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The Prevalence of Polyneuropathy in Type 2 Diabetes Subgroups Based on HOMA2 Indices of β-Cell Function and Insulin Sensitivity

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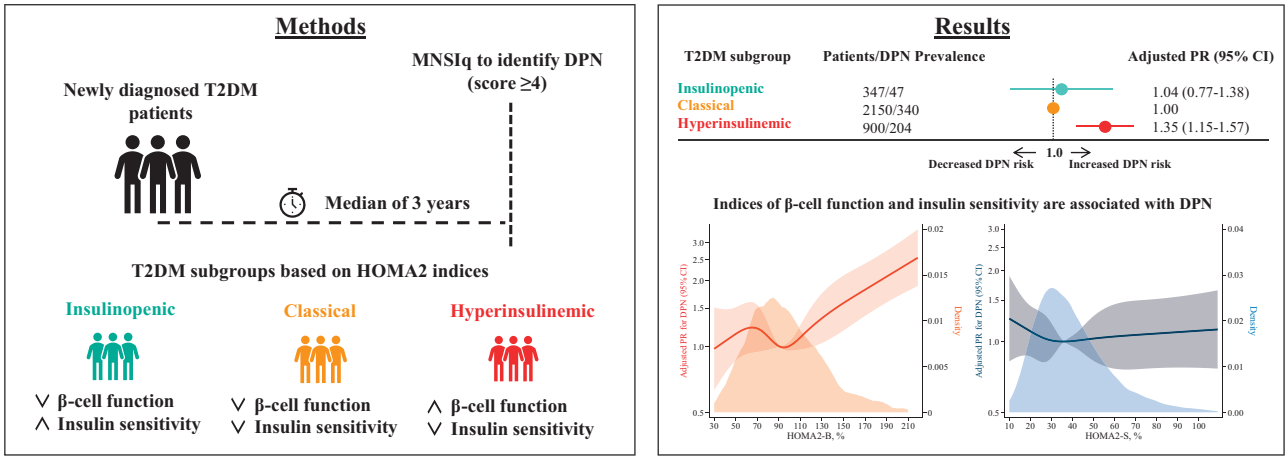

Adjusted for demographic factors, diabetes duration and therapy, lifestyle behaviors, metabolic syndrome components, and alternately for HOMA2-B when examining HOMA2-S and for HOMA2-S when examining HOMA2-B. CI, confidence interval; PR, prevalence ratio; DPN, diabetic polyneuropathy; HOMA2-B, homeostasis model assessment-2 β-cell function; HOMA2-S, homeostasis model assessment-2 insulin sensitivity; T2DM, type 2 diabetes mellitus.

ARTICLE HIGHLIGHTS

- Metabolic syndrome components may cumulatively increase risk of diabetic polyneuropathy (DPN) in type 2 diabetes mellitus (T2DM), driven by insulin resistance and hyperinsulinemia.
- Among newly diagnosed T2DM patients, we observed that the prevalence of DPN was markedly increased in patients with hyperinsulinemic T2DM (high HOMA2-B, low HOMA2-S). Higher HOMA2-B associated, in a linear relation, with higher DPN prevalence, independent of metabolic syndrome components and HOMA2-S.
- Hyperinsulinemia marked by high HOMA2-B is likely an important risk factor for DPN beyond metabolic syndrome components and insulin resistance. This should be considered when developing interventions to prevent DPN.

The Prevalence of Polyneuropathy in Type 2 Diabetes Subgroups Based on HOMA2 Indices of β-Cell Function and Insulin Sensitivity

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OBJECTIVE

Metabolic syndrome components may cumulatively increase the risk of diabetic polyneuropathy (DPN) in type 2 diabetes mellitus (T2DM) patients, driven by insulin resistance and hyperinsulinemia. We investigated the prevalence of DPN in three T2DM subgroups based on indices of β-cell function and insulin sensitivity.

RESEARCH DESIGN AND METHODS

We estimated β-cell function (HOMA2-B) and insulin sensitivity (HOMA2-S) in 4,388 Danish patients with newly diagnosed T2DM. Patients were categorized into subgroups of hyperinsulinemic (high HOMA2-B, low HOMA2-S), classical (low HOMA2-B, low HOMA2-S), and insulinopenic (low HOMA2-B, high HOMA2-S) T2DM. After a median follow-up of 3 years, patients filled the Michigan Neuropathy Screening Instrument questionnaire (MNSIq) to identify DPN (score ≥ 4). We used Poisson regression to calculate adjusted prevalence ratios (PRs) for DPN, and spline models to examine the association with HOMA2-B and HOMA2-S.

RESULTS

A total of 3,397 (77%) patients filled in the MNSIq. The prevalence of DPN was 23% among hyperinsulinemic, 16% among classical, and 14% among insulinopenic patients. After adjusting for demographics, diabetes duration and therapy, lifestyle behaviors, and metabolic syndrome components (waist circumference, triglycerides, HDL cholesterol, hypertension, and HbA_{1c}), the PR of DPN was 1.35 (95% CI 1.15–1.57) for the hyperinsulinemic compared with the classical patients. In spline analyses, we observed a linear relation of higher DPN prevalence with increasing HOMA2-B, independent of both metabolic syndrome components and HOMA2-S.

CONCLUSIONS

Hyperinsulinemia marked by high HOMA2-B is likely an important risk factor for DPN beyond metabolic syndrome components and insulin resistance. This should be considered when developing interventions to prevent DPN.

Diabetic polyneuropathy (DPN) is present in 10–20% of all individuals with newly diagnosed type 2 diabetes mellitus (T2DM) and affects 50% during the course of their disease (1–6). DPN is associated with pain, lower extremity amputation,

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cardiovascular disease, and increased mortality (4,6,7). Components of the metabolic syndrome, including central obesity, hypertension, hyperglycemia and dyslipidemia, are hallmarks of T2DM (1). Recent evidence suggests that these factors all cumulatively contribute to DPN by causing nerve inflammation and oxidative stress (1,3,6,8–11).

Patients with newly diagnosed T2DM can be categorized into three pathophysiological subgroups based on HOM2 indices of fasting β-cell function and insulin sensitivity: hyperinsulinemic, classical, and insulinopenic (12–14). Hyperinsulinemic T2DM patients have more severe metabolic syndrome components than other T2DM subgroups (12,13,15). Hyperinsulinemia per se also has been proposed to harm peripheral neurons (3,16,17), by hampering neurite regeneration and increasing their vulnerability to oxidative stress and low-grade inflammation (16–18). Yet, few studies have investigated associations between hyperinsulinemia and DPN, independent of other metabolic syndrome components. Higher levels of insulin resistance have been associated with increasing prevalence of DPN in patients with longstanding diabetes (1,19–22), but little is known about the separate effects of hyperinsulinemia and insulin resistance on DPN risk among patients with early T2DM (6,16,17).

To improve our understanding of the association of hyperinsulinemia and insulin resistance with DPN, we investigated a large population-based cohort of newly diagnosed T2DM patients with detailed phenotypic data collected during routine clinical care (23). First, we examined the prevalence of DPN in the hyperinsulinemic and the insulinopenic patients compared with the classical patients, overall and independent of metabolic syndrome components. Second, we investigated the association of estimated fasting HOMA2 indices of β-cell function and insulin sensitivity with DPN, aiming to distinguish the effects of high β-cell function (hyperinsulinemia) per se from low insulin sensitivity.

RESEARCH DESIGN AND METHODS

Setting, DD2 Cohort, and Linkage to Other Health Registries

The tax-supported Danish healthcare system provides free access to general practitioners and hospital care, and partial reimbursement for the cost of prescribed medications (24). The unique personal identifier (Civil Personal Register number) assigned to all individuals at birth or upon immigration allows linkage among Danish health registries and thereby complete follow-up (24).

The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) is an ongoing nationwide cohort of individuals with newly diagnosed T2DM (median diabetes duration = 1.3 years [interquartile range (IQR) 0.3–2.9 years]) enrolled by general practitioners and hospital clinics since November 2010 (23). At enrollment, patients undergo a short interview and physical examination, and urine and blood samples are collected and stored in the DD2 biobank (23). In the current study, linkage to the following Danish nationwide health registries provided additional information: the Danish Diabetes Database for Adults (DDDA) supplied information on biochemistry tests, anthropometric measurements, and lifestyle (23), the Danish National Patient Registry supplied a complete history of hospital contacts, and the Danish National Prescription Registry supplied information on all prescribed medications redeemed at community pharmacies in Denmark (24). A detailed description of the registries is provided in Supplementary Table 1.

Cohort Sampling

Fig. 1 shows the sampling of the cohort. We included patients enrolled in DD2 between November 2010 and February 2015 (N = 5,988). Patients were excluded if they were diagnosed with other specific forms of diabetes (N = 375), were nonfasting at the time of blood sample collection (N = 879), or had missing plasma glucose or serum C-peptide measurements in the DD2 biobank (N = 330). In total, 4,388 patients were available for further categorization. In 2016, a neuropathy questionnaire was sent out to the DD2 cohort (see below) (25). We excluded patients who died or emigrated before the questionnaire was sent out (N = 161), nonresponders (N = 739), and patients with an invalid response (N = 91) (1,23).

Indices of β-Cell Function and Insulin Sensitivity

Fasting serum C-peptide and plasma glucose values were measured at the time of DD2 enrollment and used in the homeostasis model assessment-2 (HOMA2) computational model (University of Oxford, Oxford, U.K.) to estimate β-cell function (HOMA2-B) and insulin sensitivity (HOMA2-S) (13,26,27).

We categorized patients into three pathophysiological T2DM subgroups based on HOMA2-B and HOMA2-S, as previously described (13). Detailed information on the categorization is provided in the Supplementary Material and Supplementary Fig. 1. In brief, categorization was based on cutoffs utilizing the median HOMA2-B and HOMA2-S values derived from a random cohort with normal fasting glucose measurements residing in the Southern Denmark Region (high/low HOMA2-B $\geq/<$ 115.3% and high/low HOMA2-S $\geq/<$ 63.5%) (13). Patients with hyperinsulinemic T2DM had high HOMA2-B and low HOMA2-S, patients with classical T2DM had low HOMA2-B and low HOMA2-S, and patients with insulinopenic T2DM had low HOMA2-B and high HOMA2-S (Fig. 1 and Supplementary Fig. 1). We focused on patients with hyperinsulinemic T2DM. This accorded with our main objective of investigating the effect of higher HOMA2-B on DPN, that is, beyond the effect of low HOMA2-S that was, by definition, also present in our reference group of the classical patients (Fig. 1). Thus, the median cutoff values were chosen as unbiased values to separate the effect of high HOMA2-B from low HOMA2-S without focusing on choosing the best suitable cutoff value for predictive purposes.

DPN Assessment

DPN was defined as a score of $\geq$4 for Michigan Neuropathy Screening Instrument questionnaire (MNSIq) responses. The questionnaire was sent out in June 2016 at a median of 3.0 years (IQR 2.3–3.8 years) after DD2 enrollment, as previously described (1,23,25). The 2016 questionnaire also contained additional questions on height, weight, lifestyle, mental health, and neuropathic pain. Among members of the DD2 cohort, 82% responded to the questionnaire (25).

Covariates

Supplementary Fig. 2 describes covariate assessment. Data on important DPN risk factors were obtained at DD2 enrollment. These included hip and waist circumference, alcohol consumption, physical activity, and high-sensitivity C-reactive

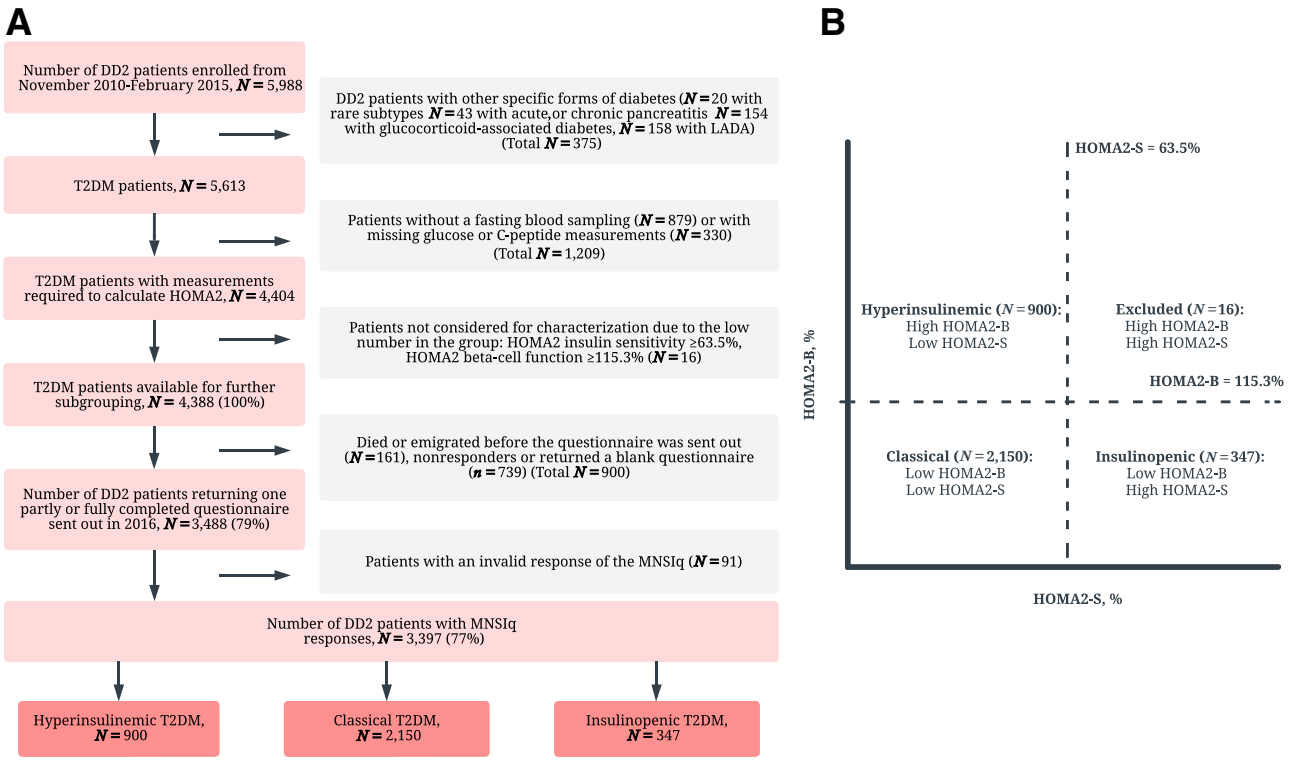

Figure 1—Flowchart of the study cohort (*A*) and outline of the study participants categorization (*B*). During 2016 (median of 3 years [IQR 2.3–3.8 years] after enrollment), follow-up questionnaires on neuropathy were sent to persons in DD2. Besides the MNSIq, this questionnaire also contained additional questions on height, weight, lifestyle behaviors, mental health, and neuropathic pain (1). See the Supplementary Material and Supplementary Fig. 1 for detailed information on categorization of T2DM patients. A small group of patients with high HOMA2-B and high HOMA2-S were excluded since the low number of patients hampered interpretation of regression coefficients (*n* = 16). GAD-ab, glutamate decarboxylase antibodies; LADA, latent autoimmune diabetes of adults.

protein (hs-CRP) level. Information on smoking, blood pressure, and additional laboratory blood tests was obtained from the DDDA. Measurements were included if they were recorded between one year before DD2 enrollment and June 2016 (when the MNSIq was filled), using the measure closest to the date of DD2 enrollment. If information on smoking was missing in the DDDA, we used self-reported data from the questionnaire sent out in 2016 (Supplementary Fig. 2).

Information on glucose-, lipid-, and blood pressure–lowering drugs was retrieved from the Danish National Prescription Registry for the year prior to enrollment. A complete hospital history of comorbidities before enrollment was ascertained from the Danish National Patient Registry to describe the study cohort and to predict missing values. All definitions, (including whether a variable was continuous or categorical and exact cutoffs), International Classification of Diseases codes, and Anatomical Therapeutic Chemical Classification System codes used in the study are provided in Supplementary Table 1

Statistical Analyses

Patient characteristics at enrollment were presented by underlying T2DM subgroup. For the first study aim, associations between the hyperinsulinemic and insulinopenic patients with DPN were analyzed by calculating crude and adjusted prevalence ratios (PRs) of DPN with Poisson regression (including robust error variance), using patients with classical T2DM as a reference. Based on previous literature on risk factors for DPN and guided by a directed acyclic graph (Supplementary Fig. 3 and Supplementary Table 2*A*–*G*) (1,6,10,11), we adjusted the regression model for the following factors that may affect HOMA2-B and HOMA2-S and also be associated with DPN risk (model 1) (1,3,8,10): demographic factors (age, sex), diabetes duration, diabetes therapy (no glucose-lowering drug [GLD] therapy, noninsulin GLD monotherapy, noninsulin polytherapy, or insulin-based regimens), and lifestyle behaviors (physical activity, smoking, and alcohol consumption). To assess the association of the T2DM subgroups beyond the effect of adverse metabolic syndrome components (28), we first stratified associations by presence or absence of central obesity (waist circumference of ≥88/102 cm [female/male]), hypertriglyceridemia (≥1.7 mmol/L or treatment with lipid-lowering medication), low HDL cholesterol (<1.0/1.3 mmol/L [male/female] or treatment with lipid-lowering medication), hypertension (≥130/85 [systolic/diastolic blood pressure] or treatment with antihypertensive medication), and elevated hemoglobin A_{1c} (HbA_{1c}; ≥53 mmol/mol [7%]). Second, we additionally included metabolic syndrome components in model 1, first individually and then all together (model 2). Supplementary Table 1 shows exact definitions of all variables used in the regression analyses. Missing values for covariates in our cohort ranged between 0.1% and 27%, with modest proportions of missingness primarily observed for triglycerides, Hba_{1c}, and blood pressure, while HDL cholesterol was an outlier,

with 52% missing values (Supplementary Table 1). We used multiple chained equations to impute missing covariates, assuming covariates were missing at random, before including the imputed values in the models. A detailed description of this procedure is provided in the Supplementary Material (29,30).

For the second study aim—to further distinguish the effect of high HOMA2-B from low HOMA2-S—we first examined associations of HOMA2-B and HOMA2-S with DPN, using model 1 adjusted restricted cubic splines with five knots (30,31). We then stratified the HOMA2-B spline model according to levels of HOMA2-S and vice versa. Finally, we adjusted our spline models for metabolic syndrome components and alternately for HOMA2-B when examining HOMA2-S and for HOMA2-S when examining HOMA2-B.

Sensitivity and Additional Analyses

First, we reran analyses while excluding HDL cholesterol from model 2 because of its strong correlation with triglycerides and waist circumference. Second, we stratified on and alternatively adjusted for hs-CRP, since low-grade inflammation could be an additional potential confounder. Third, we restricted the main analysis to patients with complete information on all covariates included in model 2 (n = 2,291, complete case analysis with no imputation). Fourth, we calculated adjusted PRs for the three T2DM subgroups, restricted to patients with no insulin use, because insulin therapy may have affected HOMA2 indices (27). Fifth, we excluded patients with a previous hospital record of any type of neuropathy at DD2 enrolment (n = 103 [3%]) to limit the risk of reverse causality. Sixth, we conducted an attrition analysis to assess baseline characteristics for MNSIq nonresponders versus responders and examined whether differential mortality in T2DM subgroups after DD2 cohort enrollment might have influenced the probability of filling the MNSIq. All data management, statistical analyses, and graphical computation were done using Stata 17 (StataCorp LLC, College Station, TX).

Research Ethics and Informed Consent

The Danish Regional Ethical Committee on Health Research for Southern Denmark (record no. S-20100082) and the Danish Data Protection Agency (record nos. 2008-58-0035 and 2016-051-000001/2514) approved the DD2 study. All DD2 participants volunteered to participate in the DD2 project and gave written informed consent.

RESULTS

Descriptive Results

Among 3,397 (77%) patients who filled in the MNSIq, we identified 900 (27%) hyperinsulinemic, 2,150 (63%) classical, and 347 (10%) insulinopenic T2DM patients (Table 1). Compared with the other T2DM subgroups, the hyperinsulinemic patients had more central obesity (hyperinsulinemic: 89%; classical: 75%; insulinopenic: 36%), had the highest median triglyceride level (hyperinsulinemic: 1.8 mmol/L; classical: 1.6 mmol/L; insulinopenic: 1.0 mmol/L), and had the lowest median HDL cholesterol level (hyperinsulinemic: 1.1 mmol/L; classical: 1.2 mmol/L; insulinopenic: 1.4 mmol/L). In contrast, the hyperinsulinemic patients had median systolic blood pressure similar to the other T2DM subgroups (130 mmHg) and similar median HbA_{1c} levels (hyperinsulinemic: 44 mmol/mol [6.2%]; classical: 48 mmol/mol [6.5%]; insulinopenic: 46 mmol/mol [6.4%]). The hyperinsulinemic patients also received more intensive blood pressure–lowering therapy (e.g., thiazides: hyperinsulinemic, 22%; classical, 18%; insulinopenic, 14%), but similar intensity of glucose-lowering therapy (noninsulin GLD polytherapy: hyperinsulinemic, 9%; classical, 12%; insulinopenic, 7%) (Table 1).

T2DM Subgroups and Association With DPN

The prevalence of DPN was 23% among the hyperinsulinemic patients, 16% among the classical patients, and 14% among the insulinopenic patients (Table 1). Correspondingly, the crude PRs of DPN were 1.43 (95% CI 1.20–1.71) for patients with hyperinsulinemic T2DM and 0.86 (95% CI 0.63–1.16) for patients with insulinopenic T2DM, compared with the classical patients (Fig. 2). The associations remained almost unchanged after adjusting for differences in demographic factors, diabetes duration and therapy, and lifestyle behaviors for patients with hyperinsulinemic T2DM (1.42 [95% CI 1.21–1.65]) and for patients with insulinopenic T2DM (0.86 [95% CI 0.65–1.14]), compared with the classical patients. The association between being in the hyperinsulinemic subgroup and increased prevalence of DPN was similar across subgroups of age and sex, whereas the association was weaker among patients without central obesity, hypertriglyceridemia, low HDL cholesterol, or hypertension. However, these subgroups were generally small, with limited statistical precision of PRs. No clear differences in associations between being in the insulinopenic subgroup and DPN prevalence were seen in stratified analyses (Supplementary Fig. 4).

After further adjusting for metabolic syndrome components (waist circumference, triglycerides, HDL cholesterol, hypertension, HbA_{1c}), DPN prevalence remained elevated for patients with hyperinsulinemic T2DM (1.35 [95% CI 1.15–1.57]). In contrast, little difference in the adjusted PR was observed for insulinopenic patients (1.04 [95% CI 0.77–1.38]) (Fig. 2). Adjustment for waist circumference alone had the greatest impact on the associations, with the PR of DPN attenuating from 1.42 (95% CI 1.21–1.65) to 1.30 (95% CI 1.12–1.52) for patients with hyperinsulinemic T2DM, in accordance with more central obesity among the hyperinsulinemic patients. In comparison, adjustment for triglycerides, hypertension, HDL cholesterol, or HbA_{1c} separately had virtually no effect on the DPN estimates in the subgroups (Fig. 2).

The Association of Estimated HOMA2-B With DPN, Beyond HOMA2-S

We observed a linear dose-response relation with high DPN prevalence for high HOMA2-B starting approximately above 110% and low HOMA2-S starting approximately below 60% (Fig. 3). Similar patterns were found in subgroups of HOMA2-B and HOMA2-S (Supplementary Fig. 5). Additional adjustment for metabolic syndrome components and HOMA2-B attenuated the association between HOMA2-S and DPN toward the null (Fig. 3). In contrast, the association between high HOMA2-B and DPN remained linearly increased after additional adjustment for metabolic syndrome components and HOMA2-S (Fig. 3).

Additional Analyses

All results were similar after excluding HDL cholesterol from model 2 and when including hs-CRP (Supplementary Table 3). Likewise, the results resembled those of the main analysis when restricting the cohort to patients with complete information on the covariates included in model 2,

Table 1—Characteristics of 3,397 newly diagnosed T2DM patients by their pathophysiological subgroup

	Hyperinsulinemic	Classical	Insulinopenic
N	900 (27)	2,150 (63)	347 (10)
DPN (MNSIq $\geq$ 4)	204 (23)	340 (16)	47 (14)
Age, median (quartiles)	63 (54–70)	62 (54–69)	65 (56–70)
Male	493 (55)	1,271 (59)	200 (58)
Year of enrollment			
2010–2012	324 (36)	753 (35)	119 (34)
2013–2015	576 (64)	1,397 (65)	228 (66)
Diabetes duration: days, median (quartiles)	430 (135–871)	566 (174–1,077)	483 (157–971)
Excessive alcohol consumption*	55 (6)	160 (7)	18 (5)
Current smoking	174 (19)	376 (17)	55 (16)
Days per week with 30 min of physical activity			
7	221 (25)	569 (26)	126 (36)
5–6	93 (10)	324 (15)	59 (17)
3–4	214 (24)	520 (24)	82 (24)
1–2	196 (22)	446 (21)	52 (15)
None	176 (20)	291 (14)	28 (8)
Waist circumference, $\geq$88/102 cm (F/M), *n* = 3,392	800 (89)	1,616 (75)	126 (36)
Waist-to-hip ratio, $\geq$0.95/1.05 (F/M), *n* = 3,391	390 (43)	679 (32)	48 (14)
Median HOMA2-B, % (quartiles), *n* = 3,397	136 (125–158)	82 (67–97)	64 (50–81)
Median HOMA2-S, % (quartiles), *n* = 3,397	27 (22–35)	38 (30–47)	74 (68–86)
Median fasting glucose, mmol/L (quartiles), *n* = 3,397	6.4 (5.9–6.9)	7.6 (6.9–8.7)	6.5 (5.8–7.3)
Median C-peptide, pmol/L (quartiles), *n* = 3,397	1,542 (1,224–1,869)	1050 (856–1,286)	556.3 (476–608)
Median HS-CRP, mg/L (quartiles), *n* = 3,342	2.3 (1.0–5.0)	1.7 (0.8–3.7)	0.8 (0.4–1.8)
Data from DDDA			
Median HbA_{1c}, mmol/mol (%) (quartiles), *n* = 2,658	44 (41–48)	48 (43–53)	46 (41–51)
Median HbA_{1c}, % (quartiles), *n* = 2,658	6.2 (5.9–6.5)	6.5 (6.1–7.0)	6.4 (5.9–6.8)
Median LDL cholesterol, mmol/L (quartiles), *n* = 2,595	2.1 (1.6–2.7)	2.2 (1.7–2.8)	2.1 (1.7–2.7)
Median HDL cholesterol, mmol/L (quartiles), *n* = 1,637	1.1 (0.9–1.4)	1.2 (1.0–1.5)	1.4 (1.2–1.7)
Median triglycerides, mmol/L (quartiles), *n* = 2,472	1.8 (1.3–2.5)	1.6 (1.1–2.4)	1.0 (0.8–1.4)
Median eGFR, mL/min/1.73 m^2 (quartiles), *n* = 2,304	85.0 (70.0–96.0)	89.0 (76.0–98.0)	90.0 (82.0–96.0)
Median systolic BP, mmHg (quartiles), *n* = 2,543	130 (120–139)	130 (125–140)	130 (125–138)
Median diastolic BP, mmHg (quartiles), *n* = 2,543	80 (71–85)	80 (75–85)	80 (72–85)
Number of metabolic syndrome components besides diabetes, *n* = 2,675			
$\leq$2	37 (4)	158 (7)	65 (19)
$\geq$3	686 (76)	1,527 (71)	202 (58)
Modified Charlson Comorbidity Index score, excluding diabetes			
0	585 (65)	1,553 (72)	269 (78)
1–2	252 (28)	515 (24)	63 (18)
3+	63 (7)	82 (4)	15 (4)
Comorbidities			
Cardiovascular disease	270 (30)	491 (23)	64 (18)
Diabetes with eye disease	80 (9)	199 (9)	29 (8)
Diabetes with kidney disease	27 (3)	28 (1)	<5 (1)
Chronic pulmonary disease	96 (11)	152 (7)	22 (6)
Hospital-diagnosed obesity	192 (21)	280 (13)	17 (5)
Alcoholism-related disorders	23 (3)	52 (2)	8 (2)
Cancer	77 (9)	185 (9)	33 (10)
Chemotherapy	66 (7)	113 (5)	17 (5)
Medication use			
No GLD use	148 (16)	341 (16)	62 (18)
Noninsulin GLD monotherapy	645 (72)	1,459 (68)	221 (64)
Noninsulin GLD polytherapy	81 (9)	255 (12)	23 (7)
Insulin therapy	26 (3)	95 (4)	41 (12)

Continued on p. 6

Table 1—Continued

	Hyperinsulinemic	Classical	Insulinopenic
Metformin	739 (82)	1,774 (83)	275 (79)
GLP1 analogs	52 (6)	110 (5)	8 (2)
SGLT2 inhibitors	<5 (0)	9 (0)	<5 (1)
DDP4 inhibitors	49 (5)	213 (10)	32 (9)
Sulfonylureas	38 (4)	167 (8)	24 (7)
Loop diuretics	126 (14)	136 (6)	15 (4)
Aspirin	291 (32)	569 (26)	75 (22)
Thiazides	199 (22)	380 (18)	48 (14)
Potassium-sparing agents	69 (8)	80 (4)	7 (2)
Renin-angiotensin-system antagonists	605 (67)	1,300 (60)	173 (50)
Ca-antagonists	280 (31)	596 (28)	78 (22)
Beta-blockers	275 (31)	481 (22)	51 (15)
Statins	652 (72)	1,564 (73)	240 (69)
Other lipid-lowering drugs	28 (3)	41 (2)	7 (2)

Data are *n* and percent unless otherwise specified. Please see definitions of covariates in Supplementary Table 1. *More than 14/21 units/week (female/male). CRP, C-reactive protein; eGFR, estimated glomerulus filtration rate; F, female; M, male.

patients without previously recorded neuropathies, and patients without insulin therapy (Supplementary Tables 3 and 4 and Supplementary Fig. 6). Our attrition analysis showed that nonresponders to the MNSIq were slightly younger and more often males, but otherwise had a similar distribution of T2DM subgroups, and similar proportions of central obesity, comorbidities, and use of comedication, compared with patients in our study cohort (Supplementary Tables 5–7). Among all patients available for T2DM categorization (n = 4,388), we observed no material difference in mortality risk during the time period from enrollment to completion of the MNSIq questionnaire, for either the hyperinsulinemic patients (age- and sex-adjusted mortality rate ratio: 1.14 [(95% CI 0.82–1.60]) or the insulinopenic patients (age- and sex-adjusted mortality rate ratio: 1.00 [95% CI 0.59–1.71]), as compared with the classical patients (Supplementary Table 8).

CONCLUSIONS

In this cohort of newly diagnosed T2DM patients enrolled from routine clinical care settings, we observed that the prevalence of DPN was markedly increased in patients with hyperinsulinemic T2DM. This association remained elevated after accounting for the effect of metabolic syndrome components. Higher HOMA2-B and lower HOMA2-S were both associated linearly with increasing DPN prevalence. However, the association with DPN remained robust only for higher HOMA2-B when we adjusted for metabolic syndrome components and HOMA2-S, but not vice versa for low HOMA2-S. Our findings indicate that higher HOMA2-B and related hyperinsulinemia is likely a more important metabolic risk factor for DPN than lower HOMA2-S. These findings improve our understanding of risk factors for DPN underlying the metabolic syndrome (6).

The relation between DPN and pathophysiological subgroups in T2DM has not been investigated before. Prior studies of small T2DM cohorts have focused mainly on the association between different measures of insulin resistance and DPN (1,19–22). Studies from Korea of patients with T2DM ($N < 100$) found that higher levels of insulin resistance were associated with higher prevalence odds ratio of DPN (age-, sex-, diabetes duration–, and smoking-adjusted odds ratio 1.67 [95% CI 1.09–2.57]) (19,20,22). Similarly, a cross-sectional study from the Shanghai Diabetic Neuropathy Epidemiology study (N = 2,035, including 534 patients with diabetes) showed that higher HOMA2 insulin resistance was associated with increased odds of clinically diagnosed DPN after adjusting for all components of the metabolic syndrome (odds ratio 1.20 [95% CI 1.10–1.40]) (21). The prior studies were mainly conducted among patients with long-standing diabetes (>10 years), among whom hyperglycemia already had damaged peripheral neurons (6). They also were limited by not considering insulin sensitivity and β-cell function simultaneously in their analyses. Accordingly, the distribution of β-cell function and insulin sensitivity indicates that the two indices are clearly correlated, and that using one measure without considering the other will still convey information on the other measure in effect estimates (13,15,27). Using methodologies aiming to separate these effects in a large cohort of newly diagnosed T2DM patients, we found evidence that high HOMA2-B associates with DPN beyond the effect of metabolic syndrome and low HOMA2-S. Our results are supported by a prior cross-sectional study based on the DD2 cohort, in which high C-peptide levels (≥1,550 pmol/L) were associated with increased DPN prevalence (age-, sex-, and diabetes duration–adjusted PR 1.72 [95% CI 1.43–2.07]) (1).

Despite cohort studies having reported an incidence rate of 24–26.9 DPN cases per 1,000 person-years in T2DM patients, the exact progression rate for DPN development has been difficult to study because of heterogenous disease presentation and nonstandardized diagnostic criteria (4,6,32). Existing evidence has indicated that obesity is a key risk factor for polyneuropathy, both in persons without diabetes and in persons with prediabetes (5,8,9), suggesting that development of DPN begins before overt T2DM (6). As high β-cell function/hyperinsulinemia may progress with increasing obesity (33,34), our findings suggest that high β-cell function/hyperinsulinemia may be an underlying driver of the association between obesity and DPN (6). Mechanistically, evidence has shown that unfortunate growth stimuli from hyperinsulinemia disrupt PI3K/AKT signaling—thereby impairing neurotrophic support and glucose uptake in peripheral neurons (6,16–18,35).

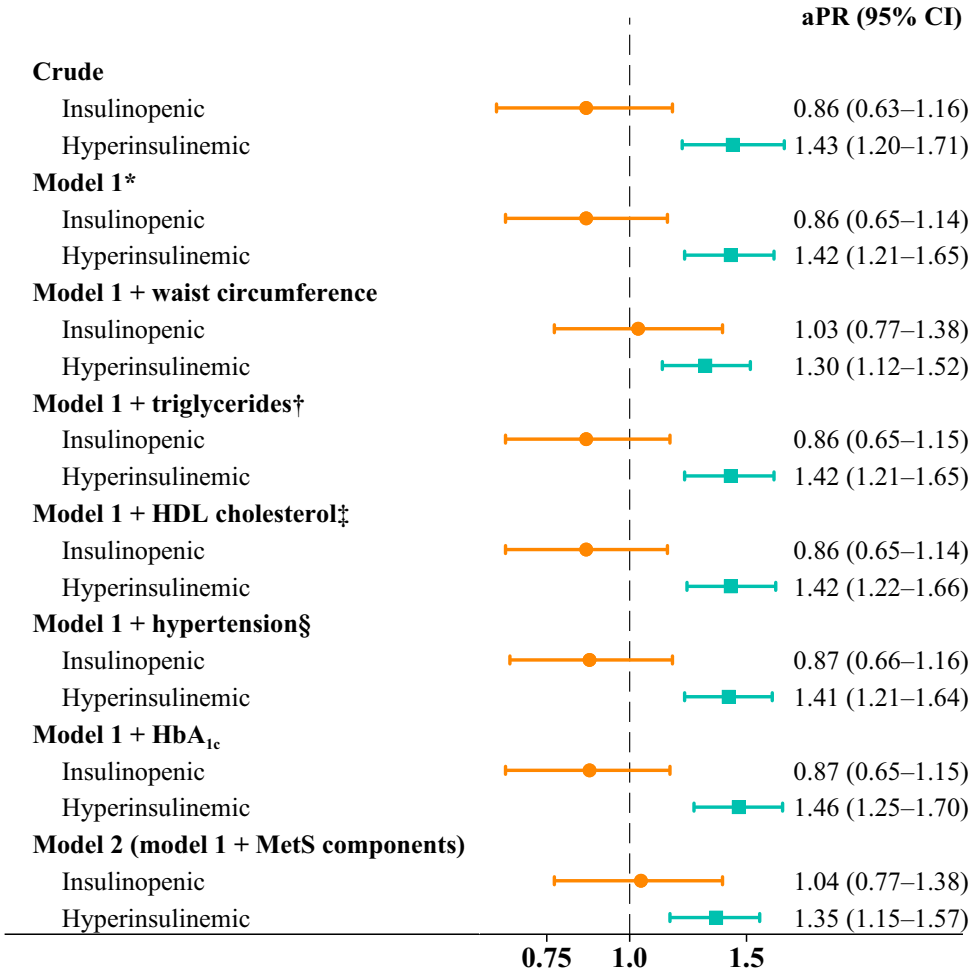

Figure 2—Crude and adjusted PRs of T2DM subgroups associated with DPN, using the classical patients as reference. Adjusted PRs for DPN are shown with adjustment for each metabolic syndrome component individually, and for all metabolic syndrome components together. Missing data were handled by multiple imputation using chained equations. A detailed description of this procedure is available in the Supplementary Material. *Model 1 was adjusted for demographic factors (age and sex), diabetes duration and therapy, and lifestyle behaviors (physical activity, smoking, and alcohol consumption). Model 2 was additionally adjusted for metabolic syndrome components: waist circumference, triglycerides, HDL cholesterol, hypertension, and HbA_{1c}. Supplementary Table 1 shows exact definitions of all variables used in the regression analyses. †Triglycerides $\geq$ 1.7 mmol/L or treatment with any lipid-lowering medication. ‡HDL cholesterol $<$1.0/1.3 mmol/L [male/female] or treatment with lipid-lowering medication. §Hypertension: systolic/diastolic blood pressure $\geq$ 130/85 mmHg or use of any antihypertensive medication. aPR, adjusted prevalence ratio; MetS, metabolic syndrome.

Thus, high concentrations of insulin might facilitate resistance and downregulation of neuronal growth pathways (6,16–18).

Our results showing no increase in DPN prevalence for the insulinopenic patients may seem at odds with a recent study conducted in the German Diabetes study cohort, which divided patients into five diabetes subgroups based on age, BMI, glycemic control, and HOMA2 indices (36,37). In that study, in a small subcohort of patients who attended a 5-year follow-up visit (n = 367), 5 of 10 patients (50%) with severe insulin deficiency had developed DPN, whereas DPN was present in 4 of 35 patients (12%) with severe insulin resistance (P value $<$ 0.0001). Outcome numbers were small and unadjusted, and it is probable that the higher DPN prevalence observed with severe insulin deficiency was driven by the very high mean HbA_{1c} level at baseline (72 mmol/mol [8.7%]) (36,37).

Recently, we directly compared our three T2DM subgroups with the T2DM subgroups proposed in the Swedish All New Diabetics in Scania (ANDIS) cohort (12). The hyperinsulinemic subgroup was the most robust, showing 70% overlap with the ANDIS severe insulin resistance subgroup, whereas the overlap of our DD2 insulinopenic subgroup with the ANDIS severe insulin deficiency subgroup was limited (12). This may contribute to the discrepant findings of the two projects and suggests the need for standardized subgroup definitions (12,15,37).

Our study has limitations. First, there is a possibility of selection bias, as we depended on the subgroup of patients who filled in the MNSIq a median of 3 years after enrollment (77% of the enrolled patients). However, in an attrition analysis, we found only minor differences in characteristics of nonresponders versus responders, and no material differences in mortality risk for T2DM subgroups up to the time of MNSIq completions. Second, our results should be interpreted in the light of the limitations of the HOMA2 calculator, which provides only indices of the steady-state insulin sensitivity and β-cell function based on

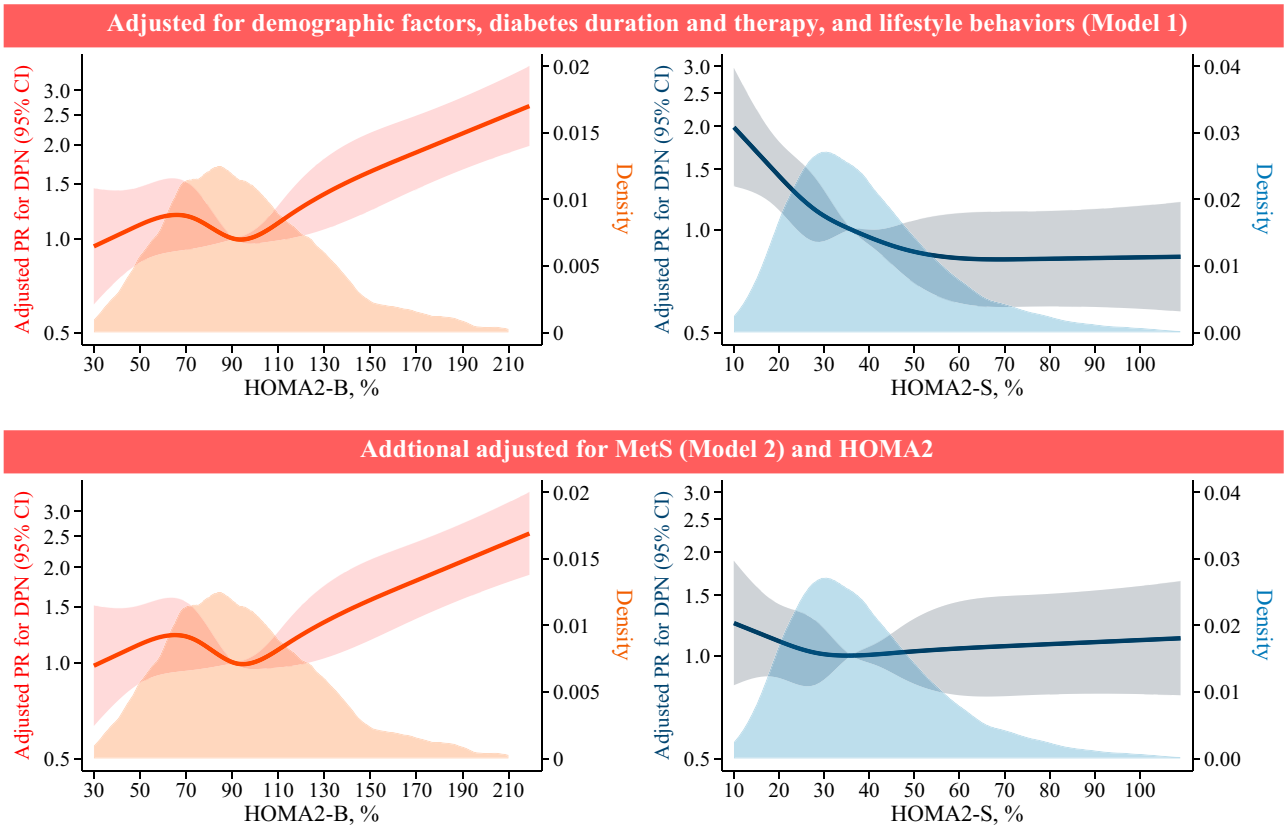

Figure 3—Adjusted prevalence ratios of DPN associated with continuous indices of β-cell function and insulin sensitivity. Splines were calculated only for patients with data on all covariates included in the models (n = 2,291). Outliers outside HOMA2 ranges were excluded, corresponding to the first and 99th percentile of the HOMA2 distribution (HOMA2-B = 28% and 218%; HOMA2-S = 13% and 107%). The two uppermost splines were adjusted for model 1: demographic factors (age and sex), diabetes duration and therapy, and lifestyle behaviors (physical activity, smoking, and alcohol consumption). The two lower splines were adjusted for model 2: model 1 + waist circumference, triglycerides, HDL cholesterol, hypertension, HbA_{1c}, and alternately for HOMA2-B when examining HOMA2-S and for HOMA2-S when examining HOMA2-B. Supplementary Table 1 shows exact definitions of all variables used in the regression analyses. The reference values were the median HOMA2 value of the total cohort (HOMA2-S = 36%; HOMA2-B = 91%). Shaded areas indicate 95% CIs.

the same fasting C-peptide and plasma glucose values. Furthermore, HOMA2 cannot measure a functional response (27,33). However, gold standard dynamic stimulatory tests like the hyperinsulinemic euglycemic clamp and the hyperglycemic clamp are not feasible for large epidemiological studies, which is why HOMA2 has been suggested for use in such studies (27). Moreover, the steady-state/nonprandial phase reflecting the basal level of β-cell function/insulin sensitivity is of clinical interest because individuals spend a considerable proportion of the day in that phase. Third, although DPN may take years to develop, we do not know with certainty that all participants were DPN naïve at enrollment, hampering calculation of incidence rates. Thus, we relied on prevalence rate ratios, which may be influenced by disease duration bias (38). Still, the similar mortality for the different T2DM subgroups between their DD2 enrollment and responding to the MNSIq questionnaire indicates that our estimates were not affected by major bias (38). Fourth, the cross-sectional design has inherent limitations in documenting temporal relationships with certainty. The median 3 years' time frame from HOMA2 assessment to DPN assessment suggests that DPN outcomes could be a mixture of new incident and preexisting prevalent DPN. However, the results were robust in additional analyses, which increased the likelihood of DPN being incident, that is, when we restricted to patients with <1 year of diabetes duration at HOMA2 assessment and excluded those with a previous diagnosis of neuropathy (n = 103 [3%]). Still, the short follow-up of median 3 years may have led to reverse causality, for example, if beginning DPN symptoms had led to less physical activity with more insulin resistance in some patients. As repeated laboratory and DPN measurements were unavailable in our study cohort, the cross-sectional analysis was the only feasible approach. Fifth, despite relying on the MNSIq without a neurologic examination, the high specificity of the MNSIq (>84%) (2) may likely produce unbiased results on the PR scale in comparative analyses (39). Finally, residual confounding could have affected our findings, as we had no information on, for example, socioeconomic factors and other causes of neuropathy. However, the potential effect of socioeconomic position may be mediated through lifestyle behaviors, which we adjusted for.

In conclusion, we provide new evidence that the prevalence of DPN clearly differs for T2DM subgroups. Higher HOMA2-B associates with DPN prevalence in a dose-response manner, independent of metabolic syndrome components and HOMA2-S. Current clinical practice provides limited guidance on preventing DPN beyond tight glycemic control (40). We suggest that

higher HOMA2-B among patients with T2DM is likely an important risk factor for DPN beyond metabolic syndrome components and insulin resistance. This should be considered when developing interventions to prevent DPN.

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Author Contributions. F.P.B.K., D.H.C., and R.W.T. conceived the study idea. F.P.B.K., D.H.C., and R.W.T. designed the study. F.P.B.K. did data management and statistical analysis. J.S.N. is the principal manager of the DD2. H.T.S. provided expert knowledge of clinical epidemiology, while B.C.C., T.S.J., and H.A. provided expert knowledge of neuropathy. J.V.S., K.H., H.B.-N., P.V., N.J., M.H.O., T.H., C.B., and A.V. contributed with expert knowledge of type 2 diabetes and pathophysiological subgroups. F.P.B.K., D.H.C., B.C.C., H.T.S., and R.W.T. prepared the first draft of the manuscript. All authors contributed to the interpretation of data and the drafting of the manuscript, as well as critically revising the manuscript draft. All authors approved the final version of the manuscript. F.P.B.K. and R.W.T. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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