Association of insulin dosage with mortality or major adverse cardiovascular events: a retrospective cohort study

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Summary

Background Existing studies have shown conflicting evidence regarding the safety of exogenous insulin therapy in patients with type 2 diabetes. In particular, observational studies have reported an increased risk of death and cardiovascular disease among users of higher versus lower doses of insulin. We aimed to quantify the association between increasing dosage of insulin exposure and death and cardiovascular events, while taking into account time-dependent confounding and mediation that might have biased previous studies.

Methods We did a cohort study using primary care records from the UK-based Clinical Practice Research Datalink (CPRD). New users of metformin monotherapy were identified in the period between Jan 1, 2001, and Dec 31, 2012. We then identified those in this group with a new prescription for insulin. Insulin exposure was categorised into groups according to the mean dose (units) per day within 180-day time segments throughout each patient's follow-up. Relative differences in mortality and major adverse cardiovascular events (non-fatal myocardial infarction, non-fatal stroke, cardiovascular-related mortality) were assessed using conventional multivariable Cox proportional hazards models. Marginal structural models were then applied to reduce bias introduced by the time-dependent confounders affected by previous treatment.

Findings We identified 165 308 adults with type 2 diabetes in the CPRD database. After applying our exclusion criteria, 6072 (mean age 60 years [SD 12·5], 3281 [54%] men, mean HbA_{1c} 8·5% [SD 1·75], and median follow-up 3·1 years [IQR 1·7–5·3) were new add-on insulin users and were included in the study cohort; 3599 were new add-on insulin users and were included in the subcohort linked to hospital records and death certificate information. Crude mortality rates were comparable between insulin dose groups; <25 units per day (46 per 1000 person-years), 25 to <50 units per day (39 per 1000 person-years), 50 to <75 units per day (27 per 1000 person-years), 75 to <100 units per day (34 per 1000 person-years), and at least 100 units per day (32 per 1000 person-years; p>0·05 for all; mean rate of 31 deaths per 1000 person-years [95% CI 29–33]). With adjustment for baseline covariates, mortality rates were higher for increasing insulin doses: less than 25 units per day [reference group]; 25 to <50 units per day, hazard ratio (HR) 1·41 [95% CI 1·12–1·78]; 50 to <75 units per day, 1·37 [1·04–1·80]; 75 to <100 units per day, 1·85 [1·35–2·53]; and at least 100 units per day, 2·16 [1·58–2·93]. After applying marginal structural models, insulin dose was not associated with mortality in any group (p>0·1 for all).

Interpretation In conventional multivariable regression analysis, higher insulin doses are associated with increased mortality after adjustment for baseline covariates. However, this effect seems to be confounded by time-dependent factors such as insulin exposure, glycaemic control, bodyweight gain, and the occurrence of cardiovascular and hypoglycaemic events. This study provides reassurance of the overall safety of insulin use in the treatment of type 2 diabetes and contributes to our understanding of the contrasting conclusions from non-randomised and randomised studies regarding dose-dependent effects of insulin on cardiovascular events and mortality.

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Introduction

Several observational studies have suggested that insulin, in a graded (linear) and dose-dependent fashion, can be harmful in the management of patients with type 2 diabetes.[1–4] Purported harms include dose-dependently increased risks of all-cause mortality and cardiovascular events.[5] Furthermore, the biological effects of insulin seem to be both proatherosclerotic and antiatherosclerotic in which the net balance is unknown but might be dependent on factors such as the level of insulin resistance, type of insulin formulation, the degree and frequency of hypoglycaemia, the magnitude of bodyweight gain, and the dose of insulin.[6] Despite these concerns, findings from randomised clinical trials such as the UKPDS and ORIGIN studies suggest that insulin does not increase overall mortality and is not cardiotoxic.[7,8]

Explanations for the discordant findings between observational studies and randomised trials in relation to the dose-dependent effects of insulin treatment are probably due to limitations in their respective methods. The randomised trials were not designed to answer hypotheses about the dose-dependent effects of insulin but rather to compare the efficacy of treatment strategies that involved a combination of antidiabetic drugs. The

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Research in context

Evidence before this study

A systematic review published in 2015 by Price and colleagues provides a comprehensive summary of the literature on the dose-related association between insulin therapy and all-cause mortality and cardiovascular morbidity in patients with type 2 diabetes as of Feb 18, 2014. We identified more recent studies using citation trackers of relevant articles from the review and conducted an updated PubMed search for articles published between Jan 1, 2014, and Sept 29, 2016, with the following search strategy: 'insulin[MesH]' AND 'diabetes mellitus, type 2[MesH]' AND 'dose OR dosage' AND 'humans[filter]' NOT 'animal[filter]'. Previous observational studies have noted that higher levels of insulin exposure are associated with an increased risk of all-cause mortality and cardiovascular morbidity and mortality. These studies used large databases such as the UK-based Clinical Practice Research Datalink (CPRD) and the Saskatchewan Administrative Healthcare Databases, and used standard multiple regression analysis with and without time-dependent variables to control for confounding. However, because these studies are probably prone to time-dependent confounding affected by previous insulin treatment, standard regression analysis is biased. Findings from previous observational studies are not corroborated with evidence from randomised clinical trials.

Added value of this study

We show that the association between higher doses of insulin and mortality is reduced and no longer significant after adjustment for time-dependent confounders affected by previous insulin exposure. Our study emphasises how sensitive to the handling of confounding factors is the magnitude and precision of the association between insulin dosage and mortality. Moreover, we did not observe an increase in major adverse cardiovascular events in those patients exposed to higher doses of insulin.

Implications of all the available evidence

This study partly reconciles opposing findings between previous observational studies and randomised clinical trials. Importantly, our study provides reassurance to prescribers and patients regarding the safety of insulin therapy in patients with type 2 diabetes. Nonetheless, more evidence is required to further understand mediating variables between insulin therapy and diabetes outcomes, as well as the relative effects of basal and prandial insulin exposure. Similar methods to ours will need to be used in larger and more detailed datasets that allow examination of basal versus prandial insulin on the one hand and standard versus novel insulins on the other.

observational studies, on the other hand, were designed to investigate the dose-dependent effects of insulin but are prone to several forms of bias. The largest threat to validity of these observational studies is confounding by disease severity, a type of channelling bias. Channelling occurs when patients differ in prognostic factors that can affect prescribing decisions. For example, prescribing decisions for insulin initiation and dosing adjustments are likely to be affected by the level of glycaemic control, frequency of hypoglycaemic episodes, frailty, and presence of microvascular and macrovascular complications. These factors are related to many adverse outcomes and can act as confounders at the start of therapy and over time (ie, time-dependent confounders).

To date, a small number of observational drug effect studies have assessed the relative effect of varying insulin dosages on safety outcomes.[9–11] These studies have generally adjusted for numerous potential confounders, measured before and after insulin initiation, using standard multiple regression analyses. However, in the situation of time-dependent confounding, when the confounder is affected by the exposure itself, standard multiple regression methods are biased;[12] marginal structural modelling is a method to handle this scenario.[13] HbA_{1c} is an example of a variable that is both a confounder and mediator. HbA_{1c} is used both to determine the dosage of insulin but is also directly affected by insulin, and HbA_{1c} is associated with survival.

To further our understanding of the relative safety of exogenous insulin exposure in type 2 diabetes, we did a retrospective, observational cohort study using both standard multiple regression models and marginal structural models to account for confounding. Our study focuses on quantifying the association between insulin dosage and mortality while taking account of the potential for time-dependent confounders that are also mediators (eg, HbA_{1c}, hypoglycaemic episodes, and bodyweight gain) that may be responsible for the harmful associations, as observed among previous observational studies.

Methods

Study design and setting

We did a cohort study using health-care records of patients attending primary care clinics participating in the UK-based Clinical Practice Research Datalink (CPRD). The CPRD database contains individual, de-identified, longitudinal data from more than 650 primary care practices in the UK and is representative of the UK population.[14] Data are available for patient socio-demographics (eg, index of multiple deprivation), health behaviours (eg, smoking status), physiological and laboratory data (eg, HbA_{1c}), diagnostic information coded using the READ classification system, and outpatient prescription records. A subset of the population (58% of our source population) is linked to hospital records (Hospital Episode Statistics [HES]) and death certificate (Office of National Statistics [ONS] mortality data)

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information. Our study received approval from the Health Research Ethics Board at Memorial University, and the study protocol was approved by the CPRD Independent Scientific Advisory Committee (ISAC 13_100R, August, 2013).

The source population for our study consisted of new users of insulin in patients aged 30 years and older who started metformin monotherapy between Jan 1, 2001, and Dec 31, 2012 (figure 1). A 365-day period was used as a washout period, whereby patients with a record of taking any antidiabetic drug within 365 days before their first metformin record were excluded. All patients were registered at an up-to-standard practice (continuous high quality data acceptable for use in research) for at least 365 days before cohort entry. We further excluded individuals with gestational diabetes or polycystic ovarian syndrome, or who were pregnant during study follow-up. We selected patients who added on insulin after starting metformin monotherapy. We excluded insulin users with greater than a 180-day gap between their first and second insulin prescriptions, patients with less than three insulin prescriptions, and patients with less than 180 days of insulin exposure. These exclusions were done to create a patient-cohort of insulin users who had continuous use for greater than 6 months. We also excluded patients with incalculable (no quantity or concentration available) and implausible insulin doses (>200 units per day on average during entire follow-up period).

Outcome

The primary outcomes of interest were all-cause mortality and major adverse cardiovascular events. Major adverse cardiovascular events were defined as the first occurrence of non-fatal myocardial infarction, non-fatal stroke, or any cardiovascular-related mortality. Secondary outcomes included individual components of the major adverse cardiovascular events composite and other individual cardiovascular events including heart failure, arrhythmias, and urgent revascularisation. We also expanded the definition of major adverse cardiovascular events to include all-cause mortality (ie, non-fatal myocardial infarction, non-fatal stroke, or all-cause mortality). In view that cardiovascular-related admissions to hospital and cardiovascular-related mortality outcomes were only available in the subcohort linked with HES/ONS, we used this linked subcohort to assess cardiovascular-specific outcomes. Outcome definitions were based on previously validated READ codes (CPRD data) or International Classification of Disease Version 10 codes (linked HES/ONS data).[15,16]

Procedures

Measurement of the intensity of insulin treatment (hereafter, referred to as insulin dose) was the primary exposure of interest. Using the total number of prescribed units and number of days between each prescription we first calculated a mean daily insulin dose for each individual between their prescriptions. We then obtained a mean daily insulin dose in each 180-day segment of individual's follow-up time. If the period of time between two prescriptions exceeded 180 days, the insulin from the first prescription was assumed to have run out after 180 days and from that point until the next prescription was considered as non-exposed. The insulin exposure was then lagged by 180 days. Given that our measure of insulin exposure is an estimate of an individual's daily dose, we categorised insulin exposure using interpretable cut-points for clinicians.

On the day a patient initiated insulin, they contributed time at risk based on their mean insulin dose in the preceding 180-day period (eg, 180-day lag period) to one of five insulin dosage categories for all-cause mortality (<25 units per day [reference group], 25 to <50 units per

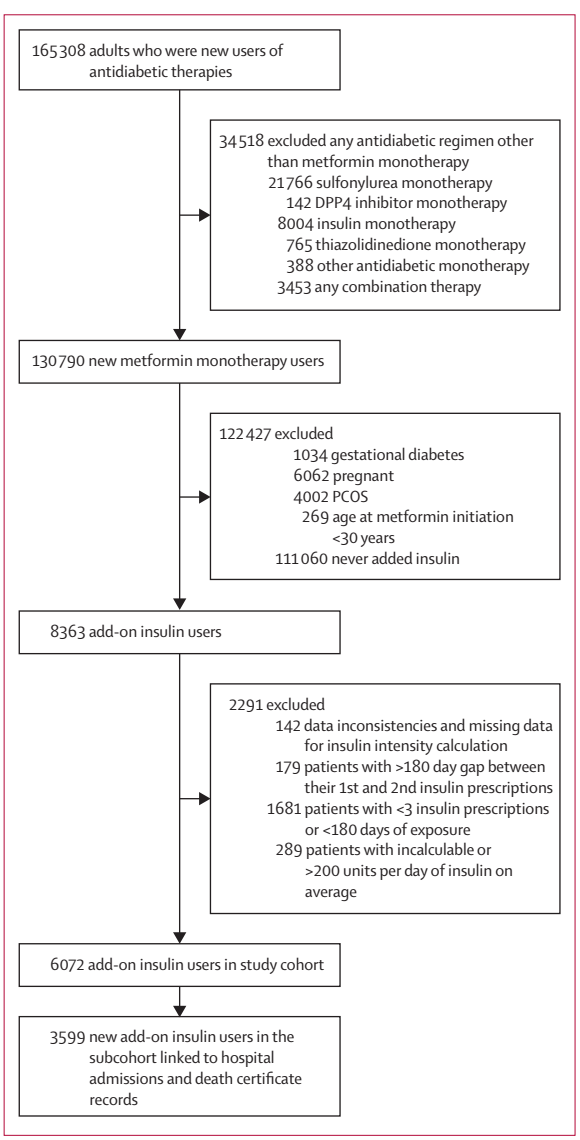

Figure 1: **Trial profile**
DPP4=dipeptidyl peptidase-4 inhibitor. PCOS=polycystic ovarian syndrome.

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	<25 units per day (n=774)	25–<50 units per day (n=2277)	50–<75 units per day (n=1491)	75–<100 units per day (n=833)	100 units per day or more (n=697)
Age (years) at insulin initiation	63·5 (13·2)	61·1 (12·8)	59·2 (12·3)	58·2 (11·5)	57·0 (10·9)
Sex					
Men	404 (52%)	1168 (51%)	804 (54%)	488 (59%)	417 (60%)
Women	370 (48%)	1109 (49%)	687 (46%)	345 (41%)	280 (40%)
Measure of deprivation*					
Least deprived	73 (9%)	276 (12%)	159 (11%)	82 (10%)	64 (9%)
Most deprived	84 (11%)	276 (12%)	189 (13%)	107 (13%)	88 (13%)
Years of anti-diabetic treatment	4·1 (2·9)	3·7 (2·8)	3·7 (2·7)	3·7 (2·6)	3·5 (2·4)
Days of metformin overlap	1014 (736)	1217 (856)	1338 (899)	1474 (924)	1585 (967)
Smoking status					
Current	145 (19%)	423 (19%)	287 (20%)	146 (18%)	109 (16%)
Former	204 (26%)	742 (33%)	485 (33%)	294 (35%)	285 (41%)
None	294 (38%)	757 (33%)	470 (32%)	255 (31%)	219 (31%)
Unknown	131 (17%)	355 (16%)	249 (17%)	138 (17%)	84 (12%)
BMI (kg/m^2)	30·6 (6·6)	30·8 (6·5)	32·5 (7·1)	33·5 (6·4)	35·8 (7·3)
Physician visits per year					
1–12	400 (52%)	1206 (53%)	828 (56%)	459 (55%)	348 (50%)
13–24	246 (32%)	724 (32%)	448 (30%)	237 (29%)	222 (32%)
25 or more	128 (17%)	347 (15%)	215 (14%)	137 (17%)	127 (18%)
History of vascular disease	234 (30%)	568 (25%)	336 (23%)	214 (26%)	194 (28%)
Charlson index					
1	643 (83%)	1992 (88%)	1314 (88%)	727 (87%)	599 (86%)
2	96 (12%)	221 (10%)	132 (9%)	83 (10%)	71 (10%)
3+	35 (5%)	64 (3%)	45 (3%)	23 (3%)	27 (4%)
HbA_{1c}					
<7·0%	106 (14%)	256 (11%)	160 (11%)	70 (8%)	83 (12%)
7·0–<8·0%	187 (24%)	475 (21%)	308 (21%)	167 (20%)	132 (19%)
8·0–<9·0%	117 (15%)	382 (17%)	281 (19%)	149 (18%)	124 (18%)
9·0–<10·0%	102 (13%)	293 (13%)	180 (12%)	123 (15%)	103 (15%)
≥10%	176 (23%)	609 (27%)	396 (27%)	215 (26%)	185 (27%)
Unknown	86 (11%)	262 (12%)	166 (11%)	109 (13%)	70 (10%)
Systolic blood pressure (mm Hg)	132·7 (17%)	133·2 (17%)	133·2 (16%)	133·5 (16%)	133·4 (16%)
eGFR (mL/min per 1·73m^2)	75·7 (27%)	78·8 (40%)	78·1 (24%)	79·8 (24%)	79·3 (27%)
Non-antidiabetic therapies					
Statins	557 (72%)	1701 (75%)	1145 (77%)	645 (77%)	550 (79%)
Calcium channel blockers	238 (31%)	619 (27%)	391 (26%)	223 (27%)	192 (28%)
β blockers	224 (29%)	573 (25%)	416 (28%)	247 (30%)	228 (33%)
Anticoagulants	58 (8%)	144 (6%)	100 (7%)	70 (8%)	61 (9%)
Antiplatelets	388 (50%)	1014 (45%)	658 (44%)	375 (45%)	317 (46%)
ACEi/ARB/renin	473 (61%)	1249 (55%)	868 (58%)	529 (64%)	479 (69%)
Diuretics	273 (35%)	754 (33%)	525 (35%)	328 (39%)	297 (43%)
Antidiabetic therapies used before insulin					
Sulfonylurea	544 (70%)	1620 (71%)	1106 (75%)	647 (78%)	547 (79%)
DPP4i	125 (16%)	396 (17%)	233 (16%)	121 (15%)	105 (15%)
GLP1RA	70 (9%)	189 (8%)	176 (12%)	105 (13%)	89 (13%)
Thiazolidinedione	170 (22%)	566 (25%)	382 (26%)	250 (30%)	219 (31%)
Other	8 (1%)	58 (3%)	49 (3%)	28 (3%)	38 (6%)

Data are mean (SD) or n (%).eGFR=estimated glomerular filtration rate. ACEi=angiotensin converting enzyme inhibitor. ARB=angiotensin receptor blocker. DPP4i=dipeptidyl peptidase-4 inhibitor. GLP1RA=glucagon-like peptide-1 receptor agonist. *Measure of deprivation is a neighbourhood-level index of material deprivation across numerous domains including housing, employment, income, access to services, education and skills, crime, and living environment.

Table 1: **Patient characteristics across insulin exposure categories of interest based on average insulin dose**

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day, 50 to <75 units per day, 75 to <100 units per day, ≥100 units per day) or one of three categories for cardiovascular events (<50 units per day [reference group], 50 to <100 units per day, ≥100 units per day). Patients stopped contributing time at risk to the insulin categories of interest at the study end date (October, 2013), date of emigration from a CPRD practice, death date, event date (in the case of cardiovascular outcome analysis), or the last date of insulin exposure, whichever was earliest. The specific insulin categories varied for all-cause mortality and cardiovascular events due to the latter having a smaller sample size. A detailed rationale for our approach to calculating insulin dosage is provided in the appendix (p 1).

Statistical analysis

We used descriptive statistics to characterise the baseline characteristics of the study cohort at the time of insulin initiation. Distribution for several a priori-defined covariates that were specified as potential confounders are presented using percentages for categorical variable and mean (SD) for continuous variable by the five insulin dose exposure categories. The independent association between insulin dose and the outcomes of interest was quantified using multivariable Cox proportional hazards models. Our main statistical model contained only baseline covariates that were measured in the year before insulin initiation. We used the abbreviated Modification of Diet in Renal Disease equation to estimate renal function.[17] Missing values for smoking status, deprivation index, HbA_{1c}, and eGFR were handled using missing indicators because of low rates of missingness. Several prespecified subgroup analyses were done to test for treatment effect modification by age, sex, history of myocardial infarction, history of heart failure, duration of diabetes treatment before insulin initiation, BMI, HbA_{1c}, and renal function; none achieved statistical significance and no interaction terms were included in final models.

Sensitivity analysis

To explore the effect of study design and potential mediating effects of specific variables we did a series of sensitivity analyses. First, we varied the operational definition of insulin exposure as the mean number of insulin units per bodyweight in kg. Second, we adjusted for patterns of oral antidiabetic use, changes in bodyweight, the occurrence of a hypoglycaemic event (admission to hospital or recorded at physician visit), and changes in HbA_{1c} during follow-up by rerunning our Cox proportional hazards model with these variables as time-dependent covariates in addition to baseline covariates. Third, we reran the main analysis using a cumulative mean dose (updated every 180 days) to measure insulin exposure. Fourth, we restricted the analysis to only the linked study cohort to measure the association between insulin dose and mortality. Fifth, we used marginal structural models to adjust for potential time-varying confounders affected by previous insulin treatment as an exploratory analysis to explore the effect of insulin dose on all-cause mortality. We used ordinal logistic regression to calculate stabilised inverse probability of treatment weights (weights were truncated at the 99.5th percentile and normalised across follow-up time) every 180 days for our insulin dose categories of interest (appendix p 2). All baseline covariates, previous insulin doses, and the following time-dependent covariates were used to calculate the treatment weights: HbA_{1c}, bodyweight, number of hypoglycaemic events, and number of cardiovascular events. A Cox proportional hazards model containing only baseline covariates was used to measure the association between insulin intensity and mortality. We did not use a marginal structural model approach for cardiovascular events given the lack of association in our primary analysis, limited sample size within exposure categories, and short follow-up time compared with the all-cause mortality analysis. Sixth, to increase the statistical power of our models to quantify the association between insulin dose and mortality, we reduced the number of covariates by choosing a subset of clinically relevant covariates and using a forward selection procedure by Akaike's Information Criterion. The standard graphical plots to test assumptions for all Cox and ordinal logistic models did not suggest any violations.

Data were analysed with R (version 3.3.0).

	Person-years of follow-up	Number of deaths	Crude incidence rate per 1000 person-years	Crude HR (95% CI)	Adjusted* HR (95% CI)
<25 units per day	2823	130	46	Reference	Reference
25 to <50 units per day	6317	245	39	0·85 (0·68–1·05)	1·41 (1·12–1·78)
50 to <75 units per day	4369	119	27	0·59 (0·46–0·76)	1·37 (1·04–1·80)
75 to <100 units per day	2369	80	34	0·73 (0·56–0·97)	1·85 (1·35–2·53)
≥100 units per day	2914	94	32	0·70 (0·53–0·91)	2·16 (1·58–2·93)

*Adjusted for baseline covariates including age at insulin initiation; sex; index of deprivation; smoking status; HbA_{1c}; chronic kidney disease stage; BMI; systolic blood pressure; number of physician visits in the year before insulin initiation; Charlson comorbidity index; previous cardiovascular disease; duration of antidiabetic treatment; duration of metformin overlap; and use of statins, non-steroidal anti-inflammatories, calcium channel blockers, beta-blockers, anticoagulants, antiplatelets, and diuretics, agents that act on the renin-angiotensin system in the year before insulin initiation, and antidiabetic drug therapies before insulin initiation.

Table 2: **Person-years of follow-up, number of deaths, and hazard ratios for all-cause mortality by levels of insulin dosage using conventional analyses**

See Online for appendix

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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	Crude incidence rate per 1000 person-years	Crude HR (95% CI)	Adjusted HR (95% CI) for baseline covariates only	Adjusted HR (95% CI) for baseline and time-varying covariates	Adjusted HR (95% CI) from marginal structural model
Insulin dose measured as average units per kg every 180 days					
<0·28 units/kg/day	40	Reference	Reference	Reference	Reference
0·28–<0·54 units/kg/day	34	0·86 (0·68–1·08)	1·16 (0·91–1·48)	0·97 (0·75–1·24)	1·28 (0·92–1·78)
0·54–<0·79 units/kg/day	32	0·81 (0·63–1·04)	1·64 (1·26–2·14)	1·31 (0·99–1·72)	1·08 (0·77–1·52)
0·79–<1·0 units/kg/day	30	0·74 (0·55–1·01)	1·41 (1·02–1·95)	1·12 (0·80–1·55)	1·12 (0·70–1·80)
>1 units/kg/day	39	0·97 (0·74–1·26)	2·13 (1·60–2·84)	1·65 (1·23–2·21)	1·48 (0·99–2·21)
Main analysis rerun in HES/ONS linked only subcohort					
<25 units per day	48	Reference	Reference	Reference	Reference
25 to <50 units per day	41	0·86 (0·65–1·13)	1·60 (1·18–2·18)	1·54 (1·13–2·12)	1·12 (0·71–1·82)
50 to <75 units per day	31	0·65 (0·47–0·89)	1·61 (1·13–2·31)	1·59 (1·10–2·30)	0·88 (0·50–1·56)
75 to <100 units per day	41	0·86 (0·60–1·21)	2·40 (1·61–3·58)	2·12 (1·39–3·21)	1·42 (0·74–2·73)
≥100 units per day	32	0·83 (0·46–0·93)	2·30 (1·52–3·46)	1·95 (1·27–2·97)	1·60 (0·85–3·02)
Insulin dose measured as an updated cumulative average every 180 days					
<25 units per day	40	Reference	..	Reference	..
25 to <50 units per day	38	0·94 (0·75–1·19)	1·76 (1·36–2·26)	1·48 (1·14–1·93)	NA
50 to <75 units per day	31	0·77 (0·59–0·99)	1·92 (1·44–2·56)	1·66 (1·24–2·23)	NA
75 to <100 units per day	37	0·93 (0·70–1·25)	2·58 (1·87–3·56)	2·21 (1·58–3·09)	NA
≥100 units per day	32	0·81 (0·59–1·11)	2·42 (1·69–3·46)	2·00 (1·38–2·89)	NA

NA=not applicable due to previous treatment highly correlated with exposure definition. HES=hospital episode statistics. ONS=Office of National Statistics.

***Table 3*: Person-years of follow-up, number of deaths, and hazard ratios for all-cause mortality by insulin dose in sensitivity analyses**

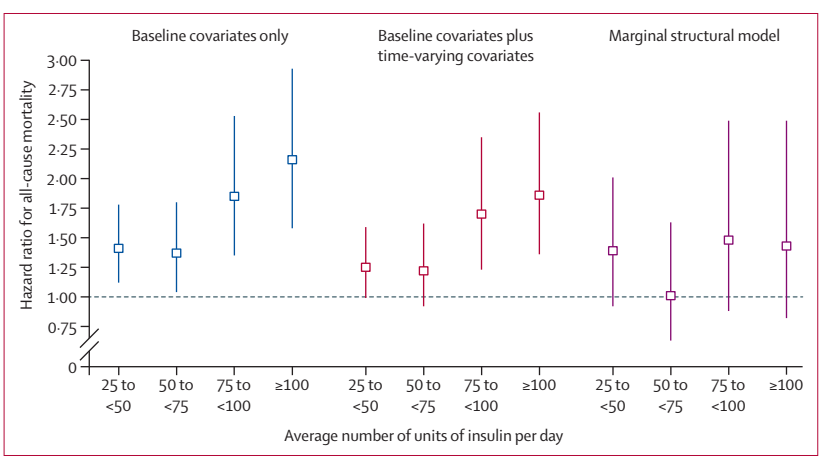

***Figure 2*: Hazard ratios for all-cause mortality across different statistical approaches**
Baseline covariates were age at insulin initiation, sex, index of deprivation, smoking status, HbA_{1c}, chronic kidney disease stage, BMI, systolic blood pressure, number of physician visits in the year before insulin initiation, Charlson comorbidity index, previous cardiovascular disease, duration of treated diabetes, duration of metformin overlap, use of statins, non-steroidal anti-inflammatories, calcium channel blockers, β blockers, anticoagulants, antiplatelets, diuretics, and agents that act on the renin-angiotensin system in the year before insulin initiation, and for antidiabetic drug therapies before insulin initiation. TVC=time-varying covariates.

Results

We identified 165 308 adults with type 2 diabetes; after applying our exclusion criteria, 6072 were new add-on insulin users and were included in the study cohort, and 3599 were new add-on insulin users and were included in the subcohort linked to hospital records and death certificate data (figure 1). Mean age of the study cohort was 60 years (SD 12·5) at the time of insulin initiation, mean HbA_{1c} was 8·5% (SD 1·75%), 3281 (54%) were men, and 394 (6%) had a previous cardiovascular event. Median follow-up time was 3·1 years (IQR 1·7–5·3) and maximum follow-up time was 12·6 years. The mean insulin dose over a patient's total follow-up time was 57·8 units per day (SD 33·5). Patients with a lower mean insulin dose per day were more likely to be older, be women, be smokers, have a lower BMI, have a longer duration of diabetes, and have pre-existing cardiovascular disease and other comorbidities (table 1).

691 deaths were reported (some deaths occurred in those who stopped insulin and no longer contributed follow-up time to an insulin exposure group for the Cox proportional hazards analysis). Crude mortality rate was 46 per 1000 person-years (95% CI 39–55) during periods of exposure to less than 25 units of insulin per day, 39 per 1000 person-years (34–44) for 25 to less than 50 units per day, 27 per 1000 person-years (23–33) for 50 to less than 75 units per day, 34 per 1000 person-years (27–42) for 75 to less than 100 units per day, and 32 per 1000 person-years (36–39) for periods of exposure to 100 or more units per day (table 2). The overall crude mortality rate was 31 deaths per 1000 person-years (95% CI 29–33). After adjustment for baseline covariates, higher insulin doses were significantly associated with increased mortality (p value for trend 0·006). Compared with those patients receiving less than 25 units per day, adjusted hazard ratios (aHR) were higher for those receiving 25 to less than 50 units per day (aHR 1·41 [95% CI 1·12–1·78]), 50 to less than 75 units per day (1·37 [1·04–1·80]), 75 to less than 100 units per day

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(1·85 [1·35–2·53], and more than 100 units per day (2·16 [1·58–2·93]; appendix p 3).

Consistent results were observed for most sensitivity analyses (table 3; appendix p 4). However, further time-dependent adjustments for changes in glycaemic control, bodyweight, frequency of hypoglycaemic events, and occurrence of cardiovascular events decreased the magnitude of the association between insulin dose and mortality; after adjustment, only the two highest insulin dose categories were associated with increased mortality compared to the lowest dose category: 25 to less than 50 units per day (aHR 1·25 [0·99–1·59]), and 50 to less than 75 units per day (1·23 [0·93–1·63]); 75 to less than 100 units per day (1·71 [1·24–2·37]), and more than 100 units per day (1·88 [1·37–2·58]). After applying marginal structural models, insulin dose was not associated with all-cause mortality (figure 2).

548 major adverse cardiovascular events occurred within the HES/ONS linked study cohort. Higher insulin doses were not associated with the composite major adverse cardiovascular event outcome. Compared with the lower dose (<50 units per day), aHRs were 1·17 (95% CI 0·94–1·44) for 50–100 units per day and 1·06 (0·78–1·44) for more than 100 units per day. Results for all secondary outcomes are shown in table 4. The only significant association observed was between insulin dose and cardiovascular-related death (aHR 1·68 [95% CI 1·17–2·42]

	Person-years of follow-up	Events (n)	Crude incidence rate per 1000 person-years	Crude HR (95% CI)		Adjusted* HR (95% CI)	
Major adverse cardiovascular events (non-fatal myocardial infarction, non-fatal stroke, or cardiovascular death)							
<50 units per day	5008	234	47	..	Reference	..	Reference
50 to <100 units per day	3551	164	46	1	(0·82–1·22)	1·17	(0·94–1·44)
≥100 units per day	1513	62	41	0·9	(0·68–1·19)	1·06	(0·78–1·44)
Major adverse cardiovascular events (non-fatal myocardial infarction, non-fatal stroke, or all-cause death)							
<50 units per day	5058	321	63	..	Reference	..	Reference
50 to <100 units per day	3598	216	60	0·95	(0·80–1·13)	1·13	(0·94–1·35)
≥100 units per day	1534	73	48	0·75	(0·58–0·97)	0·94	(0·71–1·25)
Cardiovascular-related death							
<50 units per day	5446	104	19	..	Reference	..	Reference
50 to <100 units per day	3918	62	16	0·85	(0·62–1·16)	1·68	(1·17–2·42)
≥100 units per day	1688	31	18	1	(0·67–1·51)	2·65	(1·65–4·25)
Myocardial infarction							
<50 units per day	5322	71	13	..	Reference	..	Reference
50 to <100 units per day	3818	37	10	0·72	(0·48–1·07)	0·86	(0·57–1·31)
≥100 units per day	1633	23	14	1·02	(0·63–1·66)	1·28	(0·76–2·17)
Stroke							
<50 units per day	5161	121	23	..	Reference	..	Reference
50 to <100 units per day	3684	86	23	0·98	(0·74–1·29)	1·09	(0·81–1·47)
≥100 units per day	1586	30	19	0·78	(0·52–1·17)	0·88	(0·57–1·36)
Heart failure							
<50 units per day	5182	122	24	..	Reference	..	Reference
50 to <100 units per day	3715	81	22	0·96	(0·72–1·27)	1·13	(0·85–1·52)
≥100 units per day	1585	43	27	1·24	(0·87–1·76)	1·36	(0·93–1·99)
Arrhythmia							
<50 units per day	5259	130	25	..	Reference	..	Reference
50 to <100 units per day	3804	77	20	0·76	(0·57–1·01)	0·86	(0·64–1·16)
≥100 units per day	1634	45	28	0·95	(0·68–1·35)	1·15	(0·79–1·66)
Revascularisation							
<50 units per day	3381	560	166	..	Reference	..	Reference
50 to <100 units per day	2384	376	158	1·01	(0·88–1·15)	1·05	(0·91–1·20)
≥100 units per day	895	153	171	1·17	(0·97–1·40)	1·15	(0·95–1·39)

HR=hazard ratio. *Adjusted for baseline covariates including age at insulin initiation, sex, index of deprivation, smoking status, HbA_{1c}, chronic kidney disease stage; BMI; systolic blood pressure; number of physician visits in the year before insulin initiation; Charlson comorbidity index; previous cardiovascular disease; duration of antidiabetic treatment; duration of metformin overlap; and use of statins, non-steroidal anti-inflammatories, calcium channel blockers, beta-blockers, anticoagulants, antiplatelets, and diuretics, agents that act on the renin-angiotensin system in the year before insulin initiation, and antidiabetic drug therapies before insulin initiation.

Table 4: **Major adverse cardiovascular events according to insulin dosage using conventional analyses**

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for 50–100 units per day and 2·65 [1·65–4·25] for more than 100 units per day; p>0·1 for all).

Discussion

Within a clinically relevant cohort of patients with type 2 diabetes who newly started insulin treatment, higher insulin doses were not associated with an increased risk of mortality or major adverse cardiovascular events after careful adjustment for potential confounders. For all-cause mortality, it was not until after we used a marginal structural model to account for potential time-dependent confounders within the putative causal pathways that an insulin dose-response relationship was no longer observed. These findings could partly explain, and help to reconcile, the opposing conclusions reached by previous observational studies and randomised trials with respect to the safety of insulin.

Our main analysis, whereby adjustment for baseline covariates was done using a multivariable Cox proportional hazards model, is consistent with the findings of other observational studies that found a dose-response relation between insulin and all-cause mortality and cardiovascular morbidity.[2–4] For example, using the administrative databases of Saskatchewan Health, we reported a graded relation (eg, a linear relation across exposure categories) between higher insulin doses and vascular and non-vascular mortality.[2] However, a primary limitation of that study was a lack of key clinical covariates (eg, HbA_{1c}, bodyweight, smoking status, and kidney function), which precluded our ability to adjust for important baseline and time-varying confounders. Similarly, Stoekenbrock and colleagues reported a two to three times increased risk for hospitalisation among 3853 new insulin users due to cardiovascular events in patients receiving higher versus lower doses of insulin.[4] They used a case–control design whereby 836 patients with a hospitalisation for a cardiovascular event were matched with two controls by age, sex, antidiabetic treatment duration, and type of antidiabetic treatment. However, they did not include fatal cardiovascular events in patients who did not present at a hospital and competing risk of death was a potential source of bias.

Another observational study comparable to this study also used the CPRD database and found a 1 unit per kg per day increase in insulin dose was associated with a 54% increased risk of all-cause mortality and 37% increased risk of a major adverse cardiovascular event.[3] In view that a study cohort of Holden and colleagues[3] consisted of insulin monotherapy users, the reported crude-event rates for all-cause mortality were substantially higher than in our study (61·3 *vs* 30·8 deaths per 1000 person-years). Insulin monotherapy is less common than insulin and oral combination therapy for managing hyperglycaemia in patients with type 2 diabetes; therefore, the generalisability of their findings might be limited.[18] Other studies have found an increased risk of cardiovascular events and mortality with insulin use (either monotherapy or in combination) versus oral antidiabetic drug use alone, but these studies did not assess insulin dose-response.[19,20] In such cases, residual confounding was hypothesised to be responsible for the harmful associations observed with insulin use.

To our knowledge, no previous observational studies have accounted for potential time-dependent confounders that were also intermediate variables in the putative causal pathway using marginal structural models. The primary strength of a marginal structural model approach is to adjust for confounders that both vary over time and that are affected by the exposure, because conventional Cox proportional hazards models will be biased in the presence of both time-dependent confounders and intermediate variables.[12,21]

Several randomised trials have also directly compared cardiovascular mortality and morbidity in patients using different insulin doses.[8,22–25] Our fully adjusted results are entirely consistent with evidence from these trials. For example, the University Group Diabetes Program (UGDP) trial found no differences in all-cause and cardiovascular mortality in patients randomly assigned to a fixed dose insulin group (10, 12, 14, or 16 units per day) versus a variable-dose insulin group (about half were prescribed >40 units per day and 13% >75 units per day by the end of the trial).[22] In the Hyperglycemia and Its Effects After Acute Myocardial Infarction on Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus (HEART2D) trial,[24] a prandial (mean insulin dose of 0·62 units/kg) versus basal insulin (mean insulin dose of 0·52 units/kg) regimen was compared, with no differences in cardiovascular outcomes reported. Furthermore, a recent post-hoc analysis[25] of the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial examined 10 136 patients and found no association between total, basal, or prandial insulin doses over time and cardiovascular mortality. Likewise, the Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial,[8] whereby one group was randomly assigned to insulin glargine titration and the other to standard care, found no difference in cardiovascular events after an average of 6 years of follow-up between study groups.

Despite its strengths, the first, and major, limitation of this study is the potential for residual confounding even though we adjusted for multiple socioeconomic (eg, age, sex, material deprivation), physiological (eg, bodyweight, kidney function, blood pressure, glycaemic control), clinical (eg, smoking, comorbidities), and treatment (eg, comedications) covariates. A second limitation is the potentially imprecise measurement of insulin dose used in this study. We used a similar approach in our previous work where we conceptualised insulin dose as an ordinal variable.[2] Others have used insulin exposure in units per kg per day and used a cumulative exposure framework;[3,4] however, use of cumulative exposure did not fit well into our analytical approach. A cumulative exposure definition takes into account previous insulin dosage implicitly as

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opposed to the marginal structural modelling, which takes into account previous insulin exposure using inverse-probability-of-treatment weighting. This potential limitation is common in most observational studies that used prescription or community pharmacy record data and is probably a limitation of even some randomised trials. We used the prescribed number of units and time between prescriptions to calculate a mean daily dose within 180-day periods, as others have done.[3] This probably overestimates the dose injected by patients, although exposure-validation studies are required to confirm the accuracy of insulin prescription records for measuring dose. A third limitation is the reduced sample size used to assess major adverse cardiovascular events and cardiovascular-related mortality compared with all-cause mortality, and our inability to do data-intensive marginal structural models in this subgroup. Fourth, information regarding insulin prescriptions may not have been fully captured as the CPRD data does not include prescriptions written by specialists or dispensation records from pharmacies. Fifth, we restricted our study population to insulin users who were first treated with metformin monotherapy in an effort to reflect current clinical practice guidelines; however, this might limit the generalisability of our findings to patients who have a contraindication to metformin therapy. Sixth, we excluded patients with missing or implausible insulin doses, as well as sporadic or very short (<180 days) uses of insulin, which may limit the generalisability of our findings for sporadic and short-term insulin users. Seventh, separation of basal and prandial insulin effects was not possible in our study. It is possible that differences in risk exist between basal and prandial insulin as two of the main insulin-related changes that may lead to cardiovascular and mortality risk—hypoglycemia and bodyweight gain—are mainly associated with use of prandial insulin. In addition, evidence from the UKPDS[7] and ORIGIN[8] trials suggest that there is no cardiovascular or mortality risk from basal insulin. Last, this study does not differentiate between types of insulin, such as between analogues and human insulin.

In view of the increasing number of novel insulin therapies being licensed on the market, and several novel formulations in the discovery pipeline, it will be crucial to continue to rigorously assess the risks and benefits of insulin therapies for the treatment of type 2 diabetes.[26] Overall, the result from this study reassures both patients and their physicians of the overall safety and absence of major cardiovascular harms of insulin use in the treatment of type 2 diabetes. The final result also contributes to understanding some of the potential reasons for the contrasting conclusions from non-randomised and randomised studies regarding dose-dependent effects of insulin on mortality and cardiovascular outcomes.

Contributors

JMG, EC, LKT, WKM, SWY, DM, and SRM were involved in the concept and design of the study. JMG was responsible for drafting the first version of the report. JMG and EC were responsible for data analysis. All authors contributed to the interpretation of data and provided revisions to the manuscript. JMG will act as guarantor for the study.

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Declaration of interests

SWY has received an unrestricted research grant from Sanofi-Aventis for work unrelated to diabetes. All other authors declare no competing interests.

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