Risk of Breast Cancer by Individual Insulin Use: An International Multicenter Study

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OBJECTIVE

Several studies have been published in 2009 suggesting a possible association between insulin glargine and increased risk of malignancies, including breast cancer. The objective of this study was to assess the relation between the individual insulins (glargine, aspart, lispro, and human insulin) and development of breast cancer.

RESEARCH DESIGN AND METHODS

Seven hundred seventy-five incident cases of primary invasive or in situ carcinoma breast cancer occurring in women with diabetes from 92 centers in the U.K., Canada, and France were matched to a mean of 3.9 diabetic community control subjects (n = 3,050; recruited from 580 general practices) by country, age, recruitment date, and diabetes type and management. The main risk model was a multivariate conditional logistic regression model with case/control status as the dependent variable and individual insulin use, 8 years preceding the index date, as the independent variable, controlling for past use of any insulin, oral antidiabetes drugs, reproductive factors, lifestyle, education, hormone replacement therapy and history of contraceptive use, BMI, comorbidities, diabetes duration, and annual number of physician visits. Glargine was also compared with every other insulin by computing all ratios using the variance-covariance matrix of logistic model parameters.

RESULTS

Adjusted odds ratios of breast cancer for each type of insulin versus no use of that insulin were 1.04 (95% CI 0.76–1.44) for glargine, 1.23 (0.79–1.92) for lispro, 0.95 (0.64–1.40) for aspart, and 0.81 (0.55–1.20) for human insulin. Two-by-two comparisons found no difference between glargine and the different types of insulins. Insulin dosage or duration of use and tumor stage did not change the results.

CONCLUSIONS

This international study found no difference in the risk of developing breast cancer in patients with diabetes among the different types of insulin with short- to mid-term duration of use. Longer-term studies would be of interest.

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Several studies have suggested an association between the risk of malignancies and the therapeutic use of insulin (1,2). Insulin therapies include human insulin, analogs of human insulin, and animal insulin. After the simultaneous publication of three studies comparing different insulin preparations for associated cancer risk, it was suggested that users of glargine, a long-acting insulin analog, had an increased risk of cancer and particularly of breast cancer (3–5). As a result, the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) issued an alert in July 2009 informing health care professionals and patients about a possible increase of cancer incidence in glargine users (6,7). These studies had important methodological limitations, including lack of proper control for breast cancer risk factors (8,9), and their results were not subsequently confirmed (10).

The objective of this international case-control study was to assess the relation between use of individual types of insulin (glargine, aspart, lispro, and human insulin) and development of breast cancer, controlling for breast cancer risk factors, type, severity and history of diabetes, and comorbidities (11).

RESEARCH DESIGN AND METHODS

In this case-control study, breast cancer case and community control subjects were women aged 18 years and over who had been treated for type 1 or type 2 diabetes with any type of antidiabetes drugs (oral and insulin) for at least 3 months and were alive and able to answer a telephone interview and living in the U.K. (England and Scotland), Canada (Quebec, Ontario, and New Brunswick), or France (nationwide). Women previously treated for gestational diabetes mellitus for <3 months or suffering from psychiatric or other medical conditions preventing participation were excluded. Recruitment took place between January 2010 and June 2012.

Case Subjects

Cases of breast cancer were identified in oncology clinics that treated >100 breast cancer patients annually in each participating country/region. Pathology records were searched to identify women meeting the aforementioned inclusion and exclusion criteria and who had a first lifetime pathologically confirmed diagnosis of breast cancer between 1 January 2008 and 30 June 2009, which corresponds to the 18-month period prior to the international alert issued on glargine. All hospital charts were reviewed, and patients whose records suggested a history of diabetes (type 1 or type 2) were invited to participate in the study. Information was collected from computerized oncology records on the type of breast cancer (in situ, ductal or lobular, primary invasive), tumor node metastasis (TNM) classification, examinations, and treatments. Based on TNM, cancer was secondarily staged from 0 to 4 according to the American Joint Committee on Cancer (AJCC) (version 7) classification. Types of breast cancer tumors were also classified as human epidermal growth factor receptor 2 (HER2) positive, luminal, or triple negative.

Control Subjects

Controls were identified through a pool of referents recruited by networks of general practitioners [GP] participating in the Pharmacoepidemiologic General Research eXtension (PGRx) program. This research network systematically recruits representative patients from general practice using a methodology that has previously been validated in risk-assessment studies (12). In this particular instance the PGRx recruitment system consisted, in each participating country and region, of a random sample of participating GPs instructed to identify and invite all their patients diagnosed with diabetes before 30 June 2009 (and meeting all of the inclusion and exclusion criteria mentioned above) to participate in the study.

Data Collection

History of diabetes, risk factors, and prescriptions were obtained from each participant's own diabetologist or GP involved in the treatment of their diabetes (case and control subjects). Detailed data on diabetes (type, age at diagnosis, duration, and history of antidiabetes treatments), complications (renal, vascular, ophthalmological, and neurological), and current and past HbA_{1c} results were collected for case and control subjects. All available HbA_{1c} results were computed for each participant, allowing classification of patients according to a three-class variable (mode level ≤6.5% [≤48 mmol/mol], 6.6–8% [49–64 mmol/mol], and >8% [>64 mmol/mol]).

All case and control subjects underwent an identical telephone interview specially developed for the PGRx research program using a proprietary methodology called progressive-assisted backward-active recall (PABAR) that has previously been validated (13,14). Patients were sent an interview guide ahead of time including a review of medications commonly used, listed by health problem category (cardiovascular, respiratory, metabolic, etc.). Patients were asked to provide as many prescriptions as possible. After reporting of antidiabetes drug use, patients were prompted to review the list of all antidiabetes drugs and types of insulin available on the market (trade and generic names) assisted by trained interviewers blind to the specific breast cancer hypothesis of the project. The interview also collected information on education and socioeconomic status, smoking, alcohol consumption, and physical activity; personal and first-degree relatives' history of breast, ovarian, and any other cancer; and a review of past and present medical history with a focus on diabetes-related comorbidities including retinopathy, arteriopathy, nephropathy, and peripheral neuropathy. The interview also covered lifetime reproductive and hormonal history including age at menarche, menopausal status and age at menopause, parity and age at first and last birth, breast-feeding, and use of oral contraceptive and hormonal replacement therapy (HRT). BMI corresponding to the current, highest, and lowest weights between 2001 and 2008 was computed and classified in three categories: <25, 25–29 and ≥30 kg/m^2.

Exposure to Insulin

Objective prescribing information on insulin from physicians or pharmacists was obtained in 84.4% of patients. In the remaining patients, data on insulin exposure were obtained from the

patient interview only. (See below.) Agreement between patient interviews and physicians' records for insulin use was 97% ($\kappa > 0.89$) in patients for whom information from both sources was available. Given that all patients underwent an interview, the primary analysis used the data collected during the interview, while objective data from prescriptions was used in sensitivity analyses. The time window retained a priori for the primary analysis was insulin use in the 8-year period preceding the index date defined as the date of first diagnostic biopsy confirming breast cancer for the case. An identical time window was used for the control subjects so as to match control subjects to each case subjects. (See matching rules below.) This time window corresponded to the time elapsed between the date back when glargine was first marketed (Fall 2001) and the last possible index date accepted for the study (30 June 2009). Case and control subjects were individually classified as exposed or nonexposed to each insulin type within this time window. Diabetes treatment included all types of insulin, metformin, and other oral antidiabetes drugs. Insulin use was classified as basal or prandial but also by type of molecule (human insulin, aspart, glargine, and lispro and other types such as detemir, glulisine, and animal). Detailed history for each category of treatment before the index date was collected: start/stop dates, doses, and switching. The total duration of each insulin use period was computed. Patients who reported using insulin for <3 months (total treatment duration) were classified as nonexposed to insulin. Doses were classified as either lower or higher than the median value for each individual insulin use in control subjects. Use of any insulin prior to the 8-year time window of interest was defined as "past insulin use" without distinction between individual types of insulin.

Statistical Analysis

The statistical analysis plan was finalized before the start of data collection. Participants were compared with nonparticipants for age, cancer stage (for case subjects), and antidiabetes treatments (oral therapy and insulin use). Given that some patients were dead by the time the study began, death rates according to the type of antidiabetes treatment (oral, glargine, and other individual insulins) reported in the records were computed in order to detect a potential survival bias.

Matching

Once all case and potential control subjects were interviewed, control subjects were randomly matched to case subjects on five criteria: type of diabetes (type 1 or 2), country region or province, age at recruitment (±1 year if possible; otherwise, ±2, 3, 4, or 5 years), date of recruitment (±6 months) and referral to an endocrinologist (diabetologist) for diabetes (yes/no). The objective was to obtain on average of four matched control subjects per breast cancer case subject.

Modeling Diabetes Risk Factors and Insulin Exposure

A multivariate confounding breast cancer risk score was computed to be used as an adjustment variable using sociodemographic, lifestyle (smoking, alcohol consumption, and physical activity) and reproductive factors (age at menarche, parity, breast-feeding, menopause, and use of oral contraceptives and HRT), BMI, and personal history of cancer and history of breast cancer in first-degree relatives. Individual variables associated with the case/control status were used to control for residual confounding. Unadjusted and adjusted matched odds ratios (ORs) and their 95% CI were computed using conditional logistic regression with the case/control status as the dependent variable. Individual insulin use of glargine (yes/no), lispro (yes/no), aspart (yes/no), human insulin (yes/no), and other types of insulin (yes/no) within the 8 years preceding the index date and past use of insulin (yes/no) were all entered in the models, thus allowing for mutual adjustment. In the adjusted models, OR estimates were controlled for multivariate confounding breast cancer risk score (in quartiles), BMI ($\leq$24, 25–29, and $\geq$30 kg/m^2), duration of diabetes (<10 years and $\geq$10 years), number of annual visits to a physician before the index date, cardiovascular disorder or other medication use (at least one), presence of comorbidities (<3 and $\geq$3), past use of insulin (yes/no), and any use of oral antidiabetes drugs (yes/no). ORs corresponding to each insulin product allowed for comparison between users and nonusers of each type of insulin individually, both categories containing users and nonusers of other insulins. These ORs were mutually adjusted and reflect the association of breast cancer with the insulin product considered adjusted for the use of other insulins. Sensitivity analyses were performed in users of at least one insulin treatment in the past and in users of at least one insulin treatment in the 8-year time window. For glargine specifically, a stratified analysis by duration of glargine use (<4 years and $\geq$4 years) and by maximum dose used (below or above median dose in the study population, i.e., 27 UI) was performed. Additionally, breast cancer risk estimates associated with glargine were compared with every other insulin by computing all ratios using the variance-covariance matrix of logistic model parameters.

No variable used in the analyses had >5% of missing values. In all multivariate models, missing values were imputed by median (if continuous) or mode (if categorical). The study was powered to detect an OR as small as 1.4 for glargine use and breast cancer. Recruitment was stratified by country (U.K., France, and Canada) to account for variations in glargine use and ensure sufficient exposure. Analyses were performed using the SAS software (version 9.1.3; SAS Institute, Cary, NC).

Ethics

The study protocol, consent forms, and methods for protecting the confidentiality of patients were approved by institutional review boards across the three participating countries.

Research ethics committees' approval was obtained for each participating institution and for recruitment by GPs in the U.K. and Canada. In France, ethics approval was obtained from Commission nationale de l'informatique et des libertés and from Conseil national de l'ordre des médecins. Written informed consent was obtained from

each participating patient. Physicians received fixed fees for their participation, but patients did not.

RESULTS

Description of Case and Control Subjects

Overall, 92 participating oncology centers (39 in the U.K., 38 in France, and 15 in Canada) reviewed a total of 39,558 medical records of women with a pathologically confirmed first lifetime diagnosis of breast cancer made between 1 January 2008 and 30 June 2009 (Fig. 1). Among them, 3,131 (7.9%) breast cancer patients were found to have a record of diabetes in the chart, of whom 396 (12.6%) were dead at the time of study. Death rates were higher in insulin users than in nonusers but were comparable between users of any insulin (18%) and users of glargine (17%). Thus, 2 735 (87.4%) patients were available to participate in the study. Among the latter, contact details were available for 2,408 (88.0%) patients, allowing us to seek consent for participation in the study; all patients were contacted, and 997 (41.4%) agreed to participate. Nonparticipating case subjects were of an age similar to that of participants (mean [SD] age 67.0 [11.7]) and 66.8 [9.1] years, respectively) and similarly used any type of insulin as registered in the oncology center record (16.2 vs. 14.5% [any prandial insulin] to 17.8% [any basal insulin], respectively). Participating cases were primary invasive cancers in 89% of instances and cancers in situ in the remaining 11%. According to the AJCC classification, 58.1% of patients were stages 0–1, 30.4% stage 2, and 11.5% stages 3–4. Data on hormone receptors (available in 652 cases) showed that 76.5% of cancers were estrogen- or progesterone-receptor positive and HER2 negative (luminal). Subsequently, 88 (8.8%) patients could not be reached for the interview. Of 909 interviewed case subjects, 797 were eligible for matching with control subjects after secondary exclusion due to exclusion criteria found during the interview.

For control subjects, 580 GPs across the three countries recruited 5,329 patients with diabetes agreeing to participate in the study (Fig. 2), which was estimated (using respective national statistics) to represent between 44 and 52% of the expected clientele with diabetes seen in general practices—a participation rate

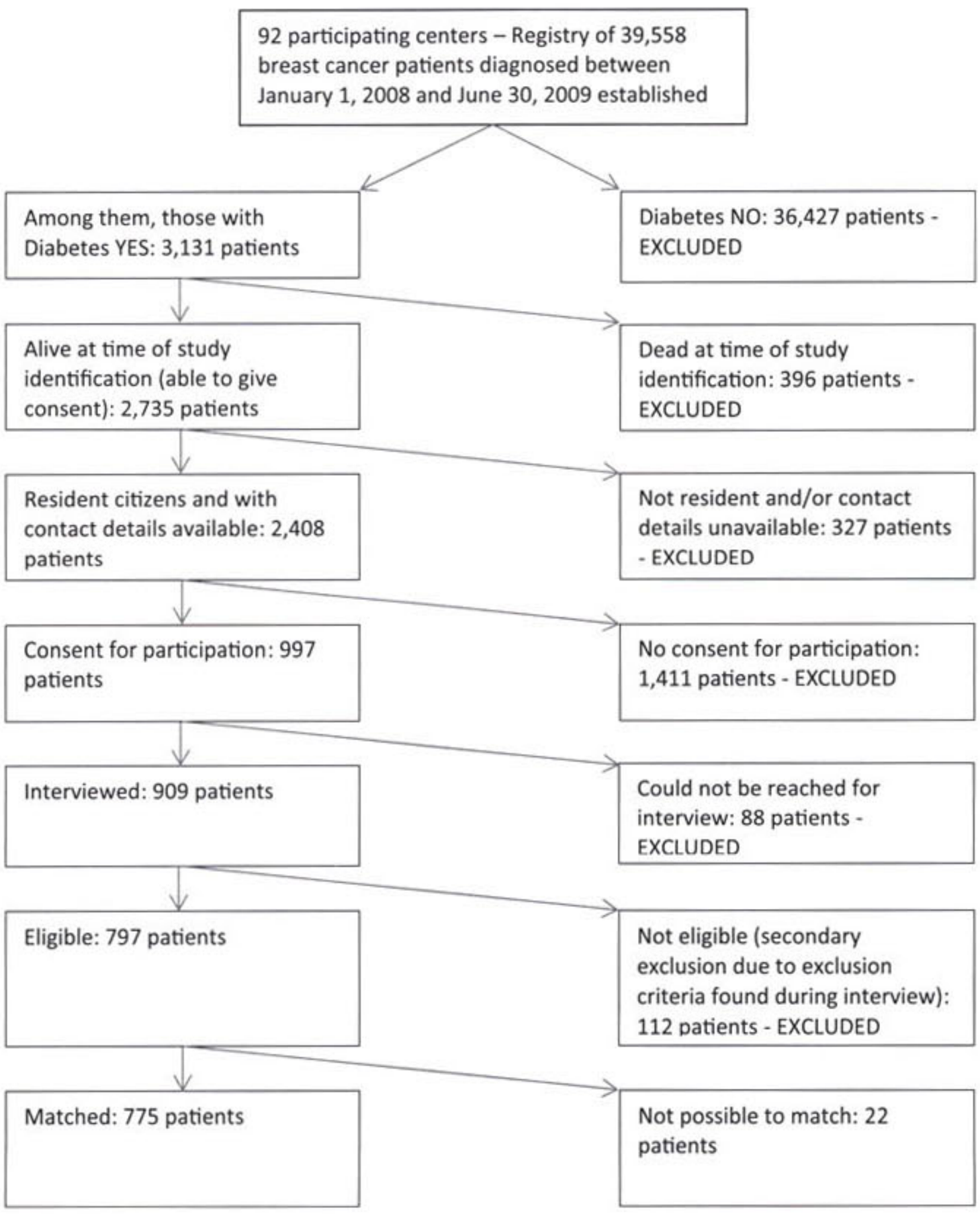

Figure 1—Flowchart: recruitment of case subjects. (A high-quality color representation of this figure is available in the online issue.)

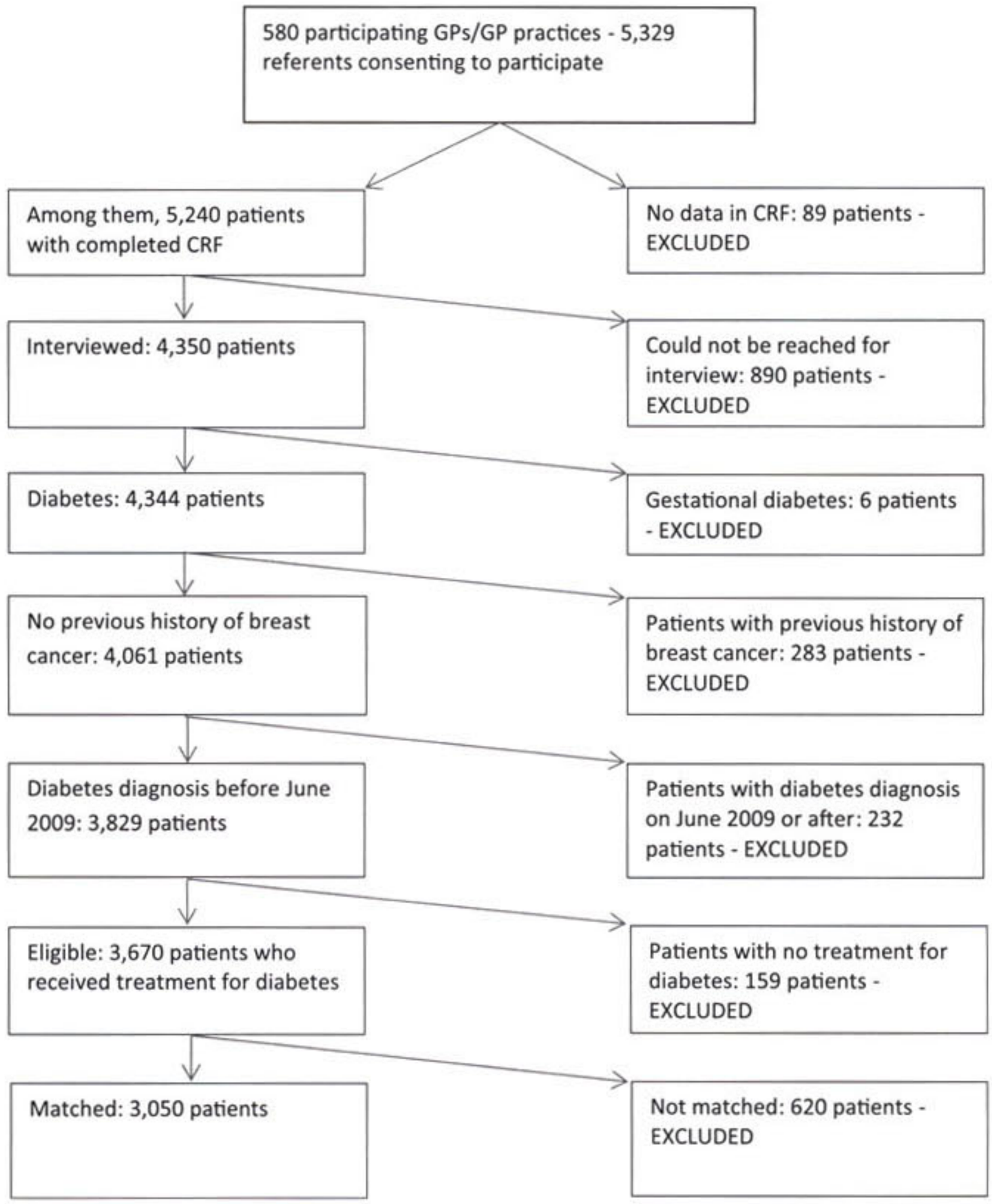

Figure 2—Flowchart: recruitment of referents (control subjects). (A high-quality color representation of this figure is available in the online issue.)

similar to that of case subjects. Subsequently, 769 patients (14.4%) were identified as not meeting the inclusion and exclusion criteria and a further 890 (16.7%) could not be reached for the interview, leaving 3,670 control patients available for matching to each case.

After the matching procedure, 22 case and 620 control subjects could not be matched, leaving a final study population of 775 breast cancer cases (21% from the U.K., 15.5% from Canada, and 63.5% from France) and 3,050 control subjects for a mean of 3.9 control subjects per case (range 1–10).

Case and control subjects did not show major differences for variables concerning lifestyle, medications use, and use of health care services (Table 1). As expected, case subjects more often had a personal and family history of breast cancer (OR 1.53 [95% CI 1.16–2.02] and 1.65 [1.34–2.01], respectively). They were also more often postmenopausal (2.65 [1.70–4.14]) and reporting current or past use of HRT (1.38 [1.10–1.73]). The probability of a case falling within the fourth quartile of the computed breast cancer risk score was much higher than for control subjects (2.74 [2.05–3.68]). Table 2 presents the main features of diabetes history and management, which were very similar between case and control subjects for all the variables studied. The distribution of the two types of diabetes by matching was identical in case and control subjects, with 6.2% for type 1 diabetes. Use of any insulin (case and control subjects combined) was independently and significantly associated with longer duration of diabetes, HbA_{1c} >8% (>64 mmol/mol), and cardiovascular comorbidities as well as recent hospitalization (≤1 year) (data not shown). No significant association with insulin use was found for current BMI, age, education, or multivariate confounding breast cancer risk score. Patients with type 1 diabetes were more likely to use glargine than any other type of insulin; no other variable was associated with glargine use comparatively with other insulins (data not shown).

Individual Insulin and Risk of Breast Cancer

None of the individual insulins were associated with an increased risk of

Table 1—General characteristics of breast cancer case and control subjects at the interview

	Case subjects (incident breast cancer with diabetes)*	Matched control subjects (no breast cancer with diabetes)*	Crude OR (95% CI)
N	775	3,050	
Age (years), mean (SD)	66.8 (9.1)	66.0 (9.9)	1.05 (1.00–1.11)
Smoking			
Current smoker	71 (9.2)	312 (10.2)	0.92 (0.69–1.23)
Former smoker	237 (30.6)	853 (28.0)	1.19 (0.99–1.43)
Alcohol intake			
Several times/week	206 (26.6)	706 (23.1)	1.24 (1.03–1.50)
Occasionally or never	569 (73.4)	2,344 (76.9)	
Exercise (min/day)			
≤30	442 (57.0)	1,628 (53.4)	
>30	333 (43.0)	1,422 (46.6)	0.88 (0.75–1.04)
Education			
High school completed	320 (41.3)	1,110 (36.4)	1.32 (1.11–1.57)
BMI (kg/m^2), mean (SD)			
Lowest in life	21.8 (3.9)	22.3 (4.3)	0.98 (0.96–1.00)
Highest in life	33.6 (7.5)	33.9 (7.6)	1.00 (0.99–1.01)
In 2003	29.8 (6.3)	30.1 (6.5)	0.99 (0.98–1.01)
Drug use at any time before index date			
Cardiovascular	715 (92.3)	2,839 (93.1)	0.80 (0.58–1.10)
NSAIDs	311 (40.1)	1,161 (38.1)	1.10 (0.94–1.30)
Antidepressants	81 (10.5)	284 (9.3)	1.22 (0.93–1.59)
HRT	139 (17.9)	400 (13.1)	1.46 (1.17–1.82)
Oral contraceptives	307 (39.6)	1,110 (36.4)	1.27 (1.06–1.53)
Use of health care services in previous year			
Hospitalization	155 (20.0)	492 (16.1)	1.23 (1.00–1.51)
No. of visits to a physician, mean (SD)	6.0 (9.0)	6.3 (5.7)	0.99 (0.97–1.00)
Any personal history of cancer	95 (12.3)	232 (7.6)	2.32 (1.75–3.06)
Family history of cancer			
Any cancer	509 (65.7)	1,805 (59.2)	1.35 (1.12–1.62)
Breast cancer	166 (21.4)	411 (13.5)	1.70 (1.38–2.09)
Multivariate confounding breast cancer risk score†			
1st quartile	104 (13.4)	852 (27.9)	
2nd quartile	125 (16.1)	832 (27.3)	1.08 (0.80–1.45)
3rd quartile	211 (27.2)	744 (24.4)	1.90 (1.42–2.53)
4th quartile	335 (43.2)	622 (20.4)	2.83 (2.12–3.77)

Data are *n* (%) unless otherwise indicated. NSAIDs, nonsteroidal anti-inflammatory drugs. *Case and control subjects from all countries combined (France, U.K., and Canada); control subjects matched to case subjects by type of diabetes (1 or 2), age, date of recruitment, region/country, and referral to diabetologist (yes/no). †Derived from multivariate regression analyses including the following variables: age at menarche, menopausal status, age at menopause, parity, age at first and last birth, breast-feeding, use of oral contraceptive and use of HRT, personal history of cancer, family history of cancer, and education.

breast cancer (Table 3). Comparisons between types of insulin use showed no significant differences in the risk of breast cancer, with OR 0.85 (95% CI 0.48–1.50) for glargine vs. lispro, 1.10 (0.64–1.89) for glargine vs. aspart, and 1.29 (0.78–2.13) for glargine vs. human insulin. No statistical difference was observed in the proportion of glargine users according to the AJCC staging for breast cancer (stage 0: 10.8% of users, stage 1: 8.5%, stage 2: 12.3%, and stage 3 or 4: 9.0%) or the tumor type (luminal: 9.0%, HER2 positive: 8.1%, and triple negative: 14.8%). Results were similar when medical information from prescriptions, rather than patient interviews, was used as the source of information on exposure. Sensitivity analyses of patients with uncertain exposure did not change the results.

The mean (SD) duration of glargine use in the whole study population (case and control subjects) was 3.2 (2.0) years. The adjusted OR for risk of breast cancer did not change with increasing duration of glargine use, with 1.15 (95% CI 0.70–1.88) for <4 years and 0.94 (0.51–1.74) for 4–7 years. Finally, in analyses restricted to insulin users, we observed that categorizing of glargine use in high and low dose returned no trend whatsoever (no use >27 IU: 1.10 [95% CI 0.61–1.97]; at least one use >27 IU: 1.02 [0.59–1.75]) (Table 3).

CONCLUSIONS

This international case-control study was specifically designed to address the question of breast cancer risk among patients with diabetes using different insulin regimens and was carefully designed to minimize the risk of biases common to this type of studies. The no-difference findings in breast cancer risk in users of any of the individually studied insulins (glargine, aspart, lispro, and human insulin) were all fully consistent in the various sensitivity analyses. Further analyses focusing on insulin glargine found no evidence to suggest that either dose or duration of glargine use influenced the risk of breast cancer. This study did not adequately explore the hypothesis that insulin could promote cancer foci development and could not address the effect of long-term exposure, since insulin analogs (glargine, lispro, and aspart) have been marketed only from 2001 onward. More recently marketed (detemir) or very infrequently used insulin types (glulisine and porcine) could not be included in this study.

Most previous studies on individual insulin use and breast cancer have been conducted by record linkage of health care databases (3–5). The first study to suggest a potential risk of glargine versus human insulin found an OR of 1.31 (95% CI 1.20–1.42) for breast cancer in high-dose users only (>50 UI) while only controlling for a limited number of factors (3). A study from the

Table 2—Diabetes history in breast cancer case and control subjects

	Case subjects (incident breast cancer with diabetes)*	Matched control subjects (no breast cancer with diabetes)*	Crude OR (95% CI)
N	775	3,050	
Diabetes			
Type 1	48 (6.2)	6.2*	N/A
Type 2	727 (93.8)	93.8*	N/A
Duration of diabetes (years), mean (SD)			
Age at diagnosis	52.4 (12.4)	52.8 (13.2)	0.99 (0.98–1.00)
Time since first diagnosis	14.5 (10.1)	13.2 (9.8)	1.01 (1.00–1.02)
Time to first insulin use	11.3 (10.1)	10.8 (10.2)	
Year of diagnosis			
Prior to 2001	470 (60.6)	1,665 (54.6)	
2002–2003	90 (11.6)	392 (12.9)	0.88 (0.68–1.14)
2004–2009	215 (27.7)	993 (32.6)	0.83 (0.69–1.00)
HbA_{1c} (mode lifetime), % (mmol/mol)			
≤6.5 (≤48)	164 (27.9)	828 (28.6)	
6.6–8 (49–64)	309 (52.6)	1,535 (53.1)	1.01 (0.81–1.26)
>8 (>64)	114 (19.4)	528 (18.3)	1.07 (0.80–1.43)
Diabetes complications and morbidity			
Retinopathy	69 (8.9)	306 (10.0)	0.82 (0.61–1.08)
Arteriopathy	32 (4.1)	137 (4.5)	0.80 (0.53–1.22)
Nephropathy	23 (3.0)	99 (3.2)	0.86 (0.54–1.38)
Peripheral neuropathy	71 (9.2)	188 (6.2)	1.51 (1.12–2.03)
At least one of the above	148 (19.1)	559 (18.3)	0.99 (0.80–1.23)
Cardiovascular disease	249 (32.1)	905 (29.8)	1.16 (0.97–1.39)
Antidiabetes treatments (lifetime)			
Oral antidiabetes drugs (without insulin)	572 (73.8)	2,299 (75.4)	0.98 (0.78–1.22)
Metformin	464 (59.9)	1,967 (64.5)	0.82 (0.69–0.99)
Insulin only	203 (26.2)	751 (24.6)	1.02 (0.82–1.28)
Basal insulin (any)	192 (24.8)	729 (23.9)	0.98 (0.78–1.22)
Prandial insulin (any)	142 (18.3)	525 (17.2)	1.02 (0.79–1.31)

Data are *n* (%) or % unless otherwise indicated. *Case and control subjects from all countries combined (France, U.K., and Canada); control subjects matched to case subjects by type of diabetes (1 or 2), age, date of recruitment, region/country, and referral to diabetologist (yes/no).

Scottish Diabetes Research Network in different subgroups of patients (5) reported hazard ratios for glargine and breast cancer varying from 1.49 (95% CI 0.79–2.83) to 3.39 (1.46–7.85), but information on dose and other important risk factors was lacking: the authors concluded that confounding by indication was likely to have occurred, as patients receiving glargine were older and exhibited higher severity of diabetes. After a preliminary study estimating the relative risk for glargine and breast cancer to be 1.99 (95% CI 1.31–3.03), another Swedish group performed two subsequent cohort follow-ups and found a relative risk of 0.87 (0.41–1.85) for glargine and breast cancer in their most recent analysis (4,15). Differences in results were attributed to random fluctuation.

A study based on the U.K. General Practice Research Database (GPRD) did not find any association between risk of breast cancer and glargine use (10), whereas another study on the same database showed that the risk of breast cancer tended to increase after 5 years of glargine use (1.8 [0.8–4.0]), and significantly so for the women who had been on insulin before starting glargine (2.7 [1.1–6.5]), indicating a possible cumulative effect (16). A retrospective nested case-control analysis in an Italian cohort of new insulin users found an elevated OR for glargine use and breast cancer (5.43 [95% CI 2.18–13.53]) (17); this study lacked controlled matching for diabetes management, undoubtedly a potentially major confounder for case-control research in pharmacoepidemiology. Finally, analyses of the French national health care insurance database found no excess of cancer (hazard ratio 0.59 [95% CI 0.28–1.25]) or cancer deaths (0.58 [0.32–1.06]) among exclusive users of glargine compared with human insulin (18). The large mortality deficit between the two populations might reflect a lack of comparability between populations.

A common issue inherent to studies conducted by record linkage of health care databases is that it usually allows access to a limited number of risk factors for the control of confounding. In our study, however, a large number of risk factors were considered and carefully evaluated. Unexpectedly, the analysis showed no impact from the inclusion of these factors into risk models on OR estimates: crude and adjusted ORs were similar.

Interpretation

Exposure to insulin was thoroughly documented within the 8-year time window (2001–2009), therefore spanning all potential exposures to glargine and other insulin analogs before the alert on insulin glargine and breast cancer was issued. Documenting past insulin use before that time window (obtained through patient interviews) allowed controlling for potential cumulative insulin effects. Still, the 8-year time window remains a relatively short period for cancer latency, but it might be sufficient to cover any potential effect on tumor growth. Moreover, very few patients had been continuously exposed to one specific type of insulin during the whole 8-year period. Among glargine users, only one-third had been exposed to that insulin during 4 years or more. For other insulins, exposure durations lasted for <4 years in the majority of users. Human insulin is a growth factor for different tumors in vitro, and elevated levels of circulating endogenous insulin produce a secondary increase of IGF-1 in vivo. This has been shown to accelerate

Table 3—Individual insulin use and risk of breast cancer

	Case subjects	Matched control subjects	Crude matched OR (95% CI)*	Adjusted matched OR (95% CI)*¥
N	775	3,050		
Use of a specific insulin in the 8-year prior to index date vs. no use of that insulin**				
Glargine	78 (10.1)	287 (9.4)	0.99 (0.74–1.34)	1.04 (0.76–1.44)
Lispro	46 (5.9)	133 (4.4)	1.24 (0.84–1.84)	1.23 (0.79–1.92)
Aspart	54 (7.0)	241 (7.9)	0.86 (0.61–1.21)	0.95 (0.64–1.40)
Human insulin	59 (7.6)	260 (8.5)	0.81 (0.57–1.13)	0.81 (0.55–1.20)
Any insulin use prior to the 8-year observation period vs. no use of any insulin	74 (9.5)	270 (8.9)	0.97 (0.69–1.35)	0.95 (0.62–1.45)
Glargine dose vs. all other users of insulin§				
N	144	410		
No glargine	70 (48.6)	207 (50.5)	1.00	1.00
Any dose	74 (51.4)	203 (49.5)	1.08 (0.71–1.64)	0.96 (0.61–1.53)
Low dose	31 (21.5)	89 (21.7)	1.17 (0.68–2.00)	1.10 (0.61–1.97)
High dose	33 (22.9)	87 (21.2)	1.05 (0.63–1.75)	1.02 (0.59–1.75)
Undefined dose	10 (6.9)	27 (6.6)	0.94 (0.42–2.14)	0.85 (0.35–2.07)

Data are *n* (%) unless otherwise indicated. *Control subjects matched to case subjects by type of diabetes (1 or 2), age, date of recruitment, region/country, and referral to diabetologist (yes/no). ¥Adjusted matched ORs obtained from conditional logistic regressions controlled for age, breast cancer risk score, BMI (≤24, 25–29, and ≥30 kg/m^2), comorbidities (<3 or ≥3), duration of diabetes (<10 years or ≥10 years), no. of visits to physician/year, and oral antidiabetes drug use. In addition, adjusted ORs for individual insulin molecules were further adjusted for other insulin use (animal, glulisine, detemir, or unclassified, as a separate category, yes/no) and past insulin use (any insulin use ≥8 years before index date). **Index date, date of first pathological confirmation of breast cancer. §High and low dose dichotomized at the median dose (27 IU) for all glargine users: low dose, no dose above the median reported; high dose, use above the median reported at least once.

the progression of cancer foci (19–22). Glargine's affinity for the IGF-1 receptor is very high, which justifies concerns about its potential ability to promote cancer growth. However, it was shown recently that glargine itself is rapidly metabolized and that its metabolites have lower affinity for IGF-1 than endogenous insulin (23). In our study, the prevalence of glargine use was similar for all tumor stages (0–4) and types (luminal, HER2 positive, or triple negative) studied, which was reassuring in this respect.

Strengths and Limitations

The main strength of this study was the comprehensive procedure by which drug exposure and information on individual risk factors (24) were collected, using a methodology that has previously been used and validated (12,13). Data were collected from clinical practice, which allowed thorough documentation of patients' history of diabetes, including severity, comorbidities, and management by either diabetologists or GP. It accurately documented the prevalence of known risk factors for breast cancer, allowing their adjustment in the analyses. Crossing data from physicians' records and standardized patient interviews allowed establishment of the lifetime history of insulin treatment, documenting the time between first diagnosis of diabetes and first insulin therapy, as well as different individual insulin regimens used during and prior to the time window of interest for this study.

The case-control design of this study is the best suited to test hypotheses on risk of relatively rare events but has several limitations. First, selection bias can occur when identification of case subjects is associated with exposure status. The large number of breast cancers screened (39,558) to identify patients exposed to antidiabetes drugs, by trained research assistants independently from the investigators, reduced this possibility. Nevertheless, based on data from oncology records, the participation of patients was not independent of exposure. Overall, 51% of patients declaring themselves glargine users agreed to participate in the study compared with 41% for users of any other type of insulin. This difference was due to case subjects from the U.K. (participation rates 56% in glargine users vs. 37% in users of other insulins, $P > 0.05$), while no difference was found in Canada and France. Participation was identical in oral antidiabetes users and nonusers (40%) and did not differ across the three countries. Chance or subtle encouragement of glargine users to take part in the study might explain this. Yet, in this case, a bias would act against our findings (null hypothesis). Excluding data from the U.K. in sensitivity analysis did not change the results (OR 1.04 [95% CI 0.72–1.50]).

Case-control studies are mostly at risk for recall bias where the exposure of interest is more likely to be reported by patients experiencing the condition of interest (i.e., cancer). Precautions to prevent this bias included collecting medical prescriptions and records from health professionals and crossing the latter information with interviews, which gave excellent agreement for insulin use (97%). Sensitivity analyses using one or the other source of information did not change the results. Also, a potential recall bias would work in favor of an association between glargine and breast cancer. Another bias could arise from a diagnosis (of cancer) that is not blinded to exposure status. This is why this study was conducted only in cases diagnosed before the issued alert. The fact that the prevalence of glargine use was similar across the different stages of cancer is also reassuring in this respect, as "lead time bias" (when early diagnosis falsely appears to prolong survival) is thus less likely.

A disadvantage of the case-control approach is that only patients alive at the time of the interview could be included. A survival effect was unlikely

to bias the comparison between different types of insulin because the death rate in cancer case subjects at the time of recruitment in the study was the same as in glargine users (17%) as opposed to other insulin users (18%). Another important potential limitation may be attributed to the relatively low participation rate. Our study was powered a priori to detect an OR of at least 1.4 for glargine use relative to nonuse of insulin based on the recruitment of 750 case and 3,000 control subjects. This required screening nearly 40,000 incident breast cancers, which is equivalent to the number of annual incident breast cancers registered in countries such as the U.K. or France. Clinical characteristics of participating breast cancer case subjects were consistent with breast cancer statistics for age, type of cancer, stage, and hormonal receptor distributions (25–28); the type and severity of diabetes, as well as the prevalence of insulin use (including by individual types) were also representative of patients with this disease in current practice, providing reasonable evidence of representativeness of the study population. The results of computation of breast cancer risk for factors such as reproductive history, HRT, and oral contraceptives were consistent with previously reported data. We did not observe, however, a risk with obesity, which has been frequently associated with a higher risk of breast cancer (29). This may be explained by the fact that the majority of our patients (cases and control subjects) were overweight or obese.

Finally, this case-control study assessed only one cancer site (breast cancer). Recent studies on individual insulin use and cancer have provided additional information on other cancers. The continuation of the ORIGIN (Outcome Reduction with Initial Glargine Intervention) trial over 6 years found no evidence of increased risk for any type of cancer (30). The same was true for a number of recent health care database studies (31,32).

In conclusion, this international case-control study specifically conducted to address the risk of individual insulin use and incident breast cancer after a mean exposure of 3.2 years did not find increased risk with any of the individual insulin studied (glargine, lispro, aspart, and human insulin). Longer-term studies are needed to further explore this issue.

Acknowledgments. The authors thank the data management, SAS programming, and operations study teams including Nadia Petiot, Rita Goorochurn-Jackson, Daniel Rochford, Darith Tran, Linda Gamal, Adeline Costa, Yann Hamon, Celine Kernaleguen, Miguel Gomes de Souza, Shajahal Mohammed, Stefanie Pohlmann, Sandra da Cruz, Agathe Legrand, Julie Fremond, Vincent Diesny, Aurélien Mantonnier, Nicolas Morisot, Souad Baguenine, Constance Charveriat, and Nadine Pelletier.

Duality of Interest. The International Study of Insulin and CAncer (ISICA) was sponsored by LA-SER and funded by an unrestricted grant from Sanofi. The funder had no input into the design, conduct, or report of the study. The study was undertaken and oversight given by an international scientific board whose members are co-authors or belong to the ISICA Group. LA-SER is an independent research organization that owns and develops the PGRx database. LA-SER has no commercial interests in any of the products studied. To study the data from PGRx or other sources, LA-SER receives funds and/or other support from regulatory agencies, public sources, academic institutions, private groups, and from the pharmaceutical industry; donor companies include, but not exclusively, the following over the past 36 months: AstraZeneca, Boiron, Expanscience, Genevrier, GlaxoSmithKline, Janssen-Cilag, Merck/Schering Plough, Negma/Wokhardt, Novartis, Pfizer, and several divisions of Sanofi.

L.G.-B. was the recipient of a research fellowship from INSERM at the time of the study and is currently employed by LA-SER, the company conducting the study, together with M.Ro. and A.K. D.C. received consulting fees and travel expenses during the design stage of ISICA from Sanofi. M.M. has received consultancy fees from Sanofi, Debiopharma, and Celgene; payment for development of educational presentations including service on speakers' bureaus from Roche and AstraZeneca; and reimbursement of travel or accommodation expenses from Sanofi. A.H.B. has received honoraria for advisory work and lectures unrelated to this work from Sanofi, Eli Lilly, and Novo Nordisk. M.P. has received consultancy fees or honoraria for work on ISICA from LA-SER and Sanofi and consultancy fees, grants, and travel expenses for other work from Novo Nordisk, Sanofi, and Pfizer. B.C. has received fees for consultancy, advisory boards, speaking, travel, or accommodation from Takeda, GlaxoSmithKline, Merck Sharp & Dohme, AstraZeneca, Bristol-Myers Squibb, Boehringer Ingelheim, Novo Nordisk, Roche, Sanofi, and Novartis. M.Ri. has received honoraria for consulting and for chairing a consulting group regarding insulin glargine, as well as research grants, speakers' fees, and travel grants from Sanofi; research grant support from Eli Lilly, Amylin, and GlaxoSmithKline; and honoraria for consulting from Eli Lilly, Amylin, and Hoffmann-La Roche. J.-F.B. has been a scientific advisory board member for Sanofi and received reimbursement for travel to board meetings; J.-F.B. also received honoraria from Sanofi and LA-SER. M.Ro. has received honoraria for advisory work and lectures unrelated to this work from Sanofi, Eli Lilly, and Novo Nordisk. J.B. and A.A. both consult for LA-SER. L.A. is a stock owner and chairman of LA-SER, the company conducting the study.

No other potential conflicts of interest relevant to this article were reported.

Author Contributions. L.G.-B. conceived both the research theme and the methods, analyzed data, interpreted the results, was in charge of the study in France and the U.K., participated in drafting and revising the manuscript, and contributed to, read, and approved the final manuscript. D.C., M.M., A.H.B., F.P.-L., M.P., B.C., M.Ri., L.M., and J.-F.B. conceived both the research theme and the methods, analyzed data, interpreted the results, and contributed to, read, and approved the final manuscript. A.K. and M.Ro. conceived both the research theme and the methods, analyzed data, interpreted the results, participated in drafting and revising the manuscript, and contributed to, read, and approved the final manuscript. J.B. and A.A. conceived both the research theme and the methods, analyzed data, interpreted the results, and contributed to, read, and approved the final manuscript. L.A. conceived both the research theme and the methods, analyzed data, interpreted the results, participated in drafting and revising the manuscript, and contributed to, read, and approved the final manuscript. L.G.-B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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