Response to Comment on: Yang et al. (2010) Associations of Hyperglycemia and Insulin Usage With the Risk of Cancer in Type 2 Diabetes: The Hong Kong Diabetes Registry. Diabetes;59:1254–1260

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We thank Carstensen (1) for his comments on our article published in the May issue of *Diabetes* (2). These comments have given us a chance to revisit some of the methodological issues in evaluating cancer risk associated with insulin use in type 2 diabetes.

Cohort study designs. Carstensen suggests that analysis that considered all follow-up time in both the insulin-user group and the noninsulin-user group might produce more reliable results. In response to this, we estimated the hazard ratio (HR) of cancer with insulin use at or before enrollment or during follow-up in the entire cohort of 4,623 nonprevalent insulin users and 1,480 prevalent insulin users. Without adjusting for covariates, the cumulative incidences of cancer were similar in ever insulin users and nonusers (Fig. 1*A*). However, using the three adjustment schemes in the sensitivity analysis as reported in our article (2), the HRs were 0.68 (95% CI 0.51–0.91; $P = 0.0085$), 0.66 (0.49–0.88; $P = 0.0047$), and 0.65 (0.48–0.87; $P = 0.0034$), respectively. These results are consistent with our sensitivity analysis, which excluded prevalent insulin users, a point agreed on by Carstensen in his comment (1). In both analyses, either including or excluding prevalent insulin users, the follow-up time was calculated as the period of enrollment to the date of cancer, death, or censoring, whichever came first. In our sensitivity analysis, we did not exclude any follow-up time except for prevalent insulin users (Fig. 2).

Sources of bias in the sensitivity analysis. There are two sources of bias during the sensitivity analysis due to definitions. Firstly, in order to ensure that use of insulin after the first cancer event was not coded, initiation of insulin usage was defined from enrollment to the date of cancer, death, or censoring, whichever came first. However, because the majority of patients started insulin therapy sometime after enrollment, many of them had a noninsulin exposure time (NIET). In other words, during NIETs, subjects were not on insulin therapy but were, by definition of insulin usage (coded yes/no), assumed to be on insulin therapy. As such, if R was the risk of cancer, e.g., incidence of cancer per 1,000 person-years, and if patients had been on insulin therapy during their NIETs, R in the insulin group would be decreased to R × [(assumed total insulin-exposure time − NIET)/assumed total insulin-exposure time], where the assumed total insulin exposure time was equal to the total follow-up time. Secondly, by definition, insulin-treated patients should not have had cancer, death or been censored during NIET, which will lead to falsely low incidences of cancer in the insulin-user group during the early phase of follow-up and thus early separation of cumulative incidence curves between the insulin nonuser and insulin-user groups (Fig. 1*B*). If the cancer risk of the insulin-user group was similar during both NIETs and other follow-up times afterward, R will be increased to R × [total follow-up time/(total follow-up time − NIET)]. After considering these two sources of bias in our sensitivity analysis, the risk of cancer in the insulin-user group would be R × [(assumed total insulin exposure time − NIET)/assumed total insulin exposure time] × [total follow-up time/(total follow-up time − NIET)]. In other words, the overall effect of the two sources of bias was largely neutral. Therefore, adding the NIET (i.e., 2,380) to the total follow-up time (i.e., 17,423) in the noninsulin-user group is not correct. Theoretically, exclusion of the NIET from the follow-up time (i.e., 5,517) of the insulin-user group seems to be the best way to remove the two sources of bias. However, this requires availability of covariates, notably A1C and lipids, measured at a time just before insulin usage. If such data are not collected (as in our case), exclusion of the NIET from the follow-up time of the insulin-user group would lead to adjustment for variables measured long before initiation of insulin therapy (Fig. 2).

Sensitivity analysis method can reproduce the known effects of statins on cardiovascular disease. We can test the validity of our sensitivity analysis method by examining known "effects" of a certain drug. We chose use of statins as a risk predictor because their cardioprotective effects had been conclusively proven in mechanistic studies and randomized clinical trials. Using the Hong Kong Diabetes Registry (2), we selected a cohort of 4,599 patients who met the following criteria: *1*) type 2 diabetes, *2*) Chinese origin, *3*) without cardiovascular disease (CVD) at enrollment, *4*) no missing values in variables used in the analysis, and *5*) not using statins at or before enrollment. During a median of 5.09 (25th–75th percentile: 2.90–7.06) years of follow-up, 365 (7.9%) developed CVD. During the follow-up period, 1,079 were started on statins,

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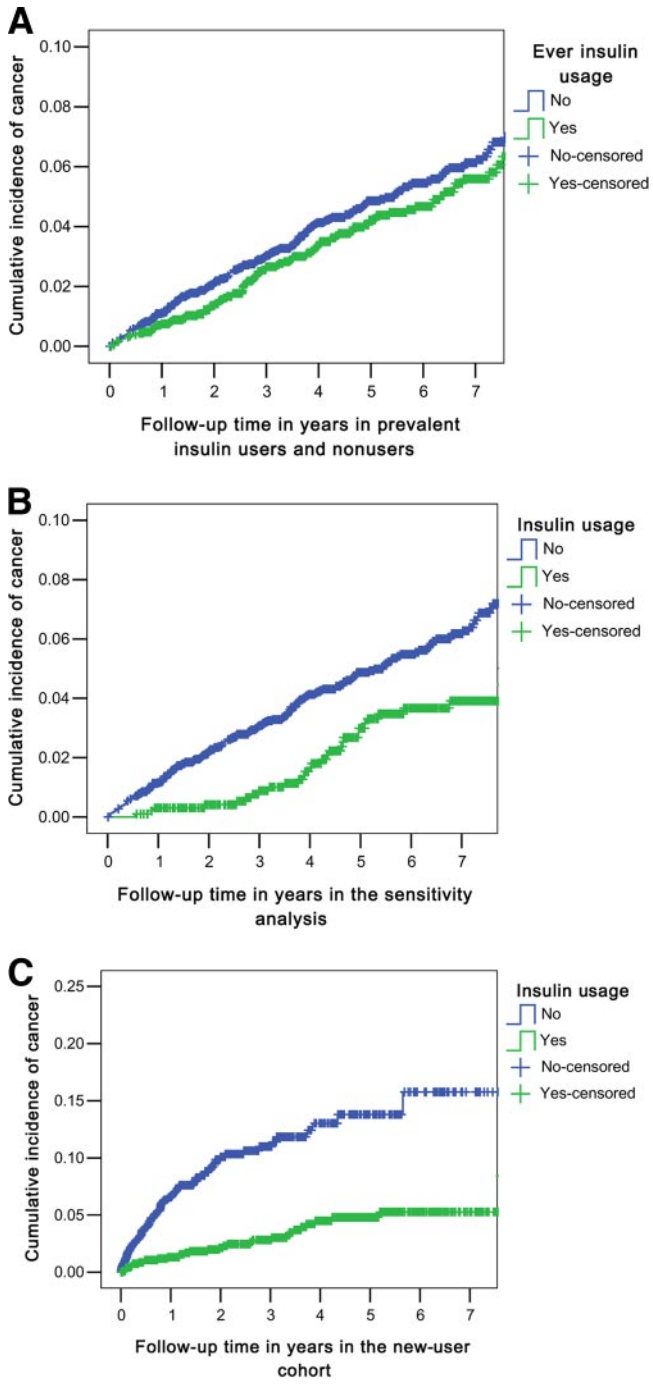

FIG. 1. Kaplan-Meier plots of cumulative incidence of cancer in insulin users and noninsulin users in type 2 diabetes. *A*: Cumulative incidence of cancer in prevalent insulin users and nonusers (*P* from log-rank test = 0.3553). *B*: Cumulative incidence of cancer in new insulin users and nonusers in the sensitivity analysis (*P* from log-rank test = 0.0075). *C*: Cumulative incidence of cancer in the new insulin-user cohort with control subjects matched for age, sex, smoking habits, and likelihood of insulin use (*P* from log-rank test <0.0001).

of whom 81 (7.5%) developed CVD. In the nonstatin users (n = 3,520), 284 (8.1%) developed CVD (P = 0.5507). After adjusting for covariates, use of statins from enrollment to the date of CVD, death, or censoring, whichever came first, was associated with reduced risk of CVD (HR 0.59 [95% CI 0.45–0.78]) (Table 1).

Time-dependent use of a drug may lead to wrong conclusions. With statin use as an example, we asked the question whether time-dependent start of statin usage in Cox regression analysis would generate better results than a simple coding of yes/no in the sensitivity analysis. After adjusting for the same group of covariates, time-dependent use of statins was associated with increased risk of CVD (HR 1.41 [95% CI 1.06–1.88]) (Table 1). It is certain that time-dependent use of statins has introduced major bias, probably due to the confounding effect of high LDL cholesterol prior to the commencement of statin therapy (which was not known and not adjusted). Similar bias might occur in the case of insulin because hyperglycemia is often associated with insulin use, and if hyperglycemia is a risk factor for cancer, this may lead to erroneous conclusions.

Dose-effect relationships in testing insulin-cancer associations. Johnson and Gale (3) suggested that "a stronger approach would [be to] employ time-varying exposure definitions and even a dose-response gradient, especially in the treatment of type 2 diabetes . . . ." Although they did not specifically define the "dose-response gradient" in their commentary, the latter was used by their study to address the associations between glucose-lowering agents and cancer mortality (4). In their study, the authors categorized insulin usage by the average cumulative insulin exposure per year to explore the effect size of insulin doses on cancer mortality. Although they reported a gradient relationship between insulin "dosage" and cancer mortality, it must be pointed out that important risk parameters, notably hyperglycemia and dyslipidemia, were not available and thus not adjusted. Without taking into consideration these important confounders, the so-called dose-response association between insulin use and cancer mortality may merely reflect an association between cancer and hyperglycemia.

New-user design. Ray (5) had pointed out that the key idea underlying new-user design is "to synchronize the beginning of study follow-up with starting the drug." When we organized the new-user cohort, we adopted two approaches to meet this suggestion. In addition to age, smoking status, and likelihood of use of insulin, we used one more matching criterion in organizing the new-user group: follow-up time in the noninsulin users must have been longer than the NIET of the insulin user in each matched pair. In other words, insulin nonusers did not have cancer, did not die, and were not censored during the NIET of the matched insulin user. The two sources of bias in the insulin-user group in the new-user cohort remained. However, the cancer risk of the noninsulin-user group has changed greatly because the matching criterion that follow-up time in the noninsulin-user group must be longer than the NIET of the insulin user in the matched pair led to exclusion of those who had developed cancer during NIETs. In other words, exclusion of noninsulin users with follow-up time shorter than the NIET of their counterpart insulin users had introduced the second source of bias. Assuming that cancer incidence in the noninsulin-user group was the same during NIET as that during other follow-up times afterward, the cancer risk of the noninsulin-user group in the new-user cohort would be R × [(total follow-up time − NIET)/total follow-up time], where R is the cancer risk of the noninsulin-user group in the cohort study design (as used in the sensitivity analysis). The additional matching criterion was more likely to decrease the cancer risk of the noninsulin-user group and led to a numerically larger HR of insulin usage versus nonusage for cancer (which is in favor of a conclusion that use of insulin is not associated with reduced cancer risk). Thus, use of

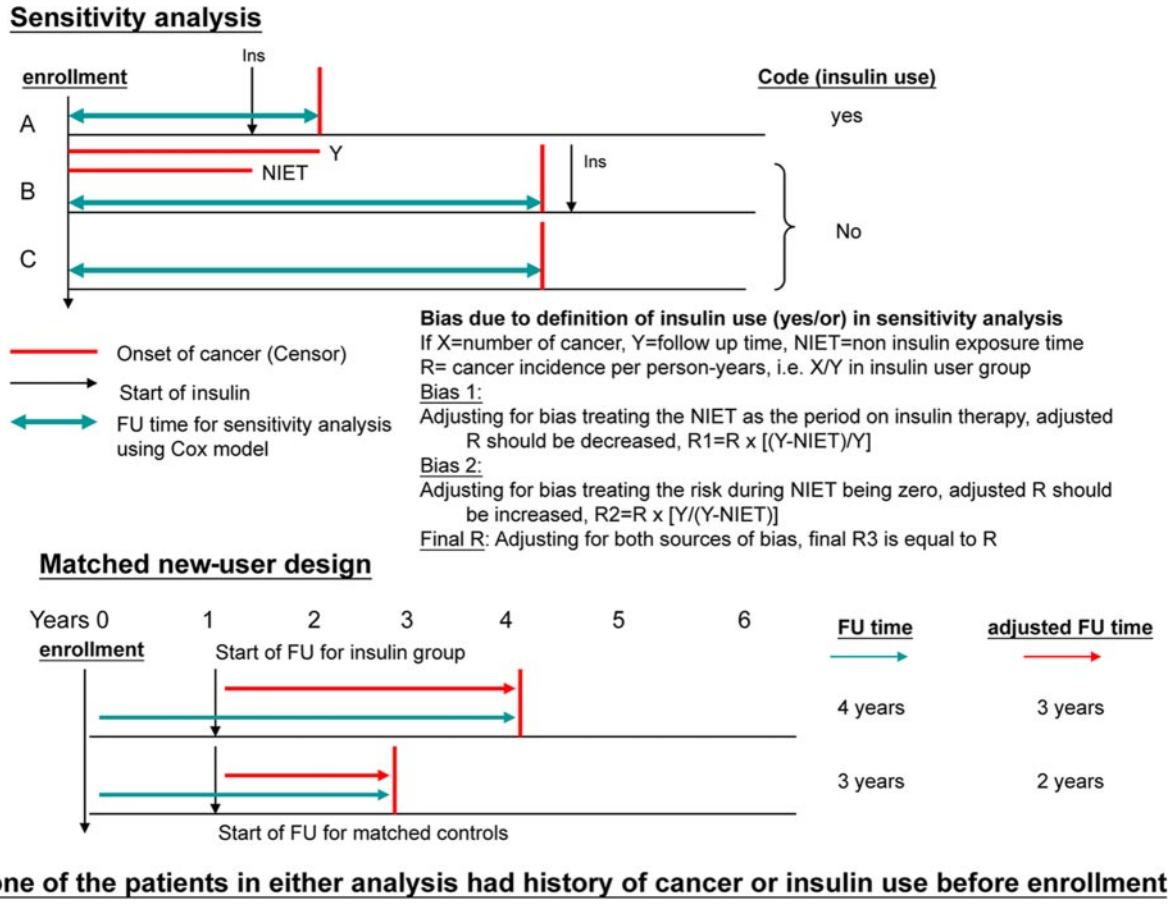

FIG. 2. Definition of insulin users and noninsulin users and their respective follow-up time in the sensitivity analysis and the matched new-user cohort analysis. FU, follow-up.

the additional matching criterion was unlikely to introduce the said bias. In the last step of organizing the new-user cohort, we further excluded the NIETs in both groups (Fig. 2). Further exclusion of the NIETs in the insulin-user group would remove the two sources of bias in the group introduced by the definition of insulin usage. Meanwhile, exclusion of the NIETs in the noninsulin-user group would also remove the second source of bias introduced by using the additional matching criterion (Fig. 1*C* and Fig. 2).

Conclusions. In summary, in both the cohort analysis with inclusion of prevalent users and the sensitivity analysis, we observed adjusted reduced cancer risk in insulin users, without removing any follow-up time. In the sensitivity analysis, we used a simple code of yes/no to define

TABLE 1
HRs of use of statins for incident CVD in patients with type 2 diabetes

	Number at risk	HR	95% CI	*P*
Initiation versus noninitiation of statins during follow-up in 4,599 nonprevalent users (code: yes/no)				
Model 1*	1,079	0.55	0.42–0.73	<0.0001
Model 2†	1,079	0.61	0.46–0.80	0.0003
Model 3‡	1,079	0.59	0.45–0.78	0.0002
Time-dependent initiation versus noninitiation of statins during follow-up in 4,599 nonprevalent users				
Model 1*	1,079	1.37	1.03–1.82	0.0308
Model 2†	1,079	1.43	1.08–1.90	0.0127
Model 3‡	1,079	1.41	1.06–1.88	0.0179

*Model 1 was adjusted for age, sex, BMI, smoking status, alcohol use, LDL cholesterol, HDL cholesterol, triglyceride, systolic blood pressure, A1C, estimated glomerular filtration rate, and Ln (urinary albumin-to-creatinine ratio + 1) at enrollment. †Based on model 1, model 2 was further adjusted for use of gliclazide and rosiglitazone from enrollment to the first date of CVD, death, or censoring, whichever came first, using a forward stepwise algorithm with $P = 0.30$ for entry and removal. ‡Based on model 2, model 3 was further adjusted for the probability of starting statin therapy, which was calculated using a logistic procedure with independent variables of age, BMI, LDL cholesterol, triglyceride, A1C, systolic blood pressure, Ln (albumin-to-creatinine ratio + 1), duration of diabetes, and retinopathy at enrollment (using the same forward stepwise algorithm).

insulin use. Although this definition can lead to two sources of bias in the insulin-user group, the overall effect of the two sources of bias seems to be neutral, and thus results from the sensitivity analysis are valid. Using CVD risk associated with statin usage as an example, we further demonstrated its validity. However, use of a time-dependent use of drugs may introduce major bias, probably because of the confounding effects of unmeasured risk factors associated with commencement of drug use (e.g., high lipid for statin or high glucose for insulin). In the matched new-user cohort, we overcame these biases by synchronizing the follow-up time in matched pairs of insulin and noninsulin users. Using these three methods of analysis (the prevalent insulin-user plus nonprevalent insulin-user cohort, the nonprevalent insulin-user cohort, and the matched new insulin-user cohort), we have generated consistent findings of the low cancer risk associated with insulin use. In a recent report, Gerstein (6) analyzed data from several large clinical trials that showed increased, neutral, or reduced risk of cancer associated with insulin. These complexities need to be addressed to avoid erroneous interpretations and thus clinical decisions. Falling short of mechanistic studies and randomized clinical trials designed to answer these questions, epidemiological findings and clinical observations continue to generate important hypothesis for testing, albeit not without limitations such as healthy volunteer bias. To this end, we agree with Johnson and Gale that we are just embarking upon a long journey to disentangle diabetes, antidiabetes therapy, and cancer.

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REFERENCES

1. Carstensen B. Comment on: Yang et al. (2010) Associations of hyperglycemia and insulin usage with the risk of cancer in type 2 diabetes: the Hong Kong Diabetes Registry (Letter). Diabetes 2010;59:e17. DOI: 10.2337/db10-0777
2. Yang X, Ko GTC, So WY, Ma RCW, Yu LWL, Kong APS, Zhao H, Chow C-C, Tong PCY, Chan JCN. Associations of hyperglycemia and insulin usage with the risk of cancer in type 2 diabetes: the Hong Kong Diabetes Registry. Diabetes 2010;59:1254–1260
3. Johnson JA, Gale EAM. Diabetes, insulin use, and cancer risk: are observational studies part of the solution—or part of the problem? Diabetes 2010;59:1129–1131
4. Bowker SL, Yasui Y, Veugelers P, Johnson JA. Glucose-lowering agents and cancer mortality rates in type 2 diabetes: assessing effects of time-varying exposure. Diabetologia 2010;53:1631–1637
5. Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. Am J Epidemiol 2003;158:915–920
6. Gerstein HC. Does insulin therapy promote, reduce, or have a neutral effect on cancers? JAMA 2010;303:446–447