resistance necessary for the growth and nourishment of the fetus [5]. Also, a study by Telejko et al. [3] found reduced ghrelin levels during pregnancy irrespective of glucose tolerance status. However, other studies have, in contrast, reported lower ghrelin levels in GDM compared with healthy pregnant women. Palik et al. [9], for example, showed that serum AG levels, as measured by a different assay from ours, were significantly lower in women with GDM vs NGT during the third trimester of pregnancy.

In fact, ghrelin levels appeared to be suppressed during pregnancy in comparison to reference levels based on healthy non-pregnant women [8]. Likewise, Tham et al. [4] showed that ghrelin levels, using a similar assay, were suppressed in relation to postpartum levels. Furthermore, those authors demonstrated that AG levels recovered after pregnancy, which implies that the low AG/UAG ratio was a result of being pregnant.

Pregnancy is characterized by increased food intakes, maternal weight gain and progressive insulin resistance and the orexigenic effects of ghrelin may have contributed to the positive energy balance, whereas adipose tissue imposes negative feedback regulation on ghrelin production [9]. Ghrelin levels decrease with hyperglycaemia and hyperinsulinaemia, reflecting the inhibitory effect of insulin on ghrelin secretion [4]. This hypothesis is supported by the increased ghrelin levels present at mid-pregnancy and decreased levels during late gestation [10]. Nevertheless, whether low ghrelin levels are a risk factor or compensatory mechanism remains unknown.

The strength of our study was that both AG and UAG were measured by highly sensitive assays. However, there are also some limitations. First, comparing our results with other study outcomes is problematic because of the different assay techniques used. Second, a larger sample size might have improved the reliability of the outcome, although our data showed no suggestion of any association between ghrelin levels in early pregnancy and the development of GDM in the studied population.

In conclusion, both AG and UAG levels are low during pregnancy regardless of the level of glycaemic control and both decrease normally after an oral glucose load.

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## Disclosure of interest

Aart Jan van der Lely is a cofounder and shareholder of Alizé Pharma, Ecully, France.

The other authors declare that they have no competing interest.

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# Hypoglycaemic episodes and risk of diabetic peripheral neuropathy in patients with type 2 diabetes

## 1. Introduction

Hypoglycaemia is one of the most serious complications of diabetes therapy, as it increases the risk of injury and death. Many studies have investigated the effects of hypoglycaemia on the central nervous system and cardiovascular system, but very few reports have focused on the peripheral nervous system (PNS) [1]. Indeed, to date, no study has evaluated whether hypoglycaemia is associated with the risk of diabetic peripheral neuropathy (DPN) in patients with type 2 diabetes (T2D).

The objective of the present study was to determine whether previous hypoglycaemic events requiring hospitalization or emergency department (ED) visits are associated with an increased risk of DPN in a large cohort of adult patients with T2D.

## 2. Methods

### 2.1. Study design and cohort

This was a cross-sectional, hospital-based observational study. Eligible participants were patients with T2D aged > 20 years

between January 2013 and October 2013, including prevalent and new cases. All patients were diagnosed by endocrinologists at the outpatient unit of a public tertiary-care hospital in central Taiwan. The diagnosis of T2D was established on the basis of American Diabetes Association (ADA) criteria. Patients' data were anonymized by a computer system before the analysis, and the study was approved by the Institutional Review Board of Taichung Veterans General Hospital (CE-16112B).

### 2.2. Anthropometric and biochemical measurements

These measurements included height and weight (both recorded using a calibrated balance scale with a stadiometer). Body mass index (BMI) was calculated from weight in kilograms divided by height in metres-squared ($kg/m^2$). Resting blood pressure was measured in a sitting position on the right arm with an automated sphygmomanometer (BP203RV-II; Nippon Colin Co., Ltd., Komaki, Japan).

Blood samples were taken from the antecubital vein after an overnight fast. Levels of fasting plasma glucose (using standard enzymatic methods) and glycated haemoglobin ($HbA_{1c}$; using high-performance liquid chromatography), and lipid profiles (using standard enzymatic methods), including triglycerides, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol, were also measured.

### 2.3. Diagnosis of hypoglycaemia and comorbidities

Hypoglycaemia was identified by International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes 251.0 (hypoglycaemic coma), 251.1 (other specified hypoglycaemia) and 251.2 (hypoglycaemia, unspecified) for the main diagnoses during ED visits or hospitalization, in accordance with the ADA diagnosis of severe hypoglycaemia, defined as severe cognitive impairment caused by hypoglycaemia and requiring external assistance for recovery, but with no specific glucose threshold [2]. Our study included hypoglycaemic episodes from 1 January 2000 through to 31 December 2012. Patients with the following ICD-9-CM codes were excluded from the study: 270.3 (leucine-induced hypoglycaemia); 775.6 (neonatal hypoglycaemia); and 775.0 (hypoglycaemia in an infant born to a diabetic mother).

Also, having any of the following histories recorded during 2010–2012 in outpatient and inpatient claims were considered to be comorbidities: hypertension (ICD-9-CM codes 401–405); hyperlipidaemia (ICD-9-CM codes 272); cardiovascular disease (ICD-9-CM codes 390–438); ischaemic heart disease (ICD-9-CM codes 410–414); and chronic kidney disease (ICD-9-CM codes 582–583).

### 2.4. Assessment of diabetic peripheral neuropathy

DPN was determined using the Michigan Neuropathy Screening Instrument (MNSI), a validated screening tool for DPN. The MNSI includes two separate assessments: a 15-item self-administered questionnaire; and a structured examination of the feet (MNSIE), which are scored for abnormalities of appearance, presence of ulcers, vibration perception and ankle reflexes. All MNSIE examinations in our study were performed by the same trained and certified health professional to reduce interobserver variability. The threshold for DPN, established by previous validation studies performed in adults, is a score $> 2$ on the MNSIE out of a total possible score of 8. Details of the examination have been described in a previous study [3].

### 2.5. Statistical analysis

Descriptive statistics are presented as means $\pm$ standard deviation (SD), numbers and percentages, and as median values with the interquartile range (IQR). Categorical variables were analyzed using Fisher's exact or the $\chi^2$ test, and continuous variables were analyzed using Student's $t$ test or analysis of variance (ANOVA). Multiple logistic regression analysis was performed with the identified independent variables, and odds ratios (ORs) with their 95% confidence intervals (CIs) were obtained between the comparative groups. All tests were two-sided, and a $P$ value $< 0.05$ was considered to indicate statistical significance. All statistical analyses were conducted using SAS statistical software for Windows (version 9.4; SAS Institute Inc., Cary, NC, USA).

## 3. Results

A total of 2733 patients were included in the study. Table 1 summarizes their demographic and clinical characteristics according to hypoglycaemic status. Mean age of patients was $63.9 \pm 13.0$ years at the time of the survey, and the female-to-male ratio was 1.37. Mean duration of diabetes was $10.1 \pm 8.3$ years, and mean $HbA_{1c}$ level was $7.5 \pm 1.5\%$ ($58 \pm 16.4$ mmol/mol). Most of the patients (96%) were being treated with some form of pharmacological therapy, and 28.1% were taking insulin (alone or in combination with oral hypoglycaemic agents).

A total of 78 patients (2.8%) had at least one episode of severe hypoglycaemia during 2000–2012. The prevalence of such episodes varied with the type of therapy: one of 90 (1.1%) patients were treated with diet alone; 32 of 1565 (2.0%) patients were treated with oral hypoglycaemic agents alone; and 37 of 605 (6.1%) patients were treated with insulin ($P < 0.001$ for trend). Patients with hypoglycaemia were more likely to be older, to have longer durations of diabetes, to be treated with insulin, to have longer durations of insulin treatment, and to also have peripheral neuropathy, hypertension, cardiovascular disease, ischaemic heart disease, chronic kidney disease and hyperlipidaemia compared with patients without hypoglycaemia ($P < 0.001$ for all).

The results of the multivariate logistic regression analysis are shown in Fig. 1. Of the 78 patients with hypoglycaemia, 54 (69.2%) had one episode and 24 (30.8%) had two or more episodes, with a total of 121 hypoglycaemic episodes identified. The risk of DPN was significantly higher in patients with hypoglycaemia than in those without hypoglycaemia (OR: 4.54, 95% CI: 2.88–7.25; $P < 0.001$). In addition, after multiple adjustments for demographic factors, comorbidities and diabetes-related factors, the risk remained statistically significantly relevant (OR: 2.03, 95% CI: 1.19–3.45; $P = 0.010$). Moreover, patients with two or more severe hypoglycaemic events appeared to be at substantially greater risk of DPN in the fully adjusted model (OR: 2.77, 95% CI: 1.06–7.25; $P = 0.038$).

A subgroup analysis stratified by $HbA_{1c}$ level (Fig. 1) was also performed. Of the 78 patients with hypoglycaemia, 39 (50%) had $HbA_{1c}$ levels $< 7\%$ and 39 had levels $\geq 7\%$. In the $< 7\%$, group, the presence of any previous hypoglycaemia did not increase the risk of DPN. In contrast, patients in the $\geq 7\%$ group with previous hypoglycaemic episodes had a significantly increased risk of DPN after controlling for confounding factors (OR: 2.62, 95% CI: 1.29–5.34; $P = 0.008$). This risk was further amplified in patients having two or more episodes of hypoglycaemia (OR: 4.74, 95% CI: 1.16–19.30; $P = 0.030$).

## 4. Discussion

The present study has demonstrated that hypoglycaemic episodes severe enough to require an ED visit or hospitalization

**Table 1**
Demographic and clinical characteristics of the study participants.

| Variable | Total ($n$ = 2773) | Hypoglycaemia | | $P$ |
|---|---|---|---|---|
| | | No ($n$ = 2695) | Yes ($n$ = 78) | |
| Sociodemographic factors | | | | |
| Age, years, mean (SD) | 63.9 (13.0) | 63.6 (13.0) | 74.9 (11.0) | < 0.001 |
| Male, $n$ (%) | 1169 (42.4) | 1138 (42.2) | 31 (39.7) | 0.662 |
| Body mass index, kg/m$^2$, mean (SD) | 25.6 (4.1) | 25.7 (4.1) | 25.3 (4.2) | 0.478 |
| Smoking, $n$ (%) | 275 (9.9) | 274 (10.2) | 1 (1.3) | 0.010 |
| Alcohol drinking, $n$ (%) | 98 (3.5) | 98 (3.6) | 0 (0.0) | 0.113 |
| SBP, mmHg, mean (SD) | 130.5 (13.6) | 130.5 (13.5) | 130.0 (18.3) | 0.799 |
| Diabetes-related condition | | | | |
| DPN, $n$ (%) | 593 (21.4) | 551 (20.4) | 42 (53.8) | < 0.001 |
| Diabetes duration, years, mean (SD) | 10.1 (8.3) | 9.9 (8.2) | 17.4 (10.5) | < 0.001 |
| Insulin use, years, mean (SD) | 4.2 (4.9) | 4.1 (4.9) | 7.0 (5.1) | 0.002 |
| Type of diabetic treatment | | | | |
| No medication, $n$ (%) | 90 (4.0) | 89 (4.1) | 1 (1.4) | < 0.001 |
| OHA only, $n$ (%) | 1565 (69.2) | 1533 (70.0) | 32 (45.7) | |
| Insulin only, $n$ (%) | 171 (7.6) | 152 (6.9) | 19 (27.1) | |
| Insulin + OHA, $n$ (%) | 434 (19.2) | 416 (19.0) | 18 (25.7) | |
| Comorbidities | | | | |
| Hypertension, $n$ (%) | 1796 (64.8) | 1724 (64.0) | 72 (92.3) | < 0.001 |
| Cardiovascular disease, $n$ (%) | 1986 (71.6) | 1911 (70.9) | 75 (96.2) | < 0.001 |
| Ischaemic heart disease, $n$ (%) | 796 (28.7) | 751 (27.9) | 45 (57.7) | < 0.001 |
| Chronic kidney disease, $n$ (%) | 153 (5.5) | 134 (5.0) | 19 (24.4) | < 0.001 |
| Hyperlipidaemia, $n$ (%) | 1852 (66.8) | 1783 (66.2) | 69 (88.5) | < 0.001 |
| Laboratory tests | | | | |
| FPG, mg/dL, mean (SD) | 143.7 (47.9) | 144.0 (47.8) | 134.4 (50.1) | 0.096 |
| $HbA_{1c}$, % (mmol/mol) | 7.5 ± 1.5 (58 ± 16) | 7.5 ± 1.5 (58 ± 16) | 7.2 ± 1.2 (55 ± 13) | 0.169 |
| TG, mg/dL, mean (SD) | 148.6 (131.2) | 148.8 (132.4) | 140.6 (76.0) | 0.399 |
| HDL-C, mg/dL, mean (SD) | 50.5 (14.2) | 50.5 (14.2) | 49.9 (14.7) | 0.725 |
| LDL-C, mg/dL, mean (SD) | 101.4 (31.1) | 101.5 (31.0) | 95.6 (35.6) | 0.123 |

Data are expressed as means ± standard deviation (SD) for continuous variables and as $n$ (%) for categorical variables; differences in continuous variables by Student's $t$ test or ANOVA; differences in categorical variables by Fisher's exact or $\chi^2$ test; DPN: diabetic peripheral neuropathy; FPG: fasting plasma glucose; $HbA_{1c}$: glycated haemoglobin; HDL-C/LDL-C: high-density lipoprotein/low-density lipoprotein cholesterol; OHA: oral hypoglycaemic agent; SBP: systolic blood pressure; TG: triglycerides.

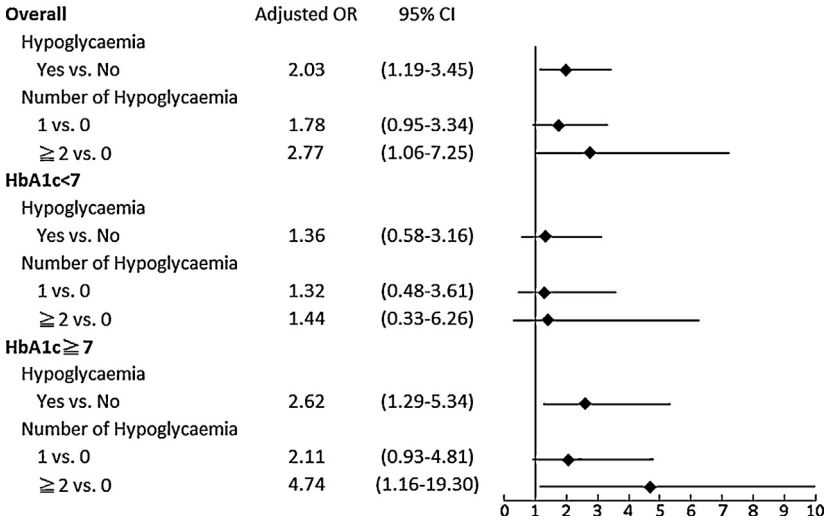


**Fig. 1.** Odds ratios (ORs) for the association of diabetic peripheral neuropathy with hypoglycaemia, adjusted for age, gender, smoking, alcohol, body mass index, hypertension, cardiovascular disease, ischaemic heart disease, chronic kidney disease, hyperlipidaemia and glycated haemoglobin ($HbA_{1c}$).

are associated with an increased risk of DPN, and that there is also a significant trend towards an increased risk of DPN as the number of hypoglycaemic events increases. To the best of our knowledge, this is the first-ever study to evaluate whether episodes of hypoglycaemia are associated with the risk of DPN in adult patients with T2D.

Many studies have investigated risk factors for the development of DPN. Multiple meta-analyses of clinical trials have shown that strictly lowering blood sugar alone does not reduce the incidence of DPN in patients with T2D [4]. One large-scale cohort study examining the cardiovascular risk factors for DPN reported an age- and gender-adjusted hazard ratio (HR) of 1.22 (95% CI: 1.10–1.34) for DPN in patients with $HbA_{1c}$ levels < 6% compared with those having $HbA_{1c}$ levels of 6–7%, suggesting that intensive glycaemic control may increase the risk of DPN [5].

However, the pathogenesis of DPN is complicated and can involve diverse complex mechanisms. Several animal studies have reported that experimental hypoglycaemia induced by injections of excess insulin in rats resulted in Wallerian-type axonal degeneration [6]. In fact, the currently available data appear to

suggest that hypoglycaemia, rather than hyperinsulinaemia, is responsible for insulin-induced hypoglycaemic peripheral neuropathy [1,7], and several possible mechanisms could explain PNS damage induced by hypoglycaemia. Depletion of energy within PNS neurons due to hypoglycaemia may result in both altered intraneural concentrations of various metabolites, leading to axonal degeneration, and myelin breakdown in Schwann cells. Endoneurial microvascular changes caused by local ischaemia may also play a role in PNS damage [7].

Results of the present study have also shown that the risk of DPN is significantly increased in patients with previous hypoglycaemic events and higher $HbA_{1c}$ levels ($\geq 7\%$), but not in those with lower $HbA_{1c}$ levels ($< 7\%$). The presence of hypoglycaemia in patients with higher $HbA_{1c}$ values may reflect greater glycaemic swings. There is growing evidence to suggest that many of the cellular processes that arise with hyperglycaemic spikes also happen during hypoglycaemic troughs [8]. In addition, fluctuating glucose levels have proved to be more deleterious to endothelial function than chronic sustained hyperglycaemic levels [9]. Indeed, our present results emphasize the importance of hypoglycaemia in glycaemic variability, and support the idea that glycaemic instability may be involved in the development of DPN, which is in agreement with the findings of previous studies [10].

The strengths of our study include its large sample size, standardized data collection, and available detailed information on confounding factors, laboratory test values and diabetes treatment. Another strength is the use of a validated clinical screening tool (MNSI), which has the advantages of being sensitive, specific, non-invasive and easy to administer. However, this study also has several limitations. First, the cross-sectional design does not permit any inferences regarding a causal relationship between hypoglycaemia and DPN. Second, the diagnosis of DPN was not confirmed by tests, including nerve conduction studies and, third, the influence of minor hypoglycaemic episodes on the risk of DPN was not assessed in this study.

## 5. Conclusion

Our study represents the first such study to examine the association between severe hypoglycaemia and DPN in patients with T2D. It was found that previous hypoglycaemic events requiring hospitalization or visits to the ED were independent risk factors of DPN in adults with T2D. Moreover, the risk of DPN increased together with the frequency of hypoglycaemic events. Further longitudinal studies to examine hypoglycaemia and DPN in patients with T2D are needed.

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## Disclosure of interest

The authors declare that they have no competing interest.

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