p < 0.01.

actigraphy is significantly associated with insulin secretion indices in sleep deprived healthy individuals [42], though the effects of changes in sleep quality were not analyzed. In the present study, we quantitatively determined sleep duration and quality using actigraphy results from a relatively large number of patients without diabetes under treatment, and show for the first time that sleep quality, though not sleep duration, is significantly associated with insulin secretion, but not with insulin sensitivity.

4.2. Sleep apnea and sleep quality

Quantitatively measured poor sleep quality, a high activity index, was associated with insulin secretion indices, but not insulin sensitivity indices in the present study. Those associations

remained significant when the parameter, wake after sleep onset, was used as another representative index for poor sleep quality. Furthermore, these associations remained significant even after adjustments for other clinical factors. These findings were rather unexpected, since sleep disordered breathing, a well-recognized cause of poor sleep quality, has been shown to be closely associated with insulin resistance [9,10,43]. In accordance with those reports, the present results also demonstrate that patients with sleep apnea with AHI greater than 5 exhibit significantly lower indices of insulin sensitivity, but not of insulin secretion, even though the significant association was lost after adjustment for other clinical factors. At present, it is not clear why poor sleep quality was not associated with insulin sensitivity in our study. Pogach et al. [43] showed that electrocardiogram-based sleep-spectrogram measurements of sleep quality were associated with alterations in insulin sensitivity in patients with sleep disordered breathing. Among the 118 patients recruited in that study, 45 (38.1%) exhibited AHI equal to or greater than 15, suggesting moderate to severe sleep apnea. In the present cohort, 75 (22.5%) of 333 had an AHI value ≥ 15 , while 104 (31.2%) showed values from 5-14.9, indicating mild sleep apnea, which may be attributed to the lack of association of sleep quality with insulin sensitivity. The different methods utilized to estimate sleep quality (actigraphy vs. polysomnography) may also be a factor.

4.3. Autonomic nervous function and sleep

Autonomic imbalance has been shown to be associated with reduced insulin sensitivity and secretion in patients with newly onset type 1 or type 2 diabetes [44]. Also, a hyperinsulinemic-euglycemic clamp study demonstrated that an autonomic blockade improves insulin sensitivity in obese insulin-resistant individuals [45], while in a basic study, pancreatic islets were shown to be innervated by both the parasympathetic and sympathetic nervous systems, which may be involved in regulation of insulin secretion [13]. Thus, autonomic nervous function may be associated with both insulin sensitivity and secretion. At the time of writing, only a single cross-sectional study has been conducted, which showed that reduced HRV is associated with lower insulin

Table 3
Multivariate linear regression analysis of factors associated with insulinogenic index and BIGTT-S.

Variables	All subjects		diabetes type excluded	
	Insulinogenic Index	BIGTT-S	Insulinogenic Index	BIGTT-S
Model 1 (n = 344, 288)				
PSQ (no = 0, yes = 1)	-0.141**	0.077	-0.106 [†]	0.063
Model 2 (n = 332, 276)				
Sleep apnea (no = 0, yes = 1)	-0.013	-0.020	0.001	-0.028
Model 3 (n = 319, 269)				
Low SDNN (no = 0, yes = 1)	0.028	-0.099*	0.023	-0.069
Model 4 (n = 324, 268)				
PSQ (no = 0, yes = 1)	-0.136**	0.075	-0.101 [†]	0.058
Sleep apnea (no = 0, yes = 1)	-0.009	-0.022	0.005	-0.029
Model 5 (n = 292, 236)				
PSQ (no = 0, yes = 1)	-0.160**	0.074	-0.121 [†]	0.045
Low SDNN (no = 0, yes = 1)	0.036	-0.103*	0.028	-0.071
Model 6 (n = 292, 236)				
Sleep apnea (no = 0, yes = 1)	-0.007	-0.047	0.002	-0.048
Low SDNN (no = 0, yes = 1)	0.022	-0.106*	0.018	-0.072
Model 7 (n = 292, 236)				
PSQ (no = 0, yes = 1)	-0.156**	0.075	-0.118 [†]	0.041
Sleep apnea (no = 0, yes = 1)	-0.001	-0.051	0.006	-0.049
Low SDNN (no = 0, yes = 1)	0.032	-0.111*	0.025	-0.075

Standardized beta coefficients are shown. For multivariate linear regression analyses, the covariates included age, gender, body mass index, current smoking, alcohol, hypertension, dyslipidemia, and HbA1c. Model 1 includes presence of PSQ, Model 2 sleep apnea, Model 3 low SDNN, Model 4 PSQ and sleep apnea, Model 5 PSQ and low SDNN, Model 6 sleep apnea and low SDNN, and Model 7 PSQ sleep apnea and low HRV, in addition to other covariates already noted. PSQ, poor sleep quality; SDNN, standard deviation of NN(RR) interval. [†]p < 0.1, *p < 0.05, **p < 0.01.

sensitivity and sensitivity, estimated by 75gOGTT in individuals without diabetes, with HRV measured during 5-min pulse recording [46]. Findings in the present study obtained with 24-h HRV measurements, which is shown to be more sensitive and reproducible than a shorter term test [22], and revealed low SDNN was significantly associated with reduced insulin sensitivity in our subjects.

Although PSQ was not significantly associated with SDNN in the present cohort, the significant association of PSQ with low insulin secretion indices was compromised in 2-way ANOVA analyses categorized by low and high SDNN, suggesting a potential interaction between sleep quality and autonomic function in regard to regulation of insulin secretion. It is noteworthy that poor sleep quality was significantly associated with reduced insulin secretion, after full adjustment for low SDNN and other clinical confounders. These discrepant results might be attributed to the fact that both PSQ and SDNN are associated with several common clinical factors, such as ageing and BMI. Subclinical autonomic dysfunction has been reported to be generally present even in patients with a prediabetes status, in which adiposity appears to be an essential factor [19–21]. Similarly, the significant association of low SDNN with reduced insulin sensitivity was lost when sleep quality was taken into account in 2-way ANOVA results, whereas the association remained significant after full adjustment with clinical confounders and PSQ. Notably, the association of low SDNN with reduced insulin sensitivity was rather enhanced following adjustment with the presence of sleep apnea. Indeed, in 2-way ANOVA results, subjects with both low SDNN and sleep apnea exhibited the lowest BIGTT-S and Matsuda index values as compared to the other groups.

4.4. Limitations

This study has several limitations, including the cross-sectional design, which negates the ability to demonstrate causal relationships, a major shortcoming of this trial. Thus far, only a single report [47] has shown that resting heart rate in individuals without diabetes is associated with future unfavorable changes in insulin levels and insulin sensitivity. Although those results were inconclusive, the authors speculated that the associations may be mediated by autonomic function. No studies that examined the impact of PSQ on future changes in insulin secretion and sensitivity shown in glucose tolerance test findings have been presented.

There are several technical issues which make us interpret the data with caution. 1) In this study, because of the cohort design, a control healthy group is absent, which may weaken our findings. 2) Because of the technical issues of actigraphy, long-term measurements with frequent changes in lifestyle of the subjects make it realistically impossible to check reproducibility of the method. 3) We did not exclude subjects with diabetes type who were revealed for the first time by 75gOGTT to avoid loss of statistical power. Furthermore, we did not analyze data for subgroups based on different glycemic abnormalities (NGT, IGT, DM type), so as to avoid reduced statistical power, and type I or II errors.

Also, the results do not reveal the potential mechanisms underlying the associations of sleep, autonomic function, and insulin secretion and sensitivity. Potential candidate factors include leptin, since it is known to be involved in both sleep [48] and autonomic nervous function [5]. In clinical studies, plasma leptin concentration has been shown to be associated with insulin secretion in both healthy [49] and obese females [50], and patients with type 2 diabetes [51]. Also, in preliminary analyses of the HSCAA cohort, plasma leptin was found to be a strong predictor of both insulin secretion and sensitivity in prediabetic patients, with the association independent of clinical factors including BMI (unpublished observations). Brain-derived neurotrophic factor (BDNF) may be

another mechanistic mediator candidate [52]. We previously reported that BDNF is associated with blood pressure fluctuation during sleep and autonomic nervous function in subjects in the HSCAA cohort [39]. We consider that the present results add important information for a better understanding of the pathophysiological significance of sleep and autonomic dysfunction in regard to glycemic abnormalities.

5. Conclusions

In patients without apparent diabetes, poor sleep quality was found to be significantly associated with reduced insulin secretion, while decreased autonomic function had a significant association with sensitivity. Those associations were shown to be independent of each other as well as other clinical parameters including sleep apnea. The development of a simple method for evaluating sleep quality and autonomic nervous function, and the resulting classification of sleep disorders, could potentially identify patients at high risk of developing diabetes.

Funding sources

This study was funded by JSPS KAKENHI grants (JP18K17399 to M.K., 19K19421 to M. Kadoya, 19K19446 to K. Kosaka, JP18K08531 to H. Koyama), Hyogo College of Medicine grant (Hyogo Innovative Challenge to H. Koyama).

Ethics

This study was approved by an appropriate institutional ethical committee (approval No. 2351) and informed written consent was obtained from each participant.

Declaration of competing interest

None of the authors have conflicts of interest to declare.

CRediT authorship contribution statement

Miki Kakutani-Hatayama: Conceptualization, Methodology, Software, Data curation, Writing - original draft. **Manabu Kadoya:** Data curation, Validation. **Akiko Morimoto:** Data curation. **Akio Miyoshi:** Data curation. **Kae Kosaka-Hamamoto:** Data curation. **Yoshiki Kusunoki:** Supervision. **Takuhito Shoji:** Supervision. **Hidenori Koyama:** Conceptualization, Methodology, Software, Writing - original draft, Writing - review & editing.

Acknowledgements

The authors are grateful for the excellent technical assistance of Sachie Koyama, Tomoe Ushitani, and Ai Matsumoto. We also wish to thank our other staff members, Masafumi Kurajoh, Akinori Kanzaki, Mariko Naka, Yonekazu Kidawara, Kosuke Nakano and Chisako Yagi, as well as the participants of the Hyogo Sleep Cardio-Atherosclerosis study for their valuable contributions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.metop.2020.100033>.

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