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The fluctuation in sympathetic nerve activity around wake-up time was positively associated with not only morning but also daily glycemic variability in subjects with type 2 diabetes

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ABSTRACT

Aims: It is known that autonomic nerve activity (ANA) affects glucose metabolism by regulating the secretion of insulin and glucagon. Sympathetic nerve stimulation results in increased blood glucose levels. ANA also showed a circadian variation, and sympathetic nerve activity was minimal at night and began to rise at arousal. Therefore, a drastic alteration in ANA around wake-up would be associated with glycemic variability (GV) known risk factor for cardiovascular disease. We investigated the relation between ANA around wake-up and either morning or daily GV.

Methods: We simultaneously performed Holter ECG and continuous glucose monitoring system in 41 patients with type 2 diabetes (T2D). ANA was assessed by heart rate variability (HRV) analysis. Delta (Δ) wake-up was defined as the difference between the maximum and minimum value during 1 h before and after wake-up time, before breakfast.

Results: Δ of low frequency/high frequency (LF/HF) around wake-up time (Δ LF/HF $_{\text{wake-up}}$) was positively associated with Δ glucose $_{\text{wake-up}}$, standard deviation (SD) glucose $_{\text{wake-up}}$, the mean amplitude of glucose excursions ($MAGE_{24h}$), and SD $glucose_{24h}$ after adjustment for age, sex, BMI, the duration of diabetes, and the prevalence of diabetic polyneuropathy ($\beta = 0.47$, $p = 0.011$, $\beta = 0.48$, $p = 0.009$, $\beta = 0.54$, $p = 0.002$ and $\beta = 0.41$, $p = 0.0025$, respectively). No association was found between Δ $LF/HF_{wake\text{-}up}$ and either mean blood glucose for 24 h, or HbA1c as parameters of chronic hyperglycemia.

Conclusions: In T2D, the fluctuation in fasting sympathetic nerve activity around wake-up was positively associated with not only morning but also daily GV.

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1. Introduction

In diabetic patients, spontaneous increase in plasma glucose from end of the nocturnal period is known as dawn phenomenon [1,2]. Major insulin counter-regulatory hormones, such as cortisol and catecholamines could account for this [3,4]. A bidirectionality exists between autonomic nerve activity (ANA) and glucose metabolism. It is also known that ANA affects glucose metabolism by regulating insulin or glucagon secretion. Sympathetic nerve stimulation in the pancreas causes a decrease in insulin secretion and an increase in glucagon secretion, resulting in elevation of blood glucose levels. On the other hand, parasympathetic nerve stimulation promotes insulin secretion and a decrease in blood glucose levels [5–8]. The activity of the sympathetic and parasympathetic nerve regulates heart rate variability (HRV) assessed by beat-to-beat modulation of heart rate, and reduced HRV has been associated with an increased risk of diabetes [9]. We have recently reported that reduced parasympathetic nerve activity and increased sympathetic nerve activity assessed by HRV analysis were associated with the decline of insulin sensitivity in a general population [10]. ANA showed circadian variation. Sympathetic nerve activity was minimal at night and began to rise at arousal [11]. Therefore, a drastic alteration in ANA around wake-up would be associated with glucose metabolism and glycemic variability (GV). Recent studies have reported that not only chronic hyperglycemia but daily GV is an independent risk factor for cardiovascular events [12,13]. To our knowledge, no study focusing on the association of the fluctuation in ANA around wake-up on GV has been reported.

During the daytime, ANA is affected by physical activity and food intake, which makes it difficult to maintain the same condition among examinations, resulting in less reproducibility. In contrast, fasting ANA around wake-up is less affected by physical activity. Sympathetic and parasympathetic nerve activity drastically changes around wake-up, suggesting that ANA around wake-up would be a representative parameter of overall ANA.

In view of this, to investigate the relation between ANA around wake-up and either morning or daily GV in type 2 diabetes (T2D), we simultaneously performed continuous glucose monitoring system (CGM) and Holter ECG recording to assess ANA over a period of 24 h.

2. Subjects, materials and methods

2.1. Study participants

Fifty-two patients who were hospitalized with T2D were recruited for the study. Subjects who had defective data (n = 7), marked autonomic failure (n = 1), spinal tumor (n = 1), hypoglycemia occurring during 1 h before and after wake-up time (n = 1), or undetermined wake-up time (n = 1) were excluded. Finally, a total of forty-one subjects (20 men and 21 women) were included in the study. Diabetic polyneuropathy was diagnosed based on the simple diagnostic criteria issued by the Diabetic Diagnostic Neuropathy Study Group [14,15]. Diabetic retinopathy was defined as simple, pre-proliferative, or proliferative retinopathy, as diagnosed by ophthalmologists. Diabetic nephropathy was defined as a urine albumin creatinine ratio(ACR) of ≧30 mg/gCr. The study protocol was approved by the Institutional Review Board of Ehime University Graduate School of Medicine. Written informed consent was obtained from all participants before they were enrolled in the study.

2.2. Assessment of daily glucose profile using the continuous glucose monitoring system

Patients were given a standardized diet (25–30 kcal/ideal body weight, 50–60% carbohydrate of total energy). CGM system

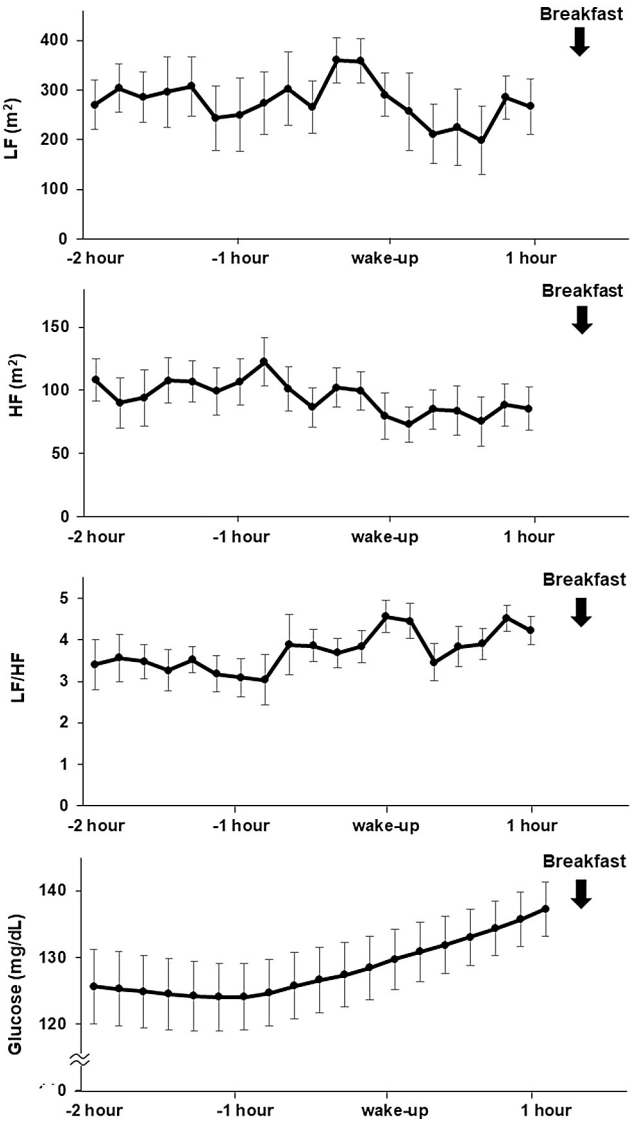

Fig. 1 – The changes in LF, HF, LF/HF and glucose from 2 h before wake-up to 1 h after wake-up in subjects with T2D. All values are expressed as mean in every 10 min, the error bars are standard error of the mean (SE).

using the iPro2 (Medtronic MiniMed, Northridge, CA, USA) with an Enlite sensor (Medtronic MiniMed, Northridge, CA, USA) was performed and data were collected every 5 min intervals in each patient at days 10 to 14, that is, at the time when the glycemic condition became stable after their admission. Since the Holter ECG was performed for 24 h simultaneous with the CGM, data was analyzed for 24 h in present study. Based on the CGM data, glucose tended to increase starting 1 h before wake-up (Fig. 1). Therefore, in each patient, the standard deviation (SD) or delta (Δ) for glucose levels during the 1 h period before and after wake-up time before breakfast were calculated as parameters as morning GV (SD glucose $_{wake\text{-}up}$ and Δ glucose $_{wake\text{-}up}$, respectively). Δ glucose $_{wake\text{-}up}$ was defined by the difference between the maximum and the minimum values during 1 h before and after wake-up time. The mean amplitude of glucose excursions (MAGE $_{24h}$) or the SD of glucose levels for 24 h (SD glucose $_{24h}$) were also calculated as parameters of daily GV. MAGE $_{24h}$ was calculated by taking the arithmetic mean of the absolute values of the differences between the adjacent peak and nadirs for all differences greater than one SD [16]. As the parameter of chronic hyperglycemia, mean blood glucose during 24 h (MBG $_{24h}$) and HbA1c were evaluated. The analyses of SD, MAGE, and MBG were performed with the Easy GV software [17].

2.3. Assessment of autonomic nerve activity

Patients underwent Holter ECG for 24 h simultaneously with CGM. Active physical exercise, smoking, and caffeine were avoided during the test period. Wake-up time was determined by daily life reports or baseline changes in the ECG in each patient. ANA was assessed by HRV analysis, which was used as a non-invasive implement to assess cardiovascular autonomic nerve activity [18]. In each patient, Spectrum analyses of HRV were performed using the Mem Calc power spectral density method, with data being collected every 5 min during 24 h period (Mem Calc system; Nihon-Kohden, Japan) [19]. Low frequency (LF) which reflected parasympathetic and sympathetic nerve activity was defined as a level of 0.04–0.15 Hz. High frequency (HF) which reflected parasympathetic nerve activity, was defined as a level of 0.15–040 Hz. The ratio of LF to HF (LF/HF) reflected sympathetic nerve dominant activity [18]. To analyze the fluctuations in ANA around wake-up time, we used the data of individuals collected every 5 min and calculated two indices, Δ during 1 h before and after wake-up time, as the fluctuation in ANA. Δ HRV $_{wake\text{-}up}$ parameters were defined by the maximum minus the minimum HRV values during 1 h before and after wake-up time before breakfast.

2.4. Statistical analysis

The characteristics of the study subjects were represented as means ± SD or number (%). In each patient, the mean, SD, and delta values for morning or daily blood glucose levels were calculated from the CGM data that had been collected every 5 min intervals during the 2 h around wake-up or a 24 h period. ANA fluctuations were calculated similarly. We then analyzed the relation between ANA fluctuation and GV in all subjects in this study. Data that were not normally distributed were natural log-transformed for statistical analyses. Multiple regression analyses were performed to examine the relation between glucose parameters and each of the HRV parameters. SD glucose $_{wake\text{-}up}$, Δ glucose $_{wake\text{-}up}$, MAGE $_{24h}$, SD glucose $_{24h}$, MBG $_{24h}$, or HbA1c was used as dependent variables, and HRV parameters, age, sex, BMI, the duration of diabetes, and the prevalence of diabetic polyneuropathy were used as independent variables. Statistical significance was defined as $p < 0.05$. All analyses were performed using the JMP 11.0 software program (SAS Institute, Cary, NC, USA).

3. Results

3.1. Characteristics of the study subjects

The characteristics of the study subjects are shown in Table 1. The mean of age was 64 years old, with 49% being male. The mean of BMI, the duration of diabetes, and HbA1c were 26, 14.6 years, and 9.4%, respectively. About 80% of the patients were being treated with insulin. The prevalence of diabetic polyneuropathy, retinopathy, and nephropathy were 61.0%, 51.2%, and 39.0%, respectively. All patients below ACR 30 mg/gCr had eGFR ≧30 ml/min/1.73 m^2.

3.2. The fluctuation in LF/HF around wake-up time was positively associated with glycemic variability in the morning in subjects with T2D

The changes in HRV parameters or glucose from 2 h before wake-up to 1 h after wake-up time are shown in Fig. 1. The HF component showed a tendency to decrease, while the LF/HF ratio tended to increase from 1 h before wake-up (p

Table 1 – Characteristics of the study subjects.

	All (n = 41)
Age (years)	64 ± 12
Men / women	20 / 21
BMI (kg/m^2)	26.0 ± 5.4
The duration of diabetes (years)	14.6 ± 10.8
Systolic blood pressure (mmHg)	121 ± 15
Diastolic blood pressure (mmHg)	68 ± 10
HbA1c (%)	9.4 ± 2.0
(mmol/mol)	80 ± 22
eGFR (ml/min/1.73 m^2)	64.0 ± 22.8
Total cholesterol (mmol/L)	4.3 ± 0.8
HDL cholesterol (mmol/L)	1.1 ± 0.4
LDL cholesterol (mmol/L)	2.5 ± 0.7
Triglycerides (mmol/L)	1.6 ± 0.8
Treatment with insulin	34 (82.9)
Treatment with GLP-1 receptor agonist	1 (2.4)
Treatment with oral hypoglycemic agent	19 (46.3)
Treatment with β–blocker	9 (22.0)
Diabetic polyneuropathy	25 (61.0)
Diabetic retinopathy	21 (51.2)
Diabetic nephropathy	16 (39.0)

Data are presented as the mean ± SD, or number (%). BMI, body mass index;
eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptid-1.

Table 2 – The fluctuation in LF/HF around wake-up time was positively associated with morning glycemic variability (GV) in subjects with T2D.

Independent variable	Dependent variable											
	Model 1				Model 2				Model 3			
	SD glucose $_{wake-up}$ (mmol/L)		Δ glucose $_{wake-up}$ (mmol/L)		SD glucose $_{wake-up}$ (mmol/L)		Δ glucose $_{wake-up}$ (mmol/L)		SD glucose $_{wake-up}$ (mmol/L)		Δ glucose $_{wake-up}$ (mmol/L)	
	Stand. β	P	Stand. β	P	Stand. β	P	Stand. β	P	Stand. β	P	Stand. β	P
Δ LF $_{wake-up}$ (ms^2)	−0.032	0.86	−0.12	0.50	−0.059	0.75	−0.15	0.42	−0.053	0.78	−0.14	0.47
Δ HF $_{wake-up}$ (ms^2)	0.001	0.95	0.027	0.87	−0.032	0.86	−0.0078	0.97	−0.026	0.89	0.010	0.95
Δ LF/HF $_{wake-up}$	0.46	0.009	0.46	0.01	0.45	0.013	0.45	0.013	0.47	0.011	0.48	0.009

Multiple regression analyses were performed involving either SD glucose $_{wake-up}$ or Δ glucose $_{wake-up}$ as a dependent variable, and each of HRV parameters as an independent variable. Model 1: adjusted for age, sex, and BMI; Model 2: adjusted for age, sex, BMI, and the duration of diabetes; Model 3: adjusted for age, sex, BMI, the duration of diabetes, and the prevalence of diabetic polyneuropathy. SD glucose $_{wake-up}$, SD of glucose during1 hour before and after wake-up time; Δ glucose $_{wake-up}$ or Δ HRV $_{wake-up}$ parameters, the difference between the maximum and minimum values of glucose or HRV during 1 h before and after wake-up time; LF, low frequency; HF, high frequency; LF/HF, ratio of LF to HF.

Table 3 – The fluctuation in LF/HF around wake-up time was positively associated with daily glycemic variability (GV) in subjects with T2D.

Independent variable	Dependent variable											
	Model 1				Model 2				Model 3			
	MAGE $_{24h}$ (mmol/L)		SD glucose $_{24h}$ (mmol/L)		MAGE $_{24h}$ (mmol/L)		SD glucose $_{24h}$ (mmol/L)		MAGE $_{24h}$ (mmol/L)		SD glucose $_{24h}$ (mmol/L)	
	Stand. β	P	Stand. β	P	Stand. β	P	Stand. β	P	Stand. β	P	Stand β	P
Δ LF $_{wake-up}$ (ms^2)	0.11	0.54	0.19	0.29	0.15	0.42	0.26	0.14	0.12	0.52	024	0.19
Δ HF $_{wake-up}$ (ms^2)	−0.088	0.60	0.038	0.82	−0.042	0.81	0.15	0.39	−0.078	0.67	0.12	0.51
Δ LF/HF $_{wake-up}$	0.52	0.003	0.36	0.050	0.57	0.001	0.43	0.016	0.56	0.002	0.41	0.025

Multiple regression analyses were performed involving either MAGE $_{24h}$ or SD Glucose $_{24h}$ as a dependent variable, and each of HRV parameters as an independent variable. Model 1: adjusted for age, sex, and BMI; Model 2: adjusted for age, sex, BMI, and the duration of diabetes; Model 3: adjusted for age, sex, BMI, the duration of diabetes, and the prevalence of diabetic polyneuropathy. MAGE $_{24h}$, mean amplitude of glucose excursions during 24 h; SD glucose $_{24h,}$ SD of glucose values during 24 h; Δ HRV $_{wake-up}$ parameters, the difference between the maximum and minimum values of HRV during 1 h before and after wake-up time; LF, low frequency; HF, high frequency; LF/HF, ratio of LF to HF.

for trend = 0.027 and 0.013, respectively). Glucose was also increased during this period (p for trend = 0.0004). To evaluate the influence of the fluctuation in ANA around wake-up on GV, we first analyzed the relation between ANA and GV in the same time period, namely during 1 h before and after wake-up time (Table 2). In multivariable regression analyses, Δ LF/HF $_{wake\text{-}up}$ was positively associated with SD glucose $_{wake\text{-}up}$ and Δ glucose $_{wake\text{-}up}$ after adjustment for age, sex, BMI, the duration of diabetes, and the prevalence of diabetic polyneuropathy. When further after adjustment for β–blocker treatment, Δ LF/HF $_{wake\text{-}up}$ was found to be positively correlated with SD glucose $_{wake\text{-}up}$ ($\beta = 0.46$, p = 0.015) and Δ glucose $_{wake\text{-}up}$ ($\beta = 0.47$, p = 0.012). Neither LF $_{wake\text{-}up}$ nor HF $_{wake\text{-}up}$ was associated with GV around wake-up.

3.3. *The fluctuation in LF/HF around wake-up was positively associated with daily glycemic variability in subjects with T2D*

We then analyzed the relation between ANA during 1 h before and after wake-up time and daily GV (Table 3). Δ LF/HF $_{wake\text{-}up}$ was positively associated with parameter of daily GV such as MAGE $_{24h}$ and SD glucose $_{24h}$. When further adjusted for β–blocker treatment, Δ LF/HF $_{wake\text{-}up}$ was positively associated with MAGE $_{24h}$ ($\beta = 0.52$, p = 0.003), and SD glucose $_{24h}$ ($\beta = 0.38$, p = 0.03). Neither LF $_{wake\text{-}up}$ nor HF $_{wake\text{-}up}$ was associated with indices of daily GV.

3.4. *The fluctuation in LF/HF around wake-up was not associated with chronic hyperglycemia in subjects with T2D*

We further analyzed the relation between ANA around wake-up and chronic hyperglycemia (Table 4). Δ LF/HF $_{wake\text{-}up}$ was not associated with MBG $_{24h}$ or HbA1c in multiple regression analyses. Neither LF $_{wake\text{-}up}$ nor HF $_{wake\text{-}up}$ was associated with indices for chronic hyperglycemia.

4. Discussion

In the present study, we demonstrated that the fluctuation in fasting sympathetic nerve activity around wake-up assessed by HRV was positively associated with both morning and daily GV in patients with T2D.

Several studies have shown the association between HRV parameters and GV in patients with T2D. The nocturnal hypoglycemia was positively associated with fasting LF/HF, and increased nocturnal glycemic variability was inversely correlated with fasting HF evaluated by HRV analysis for 5 min [20]. A correlation between nocturnal LF/HF and MAGE $_{24h}$ was found in patients with T2D treated with diet and/or metformin [21]. However, no study dealing with ANA around wake-up time has been reported previously.

A previous study reported that the dawn phenomenon could affect overall glycemic control, resulting in an elevation in HbA1c by 0.4% in T2D [22]. We found that Δ LF/HF $_{wake\text{-}up}$ was positively associated with fasting serum cortisol and plasma adrenaline, which are known factors associated with the dawn phenomenon (data not shown). In addition, the pancreas is doubly innervated by sympathetic and parasym-

Table 4 – The fluctuation in LF/HF around wake-up time was not associated with chronic hyperglycemia in subjects with T2D.

Independent variable	Dependent variable											
	Model 1				Model 2				Model 3			
	MBG $_{24h}$ (mmol/L)		HbA1c (mmol/mol)		MBG $_{24h}$ (mmol/L)		HbA1c (mmol/mol)		MBG $_{24h}$ (mmol/L)		HbA1c mmol/mol)	
	Stand. β	P	Stand. β	P	Stand. β	P	Stand. β	P	Stand. β	P	Stand. β	P
Δ LF $_{wake\text{-}up}$ (ms^2)	0.012	0.95	0.077	0.67	0.0051	0.98	0.077	0.68	0.012	0.95	0.07	0.72
Δ HF $_{wake\text{-}up}$ (ms^2)	0.27	0.10	0.18	0.28	0.29	0.10	0.20	0.28	0.31	0.091	0.19	0.30
Δ LF/HF $_{wake\text{-}up}$	0.20	0.28	0.074	0.69	0.20	0.30	0.073	0.70	0.21	0.28	0.067	0.74

Multiple regression analyses were performed involving either MBG $_{24h}$, or HbA1c as a dependent variable, and each of HRV parameters as an independent variable. Model 1: adjusted for age, sex, and BMI; Model 2: adjusted for age, sex, BMI, and the duration of diabetes; Model 3: adjusted for age, sex, BMI, the duration of diabetes, and the prevalence of diabetic polyneuropathy. MBG $_{24h}$, mean blood glucose during 24 h; Δ HRV $_{wake\text{-}up}$ parameters, the difference between the maximum and minimum values of HRV during 1 h before and after wake-up time; LF, low frequency; HF, high frequency; LF/HF, ratio of LF to HF.

pathetic nerves. Sympathetic nerve stimulation suppresses insulin secretion and increases glucagon secretion which leads to increase in blood glucose. Conversely, hyperglycemia suppresses sympathetic nerve activation, and insulin secretion is promoted by parasympathetic action, which could lead to a decrease in blood glucose [8,23]. These suggest that there would be a close relation among the fluctuation in sympathetic nerve activity around wake-up, insulin counter-regulatory hormones, and GV around wake-up.

We also found that Δ LF/HF $_{\text{wake-up}}$ was positively associated with daily GV, as presented by MAGE $_{24h}$ and SD glucose $_{24h}$. Since MAGE is defined as a variation of 1 SD or more, it is strongly affected by glycemic excursion during meals [17,24]. Although the mechanism is unclear, the fluctuation in sympathetic activity around wake-up may represent its fluctuation for 24 h, resulting in its association with daily GV, including postprandial hyperglycemia. Indeed, in the present study, we found that, Δ LF/HF $_{\text{wake-up}}$ was positively associated with SD LF/HF during 24 h after adjustment for age, sex, BMI, the duration of diabetes, and the prevalence of diabetic polyneuropathy ($\beta = 0.37$, $p = 0.042$).

In the present study, Δ LF/HF $_{\text{wake-up}}$ was found to be related to GV including MAGE $_{24h}$ and SD glucose $_{24h}$, but not related to parameters of chronic hyperglycemia such as MBG $_{24h}$ and HbA1c. It has been reported that not only chronic hyperglycemia but MAGE was associated with cardiovascular events [25]. GV was associated with the increase of circulating inflammatory cytokines more than continuous hyperglycemia [26]. GV was also associated with oxidative stress and vascular endothelial dysfunction, and it would induce cardiovascular events in T2D [27–29]. Therefore, the relation between the fluctuation in sympathetic nerve activity around wake-up and the increase in GV could explain a part of the mechanism underlying the cardiovascular events.

As the progression of diabetes, diabetic neuropathy including autonomic dysfunction develops, and fluctuations in ANA assessed by HRV would decrease [30,31]. When the condition progresses further, the response of autonomic nerves to hypoglycemia declines and severe hypoglycemia including its unawareness occurs and it may lead to increase GV.

Because the subjects in this study had a high prevalence of microvascular complications, it is necessary to consider the influence of diabetic neuropathy on HRV analyses or GV. Indeed, the duration of diabetes was inversely associated with Δ HRV $_{\text{wake-up}}$ (Supplementary Fig. 1). Δ HRV $_{\text{wake-up}}$ was reduced in the group with diabetic polyneuropathy, although LF/HF or LF did not reach statistical significance (Supplementary Fig. 2). However, even after adjustment for the duration of diabetes and the prevalence of diabetic polyneuropathy, Δ LF/HF $_{\text{wake-up}}$ was positively associated with morning or daily GV parameters (Tables 2 and 3, model 3). These results suggest that the association between the fluctuation in sympathetic nerve activity around wake-up time and GV would be observed regardless of the duration of diabetes.

It is known that hypoglycemia affects ANA [32]. In our study, we excluded one patient who showed hypoglycemia during 1 h before and after wake-up. We included two patients who showed asymptomatic level 1 hypoglycemia (3.0 to 3.9 mmol/L) during sleep. In these two patients, the LF/HF fluctuation during 1 h before and after wake-up was minimal and no reactive hyperglycemia was observed. When 39 subjects, excluding these two patients, were analyzed, the results remain essentially the same.

It has also been reported that sleep apnea or variability in blood pressure is associated with an increase in sympathetic nerve activity [33–35]. In the present study, there were no subjects with a current or past history of sleep apnea and the subjects were not evaluated sleep apnea by questionnaires or polysomnography. After further adjustment for systolic blood pressure measured on the same day as the Holter ECG, the results were still similar, although it should be noted that ABPM was not performed on the subjects.

There are several limitations in the present study. First, because we excluded the subjects with marked autonomic failure from the analyses, it is unknown whether Δ LF/HF $_{\text{wake-up}}$ is positively associated with GV even in the end stage of autonomic dysfunction. Second, our study was a cross-sectional design that did not evaluate whether autonomic nerve imbalance was a cause or consequence of GV. Third, this study was performed in a single center, and small number of subjects were analyzed. Fourth, since non-diabetic control subjects were not recruited, it is still unclear whether the findings obtained in this study are specific to patients with T2D. Further studies involving larger numbers of subjects would be desirable in terms of confirming the conclusions reached in this study.

In conclusion, the fluctuation in fasting sympathetic nerve activity during 1 h before and after wake-up was positively associated with both GV around wake-up and daily GV, but not associated with chronic hyperglycemia in patients with T2D.

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Contributors

YM, YT, RK, and HO designed the study. YM, MT, and TH collected data, and reviewed the manuscript. YM and YT analyzed data. YM, YT, and HO wrote the manuscript. All authors provided final approval of the version.

Conflicts of interest

None.

Disclosure

All authors declare that they have no competing interests.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2019.04.029.

REFERENCES

[1] Schmidt MI, Hadji-Georgopoulos A, Rendell M, Margolis S, Kowarski A. The dawn phenomenon, an early morning glucose rise: implications for diabetic intraday blood glucose variation. Diabetes Care 1981;4:579–85.

[2] Monnier L, Colette C, Sardinoux M, Baptista G, Regnier-Zerbib A, Owens D. Frequency and severity of the dawn phenomenon in type 2 diabetes: relationship to age. Diabetes Care 2012;35:2597–9.

[3] Atiea JA, Aslan SM, Owens DR, Luzio S. Early morning hyperglycaemia "dawn phenomenon" in non-insulin dependent diabetes mellitus (NIDDM): effects of cortisol suppression by metyrapone. Diabetes Res. 1990;14:181–5.

[4] Mokhlesi B, Grimaldi D, Beccuti G, Van Cauter E. Effect of one week of CPAP treatment of obstructive sleep apnoea on 24-hour profiles of glucose, insulin and counter-regulatory hormones in type 2 diabetes. Diabetes Obes Metab 2017;19:452–6.

[5] Bloom SR, Edwards AV. Certain pharmacological characteristics of the release of pancreatic glucagon in response to stimulation of the splanchnic nerves. J Physiol 1978;280:25–35.

[6] Nishi S, Seino Y, Ishida H, Seno M, Taminato T, Sakurai H, et al. Vagal regulation of insulin, glucagon, and somatostatin secretion in vitro in the rat. J Clin Invest 1987;79:1191–6.

[7] Thorens B. Neural regulation of pancreatic islet cell mass and function. DiabetesObes Metab 2014;16(Suppl 1):87–95.

[8] Rizza RA, Cryer PE, Haymond MW, Gerich JE. Adrenergic mechanisms of catecholamine action on glucose homeostasis in man. Metabolism 1980;29(11 Suppl 1):1155–63.

[9] Carnethon MR, Golden SH, Folsom AR, Haskell W, Liao D. Prospective investigation of autonomic nervous system function and the development of type 2 diabetes: the atherosclerosis risk in communities study, 1987–1998. Circulation 2003;107:2190–5.

[10] Saito I, Hitsumoto S, Maruyama K, Nishida W, Eguchi E, Kato T, et al. Heart rate variability, insulin resistance, and insulin sensitivity in japanese adults: the toon health study. J Epidemiol 2015;25:583–91.

[11] Furlan R, Guzzetti S, Crivellaro W, Dassi S, Tinelli M, Baselli G, et al. Continuous 24-hour assessment of the neural regulation of systemic arterial pressure and RR variabilities in ambulant subjects. Circulation 1990;81:537–47.

[12] Kuroda M, Shinke T, Sakaguchi K, Otake H, Takaya T, Hirota Y, et al. Association between daily glucose fluctuation and coronary plaque properties in patients receiving adequate lipid-lowering therapy assessed by continuous glucose monitoring and optical coherence tomography. Cardiovasc Diabetol 2015;14:78.

[13] Su G, Mi SH, Tao H, Li Z, Yang HX, Zheng H, et al. Impact of admission glycemic variability, glucose, and glycosylated hemoglobin on major adverse cardiac events after acute myocardial infarction. Diabetes Care 2013;36:1026–32.

[14] Yasuda H, Sanada M, Kitada K, Terashima T, Kim H, Sakaue Y, et al. Rationale and usefulness of newly devised abbreviated diagnostic criteria and staging for diabetic polyneuropathy. Diabetes Res Clin Pract 2007;77(Suppl 1):S178–83.

[15] Diagnostic Neuropathy Study Group. Peripheral Nerve. 1998; 9: pp. 137–140 (in japanese).

[16] Service FJ, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, Taylor WF. Mean amplitude of glycemic excursions, a measure of diabetic instability. Diabetes 1970;19:644–55.

[17] University of Oxford, Nuffield Department of Primary Care Health Sciences. EasyGV, http://www.phc.ox.ac.uk/research/diabetes/software/easygv. [accessed 22 April 2018]

[18] Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Eur Heart J. 1996; 17: pp. 354–381.

[19] Otsuka K, Murakami S, Kubo Y, Yamanaka T, Mitsutake G, Ohkawa S, et al. Chronomics for chronoastrobiology with immediate spin-offs for life quality and longevity. Biomed Pharmacother 2003;57(Suppl 1):1s–18s.

[20] Klimontov VV, Myakina NE, Tyan NV. Heart rate variability is associated with interstitial glucose fluctuations in type 2 diabetic women treated with insulin. Springerplus 2016;5:337.

[21] Di Flaviani A, Picconi F, Di Stefano P, Giordani I, Malandrucco I, Maggio P, et al. Impact of glycemic and blood pressure variability on surrogate measures of cardiovascular outcomes in type 2 diabetic patients. Diabetes Care 2011;34:1605–9.

[22] Monnier L, Colette C, Dejager S, Owens D. Magnitude of the dawn phenomenon and its impact on the overall glucose exposure in type 2 diabetes: is this of concern? Diabetes Care 2013;36:4057–62.

[23] Niijima A. Neural control of blood glucose level. Jpn J Physiol 1986;36(5):827–41.

[24] Kovatchev B, Cobelli C. Glucose variability: timing, risk analysis, and relationship to hypoglycemia in diabetes. Diabetes Care 2016;39:502–10.

[25] Su G, Mi S, Tao H, Li Z, Yang H, Zheng H, et al. Association of glycemic variability and the presence and severity of coronary artery disease in patients with type 2 diabetes. Cardiovasc Diabetol 2011;10:19.

[26] Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. Circulation 2002;106:2067–72.

[27] Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA 2006;295:1681–7.

[28] Torimoto K, Okada Y, Mori H, Tanaka Y. Relationship between fluctuations in glucose levels measured by continuous glucose monitoring and vascular endothelial dysfunction in type 2 diabetes mellitus. Cardiovasc Diabetol 2013;12:1.

[29] Risso A, Mercuri F, Quagliaro L, Damante G, Ceriello A. Intermittent high glucose enhances apoptosis in human umbilical vein endothelial cells in culture. Am J Physiol Endocrinol Metab 2001;281:E924–30.

[30] Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care 2010;33:2285–93.

[31] Tarvainen MP, Laitinen TP, Lipponen JA, Cornforth DJ, Jelinek HF. Cardiac autonomic dysfunction in type 2 diabetes - effect of hyperglycemia and disease duration. Front Endocrinol (Lausanne) 2014;5:130.

[32] Chow E, Bernjak A, Williams S, Fawdry RA, Hibbert S, Freeman J, et al. Risk of cardiac arrhythmias during hypoglycemia in patients with type 2 diabetes and cardiovascular risk. Diabetes 2014;63(5):1738–47.

[33] Kohara K, Nishida W, Maguchi M, Hiwada K. Autonomic nervous function in non-dipper essential hypertensive subjects. Evaluation by power spectral analysis of heart rate variability. Hypertension 1995;26(5):808–14.
[34] Gong X, Huang L, Liu X, Li C, Mao X, Liu W, et al. Correlation analysis between polysomnography diagnostic indices and heart rate variability parameters among patients with obstructive sleep apnea hypopnea syndrome. PLoS ONE 2016;11(6):e0156628.
[35] Kim YS, Kim SY, Park DY, Wu HW, Hwang GS, Kim HJ. Clinical implication of heart rate variability in obstructive sleep apnea syndrome patients. J Craniofac Surg 2015;26(5):1592–5.