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High daily insulin exposure in patients with type 2 diabetes is associated with increased risk of cardiovascular events

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ABSTRACT

Aims: Intensive glucose control, often involving insulin treatment, failed to improve cardiovascular outcomes in several clinical trials. Observational studies reported an association between insulin use and cardiovascular disease (CVD) risk. It has therefore been suggested that insulin adversely affects CVD risk. To investigate the feasibility of this hypothesis, we studied the association between insulin dose and CVD risk in type 2 diabetes.

Methods: A case-control study was conducted of new users of oral antidiabetics who were prescribed insulin, using the Dutch Pharmo database. Cases were hospitalized for a cardiovascular event (CVE) and matched 1:2 to patients who were not hospitalized for a CVE, by sex, age, duration of diabetes and type of oral antidiabetic. Patients were divided into tertiles according to mean daily insulin dose. Conditional logistic regression analyses were used to explore the association between insulin exposure and CVE risk.

Results: We included 836 patients (517 (62%) male, mean age 66 years). After adjusting for available potential confounders, including HbA1c and triglycerides, insulin exposure was positively related to CVE risk (odds ratios for high (≥53.0 U/day) and intermediate (24.3–52.9 U/day) vs. low exposure (≤24.2 U/day): 3.00 [95% confidence interval (CI) 1.70 to 5.28] and 2.03 [95% CI 1.17 to 3.52].

Conclusion: Our findings are in line with the suggestion that high-dose insulin therapy adversely affects CVD risk, but need to be interpreted with caution due to the observational nature of the study. The role of particularly high-dose insulin in the progression of CVD warrants further investigation.

1. Introduction

Patients with type 2 diabetes (T2DM) are at increased risk of cardiovascular disease (CVD) [1]. Hyperglycemia is an established independent predictor of both micro- and macro-vascular disease [2]. Consequently, optimized glucose control is the cornerstone in the treatment of T2DM. Treatment in T2DM typically starts with oral glucose-lowering drugs, whereas insulin is added only if HbA1c targets are not reached.

Although a substantial body of evidence has demonstrated a linear relationship between HbA1c levels and CVD risk, several large-scale intervention studies failed to confirm the anticipated benefit of more stringent HbA1c targets [3–6]. Insulin was often used to achieve these lower HbA1c levels While these trials were not designed to investigate the effects of insulin therapy, post-hoc analyses indicated that both hypoglycemia and the degree of HbA1c lowering could not account for the lack of benefit in patients receiving more intensive treatment [7,8]. This has caused some to speculate on the potential harms of insulin therapy. On the other hand, a reduced risk of myocardial infarction in patients receiving intensive glucose control (consisting of either oral anti-diabetics or insulin) has also been demonstrated, in the United Kingdom Prospective Diabetes Study (UKPDS) [9]. A number of observational studies have reported an association between insulin use and increased CVD risk, which persisted after adjusting for potentially confounding factors including diabetes severity [10–16]. However,

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their results should be interpreted with caution as residual bias is likely to account for some of the observed association. Mechanistically, evidence from *in vitro* and experimental studies revealed several pathways through which insulin may exert direct pro-atherogenic effects, whereas other studies have ascribed cardioprotective effects to insulin [17–20]. These findings have added to the controversy on the potential harms and benefits of insulin therapy in T2DM [17].

Previous observational studies, which indicated an association between insulin exposure and increased CVD risk, did not quantify the relationship in terms of level-of-insulin exposure. Dose-dependency in the association between insulin exposure and CVD risk could lend further support to the concept that insulin therapy *per se* might contribute to CVD risk, and could thereby provide an explanation for the lack of insulin-established HbA1c improvement on CVD risk.

In this study, we investigated whether the level of insulin exposure exhibited a positive association with the risk of subsequent CVD events in patients with new-onset T2DM. To this end, we used a large population-based database, in which pharmacy records were linked to hospital admission data.

2. Methods

2.1. Setting

Data were obtained from the PHARMO Record Linkage System (Pharmo Institute, Utrecht, The Netherlands) which consists of multiple observational databases linked on a patient level. Data characterize more than three million people in The Netherlands. For the purpose of this study, drug prescription data from the community pharmacy database, clinical laboratory measurements from the clinical laboratory register, and hospitalization data from the Dutch national medical register (LMR) were used. Drug dispensing records were coded according to the *Anatomical Therapeutic Chemical (ATC) Classification*, and included data on the date and amount of the drug dispensed, as well as the prescribed dose regimens and duration (Appendix A). Hospitalization records included information on primary and secondary diagnoses (coded according to the *International Classification of Diseases Ninth Revision (ICD 9), Clinical Modification*), admission and discharge dates, and operations and procedures (Appendix A).

2.2. Study design and population

We conducted a nested case-control study within a cohort of individuals who started treatment with oral glucose-lowering drugs (ATC code A10B) between January 1998 and December 2008. Within this cohort, we selected all individuals who were prescribed insulin during follow-up. Individuals were excluded if they had previously received antidiabetic drugs (ATC codes A10A or A10B) or if no information was available prior to the date of first prescription of oral glucose-lowering drugs.

Cases were defined as patients who were hospitalized for a cardiovascular event (CVE) during follow-up, which included ischemic heart disease, ischemic stroke and ischemic peripheral arterial disease. For each case, two controls without a CVE were included. Cases and controls were matched by sex, age at the start of insulin treatment (within three years), duration of antidiabetic therapy (within 120 days), and the type of first oral glucose-lowering drugs (metformin, SU-derivates, other). Follow-up was defined as the time interval between the date of first prescription of insulin and the date at which a CVE occurred or the date at which drug prescription data were no longer available.

Data were obtained on clinical laboratory results, previous hospitalizations, oral glucose-lowering drugs and concomitant drug therapy. Insulin exposure was quantified according to the mean daily exposure, defined as the total units of dispensed insulin divided by the number of days of insulin treatment. Subsequently, patients were divided into insulin exposure tertiles (low, intermediate and high insulin exposure groups). In addition, information was obtained on the type of oral glucose-lowering drugs, concomitantly used antihypertensive drugs and lipid-lowering therapy (see Appendix A for a list of ATC-codes). Subjects were considered to have a cardiovascular history if they had been hospitalized for a CVE prior to the index date (see Appendix A for a list of used ICD-9 codes). Results of laboratory assessments at dates approximating the date of first prescription and the last date of follow-up were retrieved. Measurement values were included if they were obtained within three months (HbA1c), six months (ALAT, creatinine, glomerular filtration rate [GFR]) or 12 months (total cholesterol, low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C] and triglycerides) before the index date or prior to the last date of follow-up.

2.3. Statistical analyses

Patient characteristics were compared between groups (cases and controls; low-, intermediate- and high insulin exposure) using a mixed-effects model for continuous variables. Differences in dichotomous variables between groups were analyzed by conditional logistic regression.

Conditional logistic regression analysis was used to explore the association between the level of insulin exposure and CVE risk. Odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) were calculated as crude estimates, using subjects in the lowest exposure tertile as references. Multivariable conditional regression analyses was used to adjust for potential confounders for which information was present in 100% of subjects i.e., gender, age (at initiation of insulin therapy), cardiovascular history, use of antihypertensive and lipid-lowering drugs, duration of insulin therapy, type of oral glucose-lowering drug (i.e., metformin, SU-derivatives, other) and continuation of oral glucose-lowering drugs after starting insulin therapy (Model 1). In addition, we adjusted for HbA1c, LDL-c, HDL-c, GFR and triglycerides at baseline (Model 2). Subsequently, backward stepwise elimination (probability for removal $p > 0.1$) of potential confounders included in Model 2 resulted in Model 3.

Variables that were not normally distributed were log-transformed before statistical analysis. Multiple imputation was performed for missing laboratory values. For each missing value, five imputations were performed, based on age, gender, cardiovascular history, use of antihypertensive agents, lipid-lowering drugs and oral glucose-lowering drugs, lipids, HbA1c, GFR, ALAT and creatinine. These were subsequently combined into one effect estimate. Statistical analyses were performed with SPSS software (version 19.0, Chicago, Illinois, USA).

3. Results

3.1. Study population

A total of 26,258 new users of oral-glucose lowering drugs were identified, of whom 3853 were subsequently prescribed insulin. Of these, 287 (7.4%) experienced a CVE during the follow-up period (cases). These cases were matched to 549 individuals who remained free of CVE (controls).

Clinical characteristics of cases and controls are shown in Table 1. Cases more often had a history of CVD (28% vs. 14%), and

Table 1
Clinical characteristics according to case-control status.

	Controls (n = 549)	Cases (n = 287)	p-value
Start of insulin therapy			
Age, years	66 ± 11	66 ± 11	matched
Male gender	339 (61.7)	178 (62.0)	matched
CVD history	74 (13.5)	79 (27.5)	<0.001
Use of lipid-lowering drugs	408 (74.3)	251 (87.5)	<0.001
Use of antihypertensive agents	368 (67.0)	167 (58.2)	0.011
First oral anti-diabetic drug			
Metformin	171 (31.1)	90 (31.4)	matched
SU-derivatives	378 (68.9)	197 (68.6)	
Other	0 (0.0)	0 (0.0)	
HbA1c, mmol/mol	62 (54–72)	63 (55–76)	0.187
Lipids (mmol/L)			
TC	4.50 (3.80–5.30)	4.80 (4.20–5.60)	<0.001
LDL-c	2.40 (1.84–3.10)	2.61 (2.10–3.10)	0.068
HDL-c	1.10 (0.95–1.31)	1.07 (0.90–1.30)	0.077
TG	1.70 (1.17–2.58)	1.88 (1.38–3.00)	0.010
ALAT, U/l	27 (20–47)	28 (20–44)	0.986
Creatinine, μmol/L	84.0 (70.0–104.0)	85.0 (72.0–107.0)	0.189
GFR, ml/min/1.73 m^2	76.2 ± 26.9	74.0 ± 26.2	0.366
End of follow-up			
Age, years	69 ± 11	70 ± 11	matched
HbA1c, mmol/mol	54.1 (47.3–62.0)	55.2 (49.7–63.9)	0.150
Lipids (mmol/L)			
TC	4.20 (3.50–4.90)	4.20 (3.60–4.90)	0.908
LDL-c	2.27 (1.80–2.80)	2.15 (1.70–2.70)	0.278
HDL-c	1.10 (0.90–1.30)	1.09 (0.88–1.30)	0.265
TG	1.50 (1.10–2.11)	1.60 (1.10–2.32)	0.249
ALAT, U/l	27.0 (18.0–46.0)	26.0 (17.0–39.0)	0.460
Creatinine, μmol/L	91.0 (73.0–122.0)	97.0 (79.0–132.0)	0.019
GFR, ml/min/1.73 m^2	69.2 ± 27.83	62.9 ± 29.3	0.011

Values represent mean ± SD, median (interquartile range) or number (%). Abbreviations: CV, cardiovascular; TC, total cholesterol; LDL-C, low-density lipoprotein; HDL-C, high-density lipoprotein; TG, triglycerides; ALAT, alanine aminotransferase; GFR, glomerular filtration rate.

had higher trigylcerides (1.88 mmol/L vs. 1.70 mmol/L), but HbA1c was similar between cases and controls. At the end of follow-up, there were no significant differences between cases and controls with regard to HbA1c, lipids or renal function. The median time between first prescription of oral glucose-lowering drugs and insulin initiation was 2.6 years (interquartile range (IQR) 0.83–4.77), and did not differ between cases and controls (p = 0.580).

3.2. Insulin exposure

Patients in the lowest exposure tertile were treated with ≤24.2 U/day, patients in the intermediate exposure tertile with 24.3–52.9 U/day and patients in the highest tertile with ≥53.0 U/day. Cases received insulin for a median duration of 2.4 years (IQR 1.0–4.9), compared to 2.7 (IQR 1.5–4.5) for controls. Notably, the type of first

Table 2
Clinical characteristics according to insulin exposure in tertiles.

	Low ≤24.2 U/day n = 278	Intermediate 24.3–52.9 U/day) n = 279	High ≥53.0 U/day n = 279	p-value
Age, years	67.3 ± 10	66.4 ± 11	64.5 ± 12	0.011
Male gender	177 (63.7)	165 (59.1)	175 (62.7)	0.510
CV history	49 (17.6)	52 (18.6)	52 (18.6)	0.938
Statins	210 (75.5)	227 (81.4)	222 (79.6)	0.227
First oral anti-diabetic drug				
Metformin	86 (30.9)	80 (28.7)	95 (34.1)	0.388
SU-derivatives	192 (69.1)	199 (71.3)	184 (65.9)	
Other	0 (0.0)	0 (0.0)	0 (0.0)	
Anti-hypertensive drugs	181 (65.1)	169 (60.6)	185 (66.3)	0.330
HbA1c, mmol/mol	51.9 (46.7–57.4)	55.2 (49.7–61.7)	57.4 (48.6–66.1)	0.008
Lipids (mmol/L)				
TC	4.01 (3.35–4.90)	4.20 (3.60–4.90)	4.29 (3.64–5.00)	0.128
LDL-C	2.20 (1.71–2.86)	2.18 (1.80–2.79)	2.20 (1.80–2.70)	0.891
HDL-C	1.08 (0.89–1.29)	1.12 (0.90–1.40)	1.03 (0.89–1.27)	0.003
TG	1.45 (1.10–2.00)	1.46 (1.05–2.10)	1.76 (1.21–2.71)	<0.001
ALAT, U/L	25.0 (17.0–45.0)	24.0 (18.0–35.0)	31.0 (19.0–48.5)	0.103
Creatinine, μmol/L	91.0 (74.0–121.5)	91.0 (75.0–125.5)	97.5 (77.0–131.0)	0.234
GFR, mL/min/1.73 m^2	69.1 ± 28.4	67.4 ± 29.2	64.2 ± 27.8	0.260
Duration of insulin therapy, years	2.0 (1.2–3.7)	3.1 (1.8–4.8)	2.8 (0.9–5.4)	0.001

Values represent mean ± SD, median (interquartile range) or number (%). Abbreviations: CV, cardiovascular; TC, total cholesterol; LDL-C, low-density lipoprotein; HDL-C, high-density lipoprotein; TG, triglycerides; ALAT, alanine aminotransferase; GFR, glomerular filtration rate.

Table 3
Odds ratios for the association between cardiovascular events and insulin exposure tertiles.

	Low (<24.2 U/day) n = 278 OR 95% CI	Intermediate (24.3–52.8 U/day) n = 279 OR 95% CI	High (>52.9 U/day) n = 274 OR 95% CI	P for trend
No. of cases (%)	59 (21.2)	101 (36.2)	127 (45.5)	–
Crude	1.00 (ref)	2.09 (1.43–3.05)	3.00 (2.07–4.37)	<0.001
Model 1[a]	1.00 (ref)	2.14 (1.42–3.22)	3.24 (2.14–4.90)	<0.001
Model 2[b]	1.00 (ref)	1.94 (1.09–3.43)	3.02 (1.68–5.44)	<0.001
Model 3[c]	1.00 (ref)	2.03 (1.17–3.52)	3.00 (1.70–5.28)	<0.001

Values represent odds ratios and corresponding 95% confidence intervals. Abbreviations: U, units; ref, reference category; OR, odds ratio; No, number.
[a] Adjusted for sex, age at initiation of insulin therapy, cardiovascular history, use of antihypertensive drug therapy, use of lipid-lowering drugs, duration of insulin therapy, type of oral glucose-lowering drug and continuation of oral glucose-lowering drugs after insulin initiation.
[b] Adjusted for variables in model 1 and for HbA1c, LDL-C, HDL-C, GFR, TG at end of follow-up.
[c] After backward stepwise elimination of variables of model 2; adjusted for cardiovascular history, antihypertensive drug therapy, lipid-lowering drugs, GFR, type of oral glucose-lowering drug and continuation of oral antidiabetic therapy.

oral glucose-lowering drugs did not differ across patients in each insulin tertile. Patients who received higher doses of insulin were younger (p = 0.011) and had higher levels of HbA1c at the end of follow-up (p = 0.008). Oral glucose-lowering drugs, mainly metformin, were continued after initiation of insulin therapy in 74.0% of patients. The percentage of patients continuing oral anti-diabetic therapy after insulin initiation was higher among patients who were treated with lower doses of insulin (86% in the lowest, 70% in medium and 66% in the highest insulin tertile). The duration of insulin therapy was longer in patients who received higher mean daily doses (Table 2).

3.3. Association between insulin exposure and cardiovascular disease

The crude analysis of the association between insulin exposure and CVE risk showed that after a mean follow-up of 4.1 years, patients in the highest insulin exposure tertile had a three-fold increased risk of a CVE compared to patients in the lowest tertile, while patients in the intermediate exposure tertile had a 2.1-fold increased risk of a CVE (OR 3.00 [95% CI 2.07 to 4.37] and 2.09 [95% CI 1.43 to 3.05], respectively; p for trend <0.001) (Table 3). The strengths of these associations did not change after adjustment for sex, age at insulin initiation, cardiovascular history, use of antihypertensive drugs and use of lipid-lowering drugs, type of oral glucose-lowering drug and continuation of oral glucose-lowering drugs after initiation of insulin therapy (OR 3.24 [95% CI 2.14 to 4.90] and 2.14 [95% CI 1.42–3.22], respectively; p for trend <0.001). Additional adjustment for levels of HbA1c, LDL-c, HDL-c, GFR and TG at the end of follow-up did not affect these results. Also after stepwise backward elimination of the full model, level of mean daily insulin exposure remained directly and significantly associated with risk of subsequent CVE (OR 2.03 [95% CI 1.17 to 3.52] and OR 3.00 [95% CI 1.70 to 5.28], respectively; p for trend <0.001) Fig. 1.

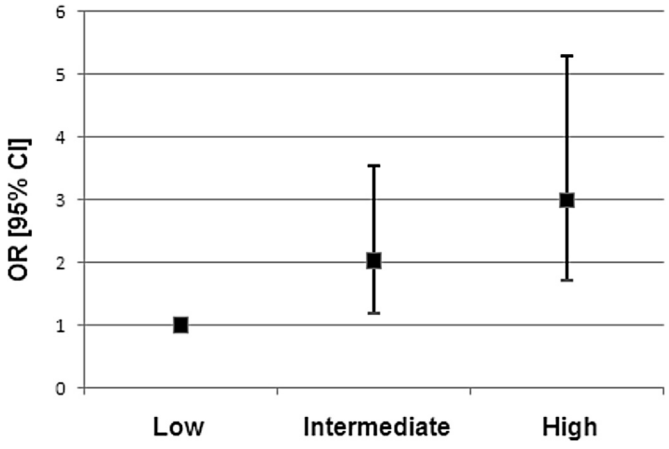

Fig. 1. Association between mean insulin exposure tertiles (low, intermediate, high) and risk of cardiovascular events. Multivariate conditional logistic regression estimates (model 3). OR, odds ratio; CI, confidence interval; CVE, cardiovascular event.

4. Discussion

4.1. Main findings

In this study, we explored the association between mean daily insulin dose and cardiovascular event risk. After adjusting for several important confounders, including achieved glycemic control, we observed a positive relationship between mean daily insulin exposure and the risk of a subsequent CVE. These findings could possibly indicate that treatment with high doses of insulin has an adverse effect on CVD risk, but need to be interpreted in light of the limitations of using retrospectively collected data.

4.2. Possible explanations

A number of explanations may account for the observed positive association between daily insulin exposure and CVE risk. The relationship could reflect a direct, adverse effect of insulin itself. On the other hand, the observed association could also reflect residual differences in risk factors, such as severity of diabetes.

Mechanistically, a direct (i.e., glucose-independent) effect of insulin on CVE risk is plausible as several (pre-)clinical studies have revealed pathways through which insulin may have a pro-atherogenic effect [17]. After binding to its receptor, insulin activates two signaling pathways. The phosphatidylinositol 3-kinase (PI3K) pathway is primarily involved in the metabolic actions of insulin, while the mitogen-activated protein kinase (MAPK) pathway stimulates various processes which are known to contribute to atherogenesis [17]. Insulin resistance is characterized by reduced activation of the PI3K-pathway, while the MAPK-pathway remains fully active. Consequently, exposure to high levels of insulin invariably results to relative hyperactivation of the pro-atherogenic MAPK pathway and a potentially pro-atherogenic state in these patients.

Alternatively, the observed association may be the result of differences in risk factors between cases and controls which have not been accounted for. Patients with more severe insulin resistance generally require higher doses of insulin, and insulin resistance itself is strongly associated with CVD risk [21]. Although we did adjust for a number of important markers of insulin resistance, such as lipids, duration of diabetes and HbA1c levels, we were unable to adjust for BMI. Yet, obesity plays a pivotal role in the association between diabetes and CVD. By inducing a systemic pro-inflammatory state, obesity increases insulin resistance and

contributes to endothelial dysfunction and causes a prothrombotic state, even before the onset of overt hyperglycemia and T2DM [21]. Therefore, the increased CVD risk among patients who used higher doses of insulin could have been mediated at least partially by differences in BMI.

In addition, glucose fluctuations may have contributed to the increased CVE risk among patients who received higher insulin doses. Recent evidence indicates that large glycemic fluctuations may have pro-atherogenic effects and could therefore increase CVD risk independent of long-term glycemic control as measures through HbA1c [22].

4.3. Findings from other studies

Comparison of the results of studies on the association between insulin therapy and cardiovascular risk is hindered by substantial differences in study designs and characteristics of the study populations, such as severity of diabetes.

Several recent observational studies have compared patients treated with insulin and patients receiving oral glucose-lowering drugs only, and have reported increased cardiovascular morbidity and mortality among patients treated with insulin [10–16]. However, insulin therapy is usually started only after failure of achieving acceptable glucose control with oral glucose-lowering drugs. Type 2 diabetic patients treated with insulin and non-insulin users therefore constitute two entirely different groups. Consequently, it is difficult to draw conclusions on the role of insulin in the increased CVD-risk observed among insulin-users based on comparison of these groups. Our study only included insulin-treated patients and compared CVD risk across different treatment doses. Although treatment with higher doses of insulin reflects increased insulin resistance, which is in itself associated with increased CVD risk, this group of patients is likely to be more homogeneous in terms of confounding CVD risk factors as compared to insulin users and non-insulin users [22]. The strength of the association persisted after adjusting for several important cardiometabolic variables associated with CVD risk, lending further support to the concept that insulin exposure could be associated with increased CVD risk. However, residual confounding can of course never be excluded.

A number of randomized controlled trials (RCTs) failed to demonstrate the anticipated incremental benefit in terms of cardiovascular outcomes of more intensive glycemic control [3–6]. These trials were not designed to investigate the effects of insulin therapy. However, intensive glycemic control often involved insulin therapy, and it could therefore be speculated that insulin may be implicated in the lack of efficacy in improving CVD outcomes. The ACCORD trial reported excess mortality in intensively treated patients. Post-hoc analyses showed that hypoglycemia and the degree of HbA1c reduction could not account for the increased mortality rates. Among patients with persistently elevated HbA1c levels, death rates were higher in those receiving intensive therapy than in those receiving standard therapy despite similar HbA1c levels [7]. Numerous factors may be implicated, but it could be hypothesized that the higher doses of insulin that were administered in an attempt to achieve low HbA1c targets in the intensive group were involved. In support, a number of other studies reported a stronger positive association between HbA1c levels and CVD risk among patients receiving insulin as compared to patients receiving oral glucose-lowering drugs only [23,24]. Although a reduced risk of major cardiovascular events was reported in a meta-analysis of RCTs, it should be noted that the individual trials were heterogeneous in terms of primary outcomes, treatment regimens and patients populations [25]. Therefore, its results should be interpreted with caution. In contrast to these trials, which aimed to investigate the benefit of more stringent glycemic targets, the ORIGIN-trial investigated specifically the effect of insulin therapy. A total of 12,537 patients with impaired fasting glucose, impaired glucose tolerance or type 2 diabetes were randomized to relatively low doses of insulin or standard care [6]. Despite achieving better glycemic control in terms of HbA1c, insulin therapy had no effect on cardiovascular outcomes after a median follow-up of 6.2 years. ORIGIN included patients early in the course of diabetes. Consequently, those patients were treated with relatively low doses of insulin (mean 26 units/day at 1 year of follow-up), which would fall into the low or intermediate tertiles in our present analysis. Our results suggest a dose-dependent association between insulin exposure and CVD risk, with the highest CVD risk in patients treated with $\geq$53 units/day. It therefore remains open to speculation whether an increased cardiovascular risk could have been observed in ORIGIN if patients with more advanced diabetes, requiring higher dosages of insulin, would have been included.

4.4. Therapeutic considerations

Although it is still too early to draw conclusions on a potential relationship between insulin therapy and CVD risk, such an association would not necessarily mean that insulin therapy should be postponed as long as possible in patients with T2DM. In fact, a number of studies have indicated that insulin administration early in the course of diabetes may improve residual β-cell function and delay diabetes progression among patients with pre-diabetes [26]. In addition, among participants of the ORIGIN trial with impaired fasting glucose or impaired glucose tolerance, early use of basal insulin reduced the incidence of overt diabetes despite a modest increase in weight gain [6]. This could indicate that initiating insulin therapy early in the disease, when lower doses are required to achieve acceptable glycemic control, may delay the necessity of treating with higher insulin doses later on. The results of our study indicate that *high* doses of insulin may adversely affect CVD risk. In addition, it is unclear whether continued uptitration of the insulin dose if glycemic targets are not met, is truly beneficial.

Several studies have shown that co-treatment with insulin and glucose sensitizing drugs, such as metformin, effectively reduces the required dose of insulin [27,28]. Compared to insulin monotherapy, this combination has been shown to reduce the incidence of CVEs in some, but not in other trials [27,29]. This discrepancy could possibly be explained by differences in study designs and patient characteristics, such as severity of disease. In our study, the proportion of patients who continued oral glucose-lowering drugs was higher among those receiving lower doses of insulin, who were also characterized by a lower CVE risk.

4.5. Strengths and weaknesses

To our knowledge, this is the first study to directly quantify the relationship between mean daily insulin dose and CVD risk among new users of insulin. The relatively large sample size enabled us to include data on a substantial number of CVD events. Although previous studies compared insulin-treated patients to patients on oral glucose-lowering drugs only, we limited the study population to insulin-treated patients, who are more likely to represent a homogeneous group. Moreover, we were able to take into account several important potential confounders, such as glycemic control and duration of antidiabetic therapy.

The major limitation of the current study is the fact that the PHARMO database contains data obtained from routine practice, which means that some potential confounders were not assessed such as BMI, smoking status and hypertension. As a consequence, the found differences in CVE risk for the different levels of insulin

exposure could possibly be explained by residual and unmeasured confounding.

While current guidelines recommend metformin as the preferred initial oral glucose-lowering drug, a substantial number of patients in our study were treated with SU-derivatives [30]. However, the proportion of patients treated with metformin versus SU-derivatives was similar across insulin exposure tertiles and therefore we do not expect that this has influenced our results.

Although the strength of the observed association and its persistence after adjusting for several important confounders suggest a possible relationship between insulin exposure and CVD risk, the observational nature of the study precludes conclusions about causality. Further studies are needed to confirm our results.

5. Conclusions

In conclusion, our results indicate that the positive relationship between mean daily insulin dose and CVD risk persists after adjusting for several important variables associated with differences in disease severity. This is in line with the hypothesis that treatment with high doses of insulin may adversely affect CVD risk. Our findings, however, need to be interpreted with caution due to the observational nature of the study. Further studies are required to determine a potential role of particularly high-dose insulin in CVD progression, and additional RCTs with sufficiently long follow-up are required to establish to which extent insulin dose should be uptitrated.

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Appendix A

ICD-9 codes used to identify events during follow-up: ICD 410, 4139, 4140, 4349, 4358, 4359, 4402.

ICD-9 codes used to define patients with a cardiovascular history at baseline: ICD 410, 4110, 4111, 4118, 412, 4139, 4140, 4148, 4149, 4331, 4349, 4358, 4359, 4370, 4371, 440.

ATC-codes used to identify new users of oral glucose-lowering drugs: ATC A10B.

ATC-codes used to identify new users of insulin: ATC A10A.

ATC-codes used to identify patients using antihypertensive drugs: ATC C02AN, C02DA, C02LB, C02LE, C02LX, C03AA, C03AB, C03BA, C03BB, C03CA, C03CB, C03DA, C03DB, C03EA, C03EB, C07FA, C07FB, C08CA, C08GA, C09AA, C09BA, C09BB, C09CA, C09DA, C09DB, C09DX, C09XA.

ATC-codes used to identify patients using lipid-lowering drugs: ATC C10A C10B.

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