
Insulin analogues and cancer risk: cause for concern or cause célèbre?

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SUMMARY

People with diabetes, particularly those with type 2 diabetes, may be at an increased risk of cancer. Furthermore, their cancer risk may be modified by treatment choices. In this respect, metformin may be protective, whereas insulin and insulin analogues can function as growth factors and therefore have theoretical potential to promote tumour proliferation. Analogues causing inappropriate prolonged stimulation of the insulin receptor, or excess stimulation of the IGF-1 receptor, are the most likely to show mitogenic properties in laboratory studies. Some recent epidemiological studies appear to be consistent with these experimental findings, suggesting that there could be different relative risks for cancer associated with different insulins, although these studies have attracted some methodological criticism. However, it is biologically plausible that hormonal factors that influence neoplasia could begin to manifest their effects in surprisingly short time-scales (within 2 years) and hence these epidemiological studies justify further research. Even if future research were to document an increase in cancer risk among insulin users, this would be unlikely to significantly diminish the favourable benefit-risk ratio for patients requiring insulin therapy. There is a need for further population studies and for the development of new laboratory models that are more sophisticated than previous experimental methods employed to assess potential tumour growth-promoting properties of insulins.

Introduction

Are diabetes and diabetes treatments associated with an increased risk of cancer? These questions have been exercising the minds of the clinical community since the recent publication of a series of epidemiologic studies (and accompanying editorial) that have investigated the relative risk for cancer incidence associated with the use of the basal insulin analogue, insulin glargine, or any insulin (1–6). These publications have resurrected awareness and focused attention on an issue that first emerged more than a decade ago, when it was shown that artificial modification of the molecular structure of insulin could result in a peptide with increased mitogenic properties in cell lines and animal models (7,8). Indeed, even earlier reports, for example Heuson et al. 1967 (9), provided evidence for links between insulin and neoplasia. This issue has been periodically studied in the intervening years and represented the *a priori* reason for conducting these observational studies.

The methodologies of each of the recent epidemiological studies have been subject to discussion, however, and published critiques and expert statements have assuaged initial concerns and successfully averted an over-reaction (10). This is important because it is clear that insulin treatment has a favourable risk-benefit ratio. However, the existence of methodological flaws in these studies does not mean there are no risk differences between different insulins; no evidence of risk is not the same as evidence of no risk and it is possible that recent communiqués on the issue have been overly reassuring. As there are different insulin products available and given the possibility that these might differentially affect the growth rates of specific cancers in specific patients (and the possibility that risk differences might increase with longer follow-up), it is important to increase our understanding of all relevant issues so that informed choices can be made.

The biological plausibility of a link between diabetes, diabetes therapies and cancer

Diabetes appears to be associated with an intrinsic increase in cancer incidence (11). Meta-analysis of six case-control and nine cohort studies, with a total of more than 2.5 million subjects, found a risk ratio (RR) for colorectal cancer of 1.30 [95% confidence interval (CI) 1.20–1.40] for subjects with diabetes vs. no diabetes (12). The findings were consistent for studies in Europe and the USA and

applied to both men and women. A further meta-analysis of 20 studies (five case-control and 15 cohort studies) showed that women with (vs. without) diabetes had a statistically significant 20% increased risk of breast cancer (RR 1.20; 95% CI, 1.12–1.28) (13). The summary estimates were similar for case-control studies (RR 1.18; 95% CI, 1.05–1.32) and cohort studies (RR, 1.20; 95% CI, 1.11–1.30). Meanwhile, Huxley and colleagues reviewed 36 studies (17 case-control, 19 cohort; $n = 9220$) suggesting a combined odds ratio for pancreatic cancer of 1.82 (95% CI, 1.66–1.99) for subjects with diabetes (14). The excess risk was highest among those with a shorter duration of diabetes, which supports the hypothesis that diabetes is often a consequence of pancreatic cancer (rather than *vice versa*). However, the existence of a residual excess cancer risk in subjects with diabetes duration longer than 5 years does support some intrinsic risk resulting from the diabetic state.

Reasons for an intrinsic elevated risk of cancers in subjects with diabetes remain to be established. Type 2 diabetes is associated with comorbidities and risk factors, such as obesity and physical inactivity, which may increase the risk of certain cancers (e.g. colorectal). However, type 2 diabetes is also associated with an altered metabolic and endocrine environment, including hyperglycaemia and hyperinsulinaemia, that may influence cancer biology. In a murine xenograft model of prostate cancer (LNCaP), a high-carbohydrate diet resulting in elevated insulin levels (1.45 vs. 0.45 ng/ml, $p = 0.039$) also resulted in significantly elevated tumour growth (0.88 vs. 0.51 g, $p = 0.04$), with evidence of increased insulin receptor levels in neoplastic tissue and increased signalling downstream of insulin and/or IGF-1 receptors (15).

There is evidence that hyperglycemia is associated with increased cancer risk (16). However, as cancer cells have active and insulin-independent glucose uptake, it is not clear that glucose availability is limiting for neoplastic growth *in vivo*. It is possible that the hyperglycemia in this study was associated with hyperinsulemia, but the insulin levels were not measured. Many cancers do, however, have a high glucose requirement as they are more dependent on glycolysis than normal tissues to generate ATP (17), and this requires more glucose than oxidative phosphorylation. If there are cases where hyperglycemia directly facilitates neoplastic growth, then it is possible that optimization of glycemic control could indeed reduce cancer risk.

A link between circulating insulin and IGF-1 levels and neoplasia has been observed [reviewed in Pollak et al. 2004 (18) and Pollak et al. 2008 (19)].

For example, a number of case-control studies have found an association between elevated IGF-1 levels, prostate cancer (20,21) and breast cancer (22–25), although some studies have failed to detect an association (26,27). High insulin or c-peptide levels have been associated with poor prognosis of breast (28) and prostate (29) cancers, among others.

Activation of insulin or IGF-1 receptors appears to be associated with poor prognosis; in a study of more than 400 cases of invasive breast cancer spanning multiple cancer subtypes, Law and colleagues found phosphorylated insulin receptor or IGF-1 receptor levels and total insulin receptor levels to be associated with poor survival (30). Cox and co-workers found expression of both insulin receptors and IGF-1 receptors in malignant prostate tissue (31).

Transgenic mice over-expressing IGF-1 in mammary tissue showed ductal hyperplasia and spontaneous mammary tumorigenesis (32), and other models show influences of insulin on experimental cancers (15,33).

A growth-promoting effect of insulin on cancer cells has been known for more than 30 years, with these findings pre-dating the commercial availability of insulin analogues or even of recombinant human insulin (34). Neoplastic cells commonly express the IR-A subtype of the insulin receptor and this foetal isoform of the insulin receptor differs in binding IGF-2 as well as insulin (35–38). Hybrid receptors are formed in cells where the insulin receptor and the IGF receptor are co-expressed, and this is common in neoplastic tissue. Thus, the relative expression levels of IR-A, IR-B, and IGF-1R will influence sensitivity to the ligands IGF-1, IGF-2 and insulin.

Investigating the possible mechanisms: insulin-like receptors, insulin analogues and mitogenicity

The most plausible hypothesis concerning the mechanism underlying the potential link between insulin and related peptide hormones and cancer growth is that these act through the insulin and IGF-1 receptors to stimulate cell growth and inhibit apoptosis (19). Studies in this area have been facilitated by the availability of a range of recombinant insulin analogues differing in receptor-binding characteristics (7,39). Of course, the goals of developing modified insulins was to favourably alter pharmacokinetic profiles, rather than alter receptor binding. Sliker and co-workers, examining a series of analogues with modifications in the B10 and B26–30 regions of the insulin molecule, found that the ability to promote growth of human mammary epithelial cells *in vitro* was related to the affinity for the IGF-1 receptor (39). Meanwhile, Hansen and colleagues found that occupancy time at the insulin receptor was also correlated with mitogenic potential: a marked increase in mitogenicity (up to sevenfold) was observed with analogues for which the dissociation constant from the insulin receptor was < 40% that of native human insulin (8). Among the insulin analogues tested by Hansen and colleagues was X10, remarkable for a low insulin receptor off-rate (14% of that of human insulin) and an IGF-1 receptor affinity almost sixfold that of human insulin (7,8). Insulin X10 has been shown to have a mitogenic potency in human osteosarcoma (Saos/B10) cells approaching 10 times that of human insulin (Table 1) (7). X10 was also more potent than insulin in promoting proliferation of the

Table 1 Differences between insulins in receptor binding properties. Data are means \pm SE except for metabolic potency data, which are means \pm 95% CI. Reproduced from Kurtzhals et al., 2000 (7)

Analogue	Insulin receptor affinity (%)	Insulin receptor off-rate (%)	Metabolic potency (lipogenesis) (%)	IGF-I receptor affinity (%)	Mitogenic potency (%)
Human insulin	100	100	100	100	100
B10Asp	205 \pm 20	14 \pm 1*	207 \pm 14	287 \pm 50	975 \pm 173
Aspart	92 \pm 6	81 \pm 8*	101 \pm 2	81 \pm 9	58 \pm 22
Lispro	84 \pm 6†	100 \pm 11†	82 \pm 3	156 \pm 16	66 \pm 10
Glargine	86 \pm 3	152 \pm 13	60 \pm 3	641 \pm 51	783 \pm 132
A21Gly	78 \pm 10	162 \pm 11	88 \pm 3	42 \pm 11	34 \pm 12
B31B32diArg	120 \pm 4	75 \pm 8	75 \pm 5	2049 \pm 202	2180 \pm 390†
Detemir	46 \pm 5‡/18 \pm 2§	204 \pm 9	ca 27¶	16 \pm 1§	ca 11¶

*Data from Hansen et al., 1996. †Data from Sliker et al., 1997. ‡Data from Markussen et al., 1996. §Binding assays for insulin detemir in albumin-free systems. ¶Potencies of free insulin detemir.

human breast cancer cell line MCF7 (40) and pre-clinical studies with X10 indicated an increased tumorigenic potential in female Sprague–Dawley rats (41), leading to discontinuation of its clinical development.

Together, these data provide evidence that mitogenic effects of insulin analogues may be mediated via inappropriate IGF-1R and/or insulin receptor signalling. Absolute proof of this mechanism would be provided by experiments that showed any cancer-promoting properties of an insulin analogue in animal models to be abolished or attenuated by IGF-1R blockade, and/or by competitive displacement from the insulin receptor. Such data do not yet exist; however, circumstantial support was provided by a study by Shukla and colleagues using human mammary cell lines sensitive to insulin (42). In this study, benign (MCF10A) and malignant (MCF7) cell lines were exposed to a series of commercially available insulins. Compared with human insulin, insulin glargine showed a significant increase in proliferative potential of 1.6- to 3.1-fold in MCF7 cells, the mitogenicity of the other insulins being similar to or lower than human insulin. Insulin glargine was seen to strongly activate the MAPK pathway in the MCF7 cell line; inhibition of this pathway using a specific MAPK inhibitor completely suppressed this proliferative activity. Importantly, knockdown of the IGF-1 receptor, but not knockdown of the insulin receptor (by transfection with IGF-1R siRNA and IR siRNA respectively), also abolished proliferation of the cell line in response to insulin glargine, strongly implicating IGF-1 receptor mediated activation of the MAPK pathway as the driver of cell proliferation (42). However, in other situations, it is conceivable that the degree of insulin receptor activation will be critical, and that peak insulin levels and/or the time course of insulin exposure will be important. There are important gaps in knowledge relating to differences between various insulins, with respect to activation of hybrid receptors, and also with respect to differences (if any) between the insulin receptor isoforms.

Given present knowledge, it is important that the receptor binding properties of insulin analogues are tested to ensure that the balance of IGF-1R:IR affinity is not disturbed, and that insulin receptor off-rate is not unduly reduced. It is of interest in the context of the findings of Shukla and colleagues (42), and of the epidemiological findings discussed below that insulin glargine has an increased affinity for the IGF-1 compared with native human insulin in cells expressing this receptor (7). Using the Saos B10 cell line, Kurtzhals and colleagues found a mitogenic potential for insulin glargine 7.83 times that of human insulin (Table 1), a finding consis-

tent with data obtained by Kohn and colleagues, who reported a 4.1-fold increased proliferation of human mammary epithelial cells with insulin glargine vs. human insulin (43).

Insulin glargine is widely prescribed and, as such, underwent rigorous testing in animal and clinical studies, and it should be noted that no increased cancer incidence was seen when it was tested in Sprague–Dawley rats (44), in contrast to the findings with insulin X10; nor was any excess cancer risk reported during the insulin glargine clinical development programme. However, carcinogenicity studies in Sprague–Dawley rats using insulin glargine could not be performed at the doses where X10 manifested increased cancer incidence, because of the prolonged hypoglycaemic effect of insulin glargine (45). Furthermore, as occult cancers are common in adults (46,47), it can be argued that it is appropriate that safety studies of hormonal agents with respect to neoplastic disease should not be confined to carcinogenesis endpoints, but should also include assessment of effects on pre-existing tumours. This is in keeping with the highly plausible view that hormones may influence neoplastic disease by stimulating proliferation, rather than by acting as mutagens.

The difficulties of performing animal studies and the need to screen a large number of candidate molecules mean that *in vitro* studies remain an important tool for evaluating the relative mitogenic potential of insulin analogues. It is important that receptor binding is appropriately tested as there are two pitfalls that may give falsely reassuring data. Firstly, mitogenic properties are manifest in some cell lines and not in others. The effects tend to be greatest in cells that express a relatively high proportion of IGF-1 receptors compared with insulin receptors (8,42). Therefore, mitogenicity must be tested in sensitive cell lines.

Secondly, cell lines responsive to insulin or IGF-1 have a maximum mitogenic response to these hormones that can be elicited by nearly all insulin-like molecules (including insulin itself and IGF-1) when given at sufficiently high concentrations, with the effective concentration differing between hormones (see Figure 1). It follows that the relative mitogenic potency of different insulins can only be assessed if full dose-response curves are constructed for parameters of metabolic and mitogenic activity. The dose required to elicit ED50 responses can then be calculated and the ratio of these for mitogenic vs. metabolic activity discerned. If dose-response curves are parallel, as is normal, this value will be a constant over the effective dose-response range of the insulins. Single-dose experiments will give very different results according to where the dose chosen relates

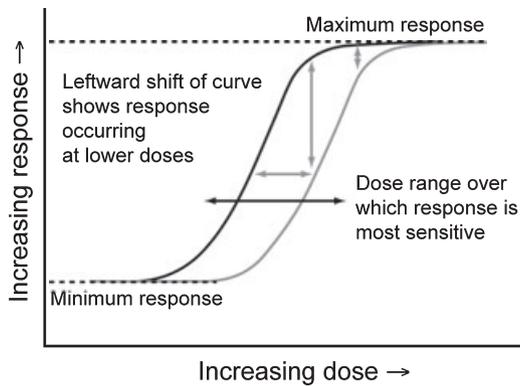


Figure 1 Schematic showing hypothetical insulin dose-response for two insulins that differ in their potency to evoke a response. This illustrates how single-dose assessments (vertical distances) will give varying results depending on the dose chosen, whereas differences based on ED50 (horizontal distance) will apply across the whole tissue-respondent portion of the dose-response curve

to the dose-response curve (Figure 1). If the concentration is close to the maximum (or minimum) response level then no difference between insulins will be discerned, and this could again be falsely reassuring. These issues were illustrated by a study reported by Weinstein and colleagues, in which three cell lines were exposed to a single 100-nM level of four insulins (human insulin, insulin lispro, insulin detemir and insulin glargine); at this supra-physiological level, few differences were evident between insulins, whereas in a separate signalling experiment performed at a 10-fold lower concentration, activation of both insulin receptor and IGF-1 receptor was seen with insulin glargine, whereas insulin detemir activated the insulin receptor alone (48).

What is the clinical evidence linking insulins and cancer?

First, it should be noted that, for patients with insulinopenic diabetes, insulin therapy is a clinical necessity to limit morbidity and mortality. With regard to cancer risk, it is therefore a matter of determining whether there are choices to be made based on any difference in risk between insulins. The available evidence is somewhat ambiguous and inconsistent. There are, however, some observations that we believe indicate the need for further study.

Hemkens and colleagues conducted a cohort study using insurance records of more than 127,000 patients treated in Germany with insulin glargine, insulin aspart, insulin lispro or human insulin (1). They found a dose-dependent association between

use of all these insulins and cancer. Compared with human insulin, the dose-dependent risk increase was steep for insulin glargine (HR 1.31 for 50 IU dose, $p < 0.0001$), but not for insulin aspart or insulin lispro. It should be noted that the finding of an excess cancer risk with insulin glargine only emerged after adjustment for dose; patients receiving human insulin had higher mean doses and the risk of malignancy before adjustment was lower with all three insulin analogues than with human insulin. This study is, nevertheless, important because of the large numbers of individuals involved. However, as with any retrospective cohort study, it is limited by the fact that patients were not randomised to treatment groups and despite corrections for all available confounders, several potentially relevant factors, such as body mass index and duration of diabetes were not available.

The serious questions raised by the analysis performed by Hemkens and colleagues and the equally serious reservations regarding its methodology and the apparent biological implausibility (to reviewers in the arena of diabetology) of cancer development in the short follow-up of 1.31 years, led to the commissioning of a number of confirmatory studies (5). Jonasson and co-workers examined Swedish registry data for almost 115,000 patients who had received insulin during a 6-month period in 2005 (2). They found that during the subsequent 2-year period, the risk of developing breast cancer among women receiving insulin glargine monotherapy was significantly higher than among those using other insulins (RR 1.99, 95% CI, 1.31–3.03). Risk of development of other forms of cancer and of malignancy overall, however, was not significantly elevated with insulin glargine compared with other insulins. As with the German study, this analysis had limitations: principally that there was no dose information available, and thus no correction for dose was possible; that all other insulins including other analogues were combined in the control group (which raises the question of what is the most appropriate reference group in studies of cancer risk with insulin) and the fact that, again, this was not a randomised study and thus some degree of selection bias is inevitable.

Colhoun and colleagues examined the national registry data of almost 50,000 insulin-treated patients in Scotland (3). Overall, exposure to insulin glargine (with or without bolus insulins) was associated with a reduced rate of total cancers (RR 0.66, 95% CI, 0.57–0.76). In common with the previous studies, however, patients receiving insulin glargine alone had a higher risk of cancer than those receiving another insulin without insulin glargine (RR 1.66, 95% CI,

1.06–2.60), with a significant increase in risk of breast cancer (RR 4.37, 95% CI, 1.64–11.7). The plausibility of a modifying effect of co-administration of another insulin on the relationship of glargine to cancer risk is open to question. A further UK analysis by Currie and co-workers examined people with type 2 diabetes treated in UK general practice and treated with insulin or oral antidiabetic agents (4). Patients treated with metformin had significantly lower risk of development of solid tumours, compared with those treated with sulphonylurea (RR 1.36, 95% CI, 1.19–1.54) or insulin-based therapy (RR 1.42, 95% CI, 1.27–1.60). No significant differences in cancer risk were detectable among the four insulin categories, namely insulin glargine, human basal insulin, human biphasic insulin and analogue biphasic insulin. The apparent protective effect of metformin is consistent with other research, including an early study by Evans et al (49) and a more recent one by Libby and colleagues (50), in which patients with type 2 diabetes treated with metformin had a lower risk of cancer than those without record of metformin use; it is plausible that the effects of metformin are mediated by the reduction of insulin levels observed when this agent is given to hyperinsulinaemic patients, and/or to direct antiproliferative actions on cancer cells involving activation of AMP kinase (19,51–54).

A prospective trial assessing the effect of insulin glargine on proliferative retinopathy was also recently reported (55). This 5-year study is of interest because IGF-1 is implicated in the pathophysiology of proliferative retinopathy (56). The study reported by Rosenstock and colleagues showed no difference between insulin glargine and NPH insulin with regard to progression of retinopathy. When the database used for the retinopathy analysis was retrospectively analysed for cancer endpoints, no increase was seen with insulin glargine, although it is to be noted that the study was not designed nor powered to detect relatively infrequent cancer endpoints, the comparison of serious malignancies being based on up to 31 events per group. The findings regarding retinopathy are reassuring and robust; however, as the mechanism of IGF-1 involvement with retinopathy is likely to be paracrine, no extrapolation should be made from these results *per se* to systemic cancer risks.

Data from this study have, however, also been combined with those from a further 30 studies (mostly of 6 months' duration) from the manufacturer's database to enable a comparative assessment of glargine vs. pooled comparators (mostly NPH insulin) to be made in a total of 9235 patient-years

of treatment exposure (57). In this analysis, 52 treatment-emergent malignancies were documented in 45 of 5675 glargine-treated patients (0.8%), vs. 48 cases in 46 of 5223 patients from comparator groups. Most of these cases occurred in patients with type 2 diabetes and there were no significant differences in the overall or prespecified cancer rates between the insulin glargine and pooled comparator groups. Dose–response relationships or comparisons by regimen were not assessed in this analysis.

Further epidemiological data are available from a meta-analysis of the Novo Nordisk database of clinical trials involving insulin detemir (58). Here, the data again suggest very low absolute event rates, with the relative risk for malignancy with insulin detemir being statistically significantly lower than for NPH insulin: 0.36 vs. 0.92 events per 100 patient-years of exposure, $p < 0.05$ (based, respectively, on 2252 and 1420 patient-years of exposure). A comparison of insulin detemir with insulin glargine (most data in type 2 diabetes) gave respective values of 0.87 and 1.27 events per 100 patient-years (NS), but this was based on only 917 and 628 patient-years of exposure.

Is a difference in the incidence of cancer plausible in less than 2 years of follow-up?

One of the criticisms levelled against these studies is that they involved short follow-up periods and, where differences in cancer incidence emerged, these were seen in time intervals of less than 2 years, a fact that seemed implausible to some commentators. However, it is our opinion that it is indeed biologically possible for differences in the cancer diagnosis rate to arise in this timespan, as the observed effects are likely to reflect not the initiation of new tumours but the growth of subclinical tumours into diagnosable volumes. It is considered likely that such subclinical lesions are common in most adults – autopsy studies suggest that undetected cancers are present in a sizeable proportion of adult men (46) and women (47). Evidence of the plausibility of rapid changes in risk of clinical cancer diagnosis is provided by the rise in breast cancer incidence in the months that follow pregnancy (59) and, conversely, by the reduction in breast cancer incidence that was observed in the immediate aftermath of the fall from grace of oestrogen replacement therapy (60), as well as by the reduced risk of cancer that is observable in the early months following bariatric surgery (61,62).

What next?

More questions have been raised than have been answered by the data now available to us and we hardly need state that further research is needed. Further independent observational and epidemiological studies are indicated, but expectations should be tempered; these may not be able to provide a reliable quantification of relative risks unless the effect size is large and will always be subject to confounding issues. On the other hand, the effect of insulin analogues on cancer risk or development is unlikely to form a suitable subject for a prospective interventional controlled trial. We therefore need imaginative alternatives. These might include careful studies of tumour biology and cell signalling pathways in neoplastic tissue from cancer patients who also have diabetes and are receiving insulin, and the development of improved animal models.

In the past, carcinogenicity of insulins was assessed experimentally in large part by using 2-year exposures in non-diabetic rodents; this method led to some deaths because of hypoglycaemia and is sub-optimum for assaying the effects of treatment on pre-existing cancers. Therefore, it will be of interest to compare tumour growth in control hosts, untreated diabetic hosts and diabetic hosts receiving various insulins, as an example. There is also a need to develop a validated functional assay of the mitogenicity of human serum and to determine if this is influenced by type 1 or type 2 diabetes or by various diabetes treatments.

It must be emphasised that type 2 diabetes has been associated with increased cancer risk, as discussed above. This may be related to elevated endogenous insulin levels seen in the setting of insulin resistance of classic insulin target organs, while tumors remain insulin sensitive, but other mechanisms have not been ruled out. Similarities and differences in the effects of endogenous insulin and various administered insulins on cancer endpoints in experimental models requires study.

In the meantime, in agreement with statements issued by the major diabetes associations, there is no need to panic; insulin has an excellent risk-benefit ratio and any absolute risk differences between analogues are likely to be small. However, careful consideration of the choices available might be considered wise for patients who are at high biological risk of cancer (for example, those with family or personal history of cancer) and many of these patients will appreciate discussion of these issues if they require pharmacotherapy for diabetes. The point at which insulin initiation takes place might also be usefully reconsidered, although there are cur-

rently few risk-benefit data to guide decisions on this issue. An increased risk of cancer associated with insulin therapy in general or with certain modified insulins cannot be excluded on the basis of available data, but this must be considered in the context of the relatively high and well-documented risks of morbid endpoints encountered when diabetes is poorly controlled.

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