Expert Opinion

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Controversies in the use of insulin analogues

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Introduction: In recent years, insulin analogues have been developed in order to improve the pharmacological parameters of insulin and to better mimic endogenous insulin output. Given that some of the modifications introduced into insulin analogues are located in a domain involved in a potential interaction with the insulin-like growth factor-I receptor (IGF-IR), it has been postulated that certain analogues may display IGF-I-like activities.

Areas covered: We review the recent literature investigating the risk of malignant neoplasms and mortality in diabetic patients treated either with human insulin or with one of three insulin analogues (lispro, aspart, and glargine). We examine how critical analyses are consistent with the notion that the use of insulin glargine is associated with a possible increased risk of tumors in humans.

Expert opinion: The introduction of insulin analogues has had a major impact in diabetes care. However, the benefit of some of these new insulins for the patient has yet to be demonstrated. Furthermore, research is needed to clarify whether insulin glargine is more strongly associated with cancer risk compared with other insulins.

Keywords: cancer, detemir, glargine, IGF-I, insulin, insulin analogues

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1. Historical background

Endogenous insulin secretion is composed of two distinct patterns of discharge, that is bolus (postprandial) and basal (constitutive) secretions. Each form of insulin secretion exhibits typical regulatory features and is responsible for the control of specific metabolic events. Exogenous insulin administration is the only therapy available for type 1 diabetes mellitus and is also a valuable therapeutic tool in the treatment of type 2 diabetes mellitus. To overcome the difficulties in the treatment of diabetes mellitus a continuous search aimed at improving the chemical formulations of human insulin preparations was and remains one of the central goals of the pharmaceutical industry. As regular insulin used alone between 1922 and the 1940s had a short half-life, one of the early objectives was to design a long-acting preparation. This led Hagedorn *et al.* [1] to formulate 'protamine insulin', which was modified to 'protamine zinc insulin' by Scott and Fischer [2], and later to 'neutral protamine Hagedorn' (NPH) insulin by Krayenbühl and Rosenberg [3]. NPH insulin is used until today as one or two daily injections. Given that regular insulin does not act fast enough on the metabolism of ingested carbohydrates and proteins at meals, and in view of the fact that the action of NPH insulin is not longer than 10 – 12 h, the search for quicker, short-acting and long-acting insulins continued. Advancing technologies, including recombinant DNA methodologies, enabled the manipulation of the insulin molecule and opened the new era of 'biotech insulin analogues' [4].

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Article highlights.

- Insulin analogues were developed in order to improve the pharmacological parameters of insulin.
- Insulin analogues are divided into two major subgroups: short- and long-acting analogues.
- Recent epidemiological studies suggest that the use of insulin glargine, a long-acting analogue, in type 2 diabetes is, after adjusting for dose, associated with a possible increased risk of tumors in humans.
- Several authors have criticized the reports linking glargine therapy with increased cancer prevalence, mainly on methodological grounds.
- *In vitro* studies are consistent with the notion that insulin glargine (and probably other analogues as well) display IGF-I-like proliferative and antiapoptotic activities.
- Insulin glargine exhibits atypical signaling activities, including strong activation of the IGF-I receptor.
- Further clinical and basic research is needed to clarify whether insulin analogues and, in particular, insulin glargine are more strongly associated with cancer risk compared with native insulin.

This box summarizes key points contained in the article.

One of the first analogues to be synthesized was insulin Asp B10, in which a histidine residue was exchanged for an aspartic acid at B10, a position important for the affinity of the molecule to the insulin-like growth factor-I receptor (IGF-IR). This analogue, however, was found to have a carcinogenic effect in female rats and, therefore, abandoned for clinical use [5]. Subsequently, Novo-Nordisk synthesized a fast-acting analogue termed Asp B28 in which a proline residue was substituted by a charged aspartic acid at position B28 [6]. Another example of a fast-acting insulin is the 'LysPro' insulin in which the sequence of B28 and B29 residues is reversed (**Figure 1**) [7]. Another fast-acting insulin is 'Glulisine', obtained by substituting lysine at position B29 for glutamine and aspartic acid at position B3 for lysine [8]. **Table 1** shows some of the milestones in the development of insulins and insulin analogues.

Among the long-awaited, long-acting insulins there are at present two clinical formulations with only a few years of clinical experience. The first one is insulin glargine (Lantus, Sanofi-Aventis) in which two arginine residues have been added to the B chain at positions 31 and 32 and glycine has been substituted for histidine at position 21 in the A chain (**Figure 2**) [9]. The slow dissolution of hexamers into the blood results in a flat 24 h lasting effect [10]. The second long-acting analogue synthesized is insulin detemir (Levemir, Novo-Nordisk) in which the 14-carbon myristic acid is acylated to the B29 lysine position [11]. The myristic acid of insulin detemir binds to albumin and forms a stable but reversible complex. The dissociation of this complex delays the absorption of insulin from the subcutaneous tissues and, therefore, its duration of activity ranges between 12 and 14 h [12].

2. Clinical aspects

The few experimental reports documenting the putative mitogenic activity of a number of insulin analogues, in particular insulin glargine and detemir, raised a worldwide curiosity, tension, and a rush to summarize present available clinical experience. The publication of the first summaries raised controversial discussions at scientific meetings and in published reports [13] and alarm in the public media. Before presenting the 'pros' and 'cons' data, we would like to emphasize the difficulties in assessing and judging the findings:

- The long-acting insulin analogues are used as basal secreted hormone and rapid-acting insulins are injected at meals. Only rarely are they injected alone in type 2 diabetic patients.
- The experience with the new analogues is rather short, not longer than 5 years.
- The genetic predisposition to cancer in the populations reported is usually not mentioned in most studies published so far.
- Use of insulin analogues in type 1 as compared with type 2 diabetes mellitus may lead to different outcomes.
- Confounding effects of glucose control are missing in the publications.
- There is lack of consistency between methods of assessment.
- These, and probably other, limitations should be kept in mind when reading the present available literature, summarized forthwith.

3. Epidemiological reports – the search for a link between insulin, glargine and cancer

The recent seminal paper published by Hemkens *et al.* [14] in Diabetologia investigated the risk of malignant neoplasms and mortality in patients with diabetes treated either with human insulin or with one of three insulin analogues. The study comprised 127,031 patients studied between January 1998 and June 2005, and was based on data provided by Germany's largest statutory health insurance fund, the Allgemeine Ortskrankenkasse. Of these patients, 27,347 received insulin glargine and 95,804 human insulin. The mean follow up was 1.63 years (maximum 4.41 years). Analysis by multiple Cox regression models adjusted for potential confounders such as sex, age and dose revealed a statistically significant increased cancer risk in those treated with insulin glargine compared with those treated with human insulin ($p < 0.0001$). Considering that the glargine dose was lower than that of human insulin proved, according to the authors, that the mitogenic properties of insulin glargine are greater than those of human insulin. Several authors criticized this report, mainly on methodological grounds. For example, Pocock and Smeeth [15] criticized the study because the allocation to treatment groups and drugs doses was not determined before follow up. Our

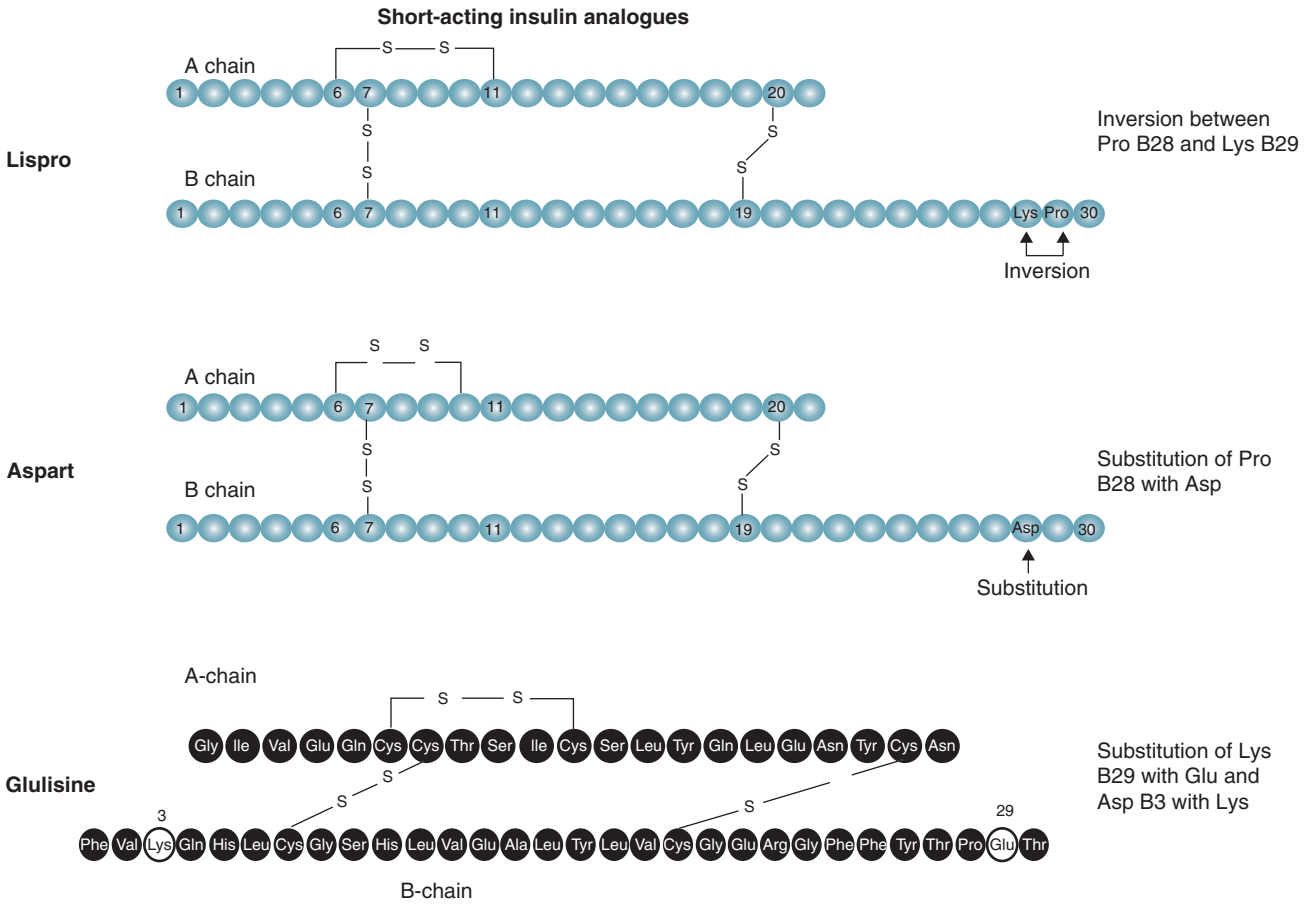

Figure 1. Typical short-acting insulin analogues currently in clinical use. Short-acting insulin analogues are designed to mimic postprandial insulin secretion. Usually, they exhibit an onset in less than one hour and the duration of their effect is less than four hours. Ideally, they should be nonimmunogenic, chemically stable, and mixable with other insulins and insulin analogues. The hypoglycemic potency of the analogues should be equal to or greater than that of human insulin. Schematic representations of analogues lispro, aspart, and glulisine are shown in the figure.

opinion is that it is impossible to predict in long-term clinical circumstances that no change in drug or dose will be needed. It is of note that besides the mostly harsh criticism published in response to the Hemkens *et al.* paper and the accompanying editorial in Diabetologia, Peter Butler, Editor-in-Chief of Diabetes, congratulated the editorial team at Diabetologia for writing a balanced editorial and for obtaining the additional studies (see below) linking the use of insulin and insulin analogues with cancer [13].

In the same issue of Diabetologia, Jonasson *et al.* [16] reviewed 114,841 patients with diabetes mellitus in Sweden treated with insulin between July and December 2005 and studied for cancer prevalence between January 2006 to December 2007. Patients who had been diagnosed with cancer before the initiation of the study were excluded. Poisson regression analyses were used to evaluate the association between the patients using glargine alone or other types of insulin and malignancies. After adjustment for age at onset of diabetes, sex, smoking, etc., the main finding was that women using glargine monotherapy had an increased incidence of breast cancer when compared with women using other insulins [relative risk (RR) 1.99 (95% CI, 1.31 – 3.03)], or glargine in combination with other insulins [RR 1.10 (95% CI, 0.77 – 1.56)].

In addition, Colhoun *et al.* [17] reviewed the registry data of almost 50,000 insulin-treated diabetic patients in Scotland. Patients receiving insulin glargine alone had a higher risk of cancer than those receiving other insulins without glargine (RR 1.66 [95% CI 1.06 – 2.60]). Of interest, a highly significant increase was seen in the risk of breast cancer [RR 4.37 (95% CI 1.64 – 11.7)]. In a study sponsored by Sanofi-Aventis, Rosenstock *et al.* [18], summarizing an open-label 5-year trial of insulin glargine versus NPH insulin in 1024 diabetic patients designed to study retinopathy, reported 57 cancer cases in the glargine-treated group and 62 cancer cases in the NPH-treated patients. Hence, no differences in cancer incidence between treatments were observed in this study.

Table 1. Milestones in the development of insulins.

Year	Milestone in insulin development	Ref.
1922	Insulin discovered	[47]
1922	Regular human insulin first used	[47]
1936	Protamine insulin	[1]
1936	Protamine zinc insulin	[2]
1946	NPH insulin	[3]
1981-88	Biosynthetic insulins	[4]
1990	Insulin Aspart B28	[6]
1992	First insulin analogue reported to be carcinogenic	[5]
1992	Lys Pro insulin	[7]
1997	Insulin detemir	[48]
2000	Insulin glargine	[9]
2003	Insulin glulisine	[8]
2009	Epidemiological studies show correlation between glargine use and cancer incidence	[14,16,17]
2010	Consensus statement on Diabetes and Cancer by the American Diabetes Association and the American Cancer Society	[6]

Recently, Mannucci *et al.* [19] reported a case control study involving 1340 insulin-treated diabetic patients starting insulin between January 1998 and December 31, 2007. For each patient, up to five control patients matched by age and sex were selected. Chi-square test was used for comparison between the five age groups designed. During a median follow up of 75.9 months (6.3 years), 112 patients with cancer were observed (60 males, 52 females). Their mean age was 68.9 ± 9.9 years; mean duration of diabetes was 8.4 (0.3 – 21) years. The length of the follow-up in the controls was 10 years. Cancer was significantly associated with a high dose of glargine (> 0.3 IU/kg) and was greater than with other types of insulin.

Finally, analyzing the manufacturer's (Sanofi-Aventis) database of insulin glargine clinical follow-up, consisting of 5657 insulin glargine-treated patients and 5223 controls using mostly human insulin preparations, Home and Lagarenne [20] reported 52 cases of cancer in the glargine-treated groups and 48 patients with cancer in the control group. The study durations were from 4 to 48 weeks with one longer exception in the control group. No ages are stated. In summary, the authors concluded that this study doesn't show an increased prevalence of cancer in glargine-treated patients. The main limitations of this study were the use of the manufacturer's data and the short duration of treatment.

4. Insulin glargine and retinopathy

In addition to the controversies generated by the use of insulin analogues in the specific context of cancer, debate took also place regarding other potential (non-malignant) complications, including retinopathy. Four of the randomized multinational insulin glargine trials of 28 – 52 weeks duration were reviewed for development of retinopathy [21]. During the treatment period, retinal examinations (including evaluation of proliferative diabetic retinopathy, macular oedema and other adverse events) were done in 2207 patients by fundoscopic clinical examination and also fundus photographs. In one of the four studies (Early Treatment Diabetic Retinopathy Study) more patients in the insulin glargine group had a three-step or greater progression on the standard scale for evaluation of retinopathy (7.5 versus 2.7%, $p < 0.05$). Taken as a whole, however, the authors concluded that results do not suggest any increased risk in the development or progression of retinopathy in patients treated with insulin glargine compared with NPH insulin. In another study, a higher incidence of new onset macular oedema (11.2 versus 6.5%) was observed [22]. Rosenstock *et al.* [18], summarizing a five-year follow-up study of 1024 patients with type 2 diabetes sponsored by Sanofi-Aventis, concluded that the 515 patients receiving once daily glargine showed no evidence of greater risk of the development or progression of retinopathy than the 509 patients receiving twice daily NPH insulin. It needs to be mentioned that the various studies display a lack of correlation between methods of assessment.

5. Insulin determir versus insulin glargine

As mentioned above, the particular feature that differentiates insulin detemir from all other analogues and unmodified natural insulin is the coupling of the analogue to albumin, which occurs immediately after injection. It is estimated that approximately 97% of the applied insulin detemir is bound to albumin. Tumor cells actively metabolize albumin to supply their increased needs for amino acids and energy [23]. Hence, insulin detemir could be transported into tumor cells by its coupling to albumin. As a corollary, the insulin content of tumor cells might be relevant for tumor growth. Dejgaard *et al.* [24] performed a meta-analysis in a population of 8693 patients with diabetes, part of a Novo-Nordisk sponsored randomized and controlled trial. The number of patients treated with insulin determir was 1219 (44% females) and 830 for insulin glargine (44% females). The total number of malignancies registered for determir as well as for glargine was 16 whereas the number of cancers in the 6644 patients treated with human insulin was 26. Hence, the prevalence of malignancies in the long-acting insulin analogues group was greater than that in the human insulin group (32 out of 2049 versus 26 out of 6644). The median treatment duration was 51 weeks for determir or glargine.

6. Insulin analogues and gestational diabetes

Of interest is the paper by Singh *et al.* [25] who analyzed 68 randomized controlled studies with the aim of comparing the efficacy of the new short- and long-acting insulin analogues with that of conventional insulin in the treatment of both type 1 and type 2 and gestational diabetes mellitus.

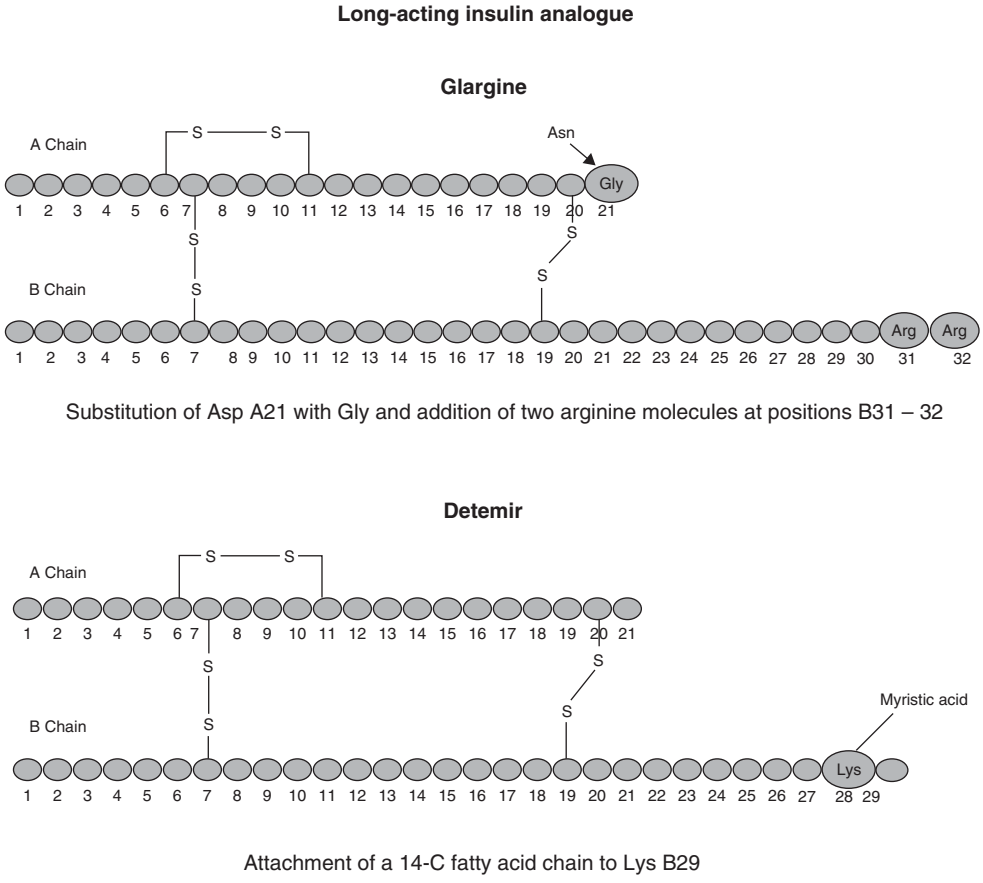

Figure 2. Typical long-acting insulin analogues currently in clinical use. The two arginine residues added to the B-chain in insulin glargine result in an insulin which is soluble at the acidic pH 4.0 – 5.0 of the injection medium but precipitates once injected into the subcutaneous tissue where pH 7.4 is physiological. Less soluble insulin is absorbed slowly, but is therefore more susceptible to degradation before it is absorbed. The A21 arginine to glycine substitution retards this degradation. Insulin detemir is 98% reversibly bound to free fatty acid binding sites on albumin in plasma and interstitial fluid. This unique mechanism of albumin binding prolongs its duration of action and contrasts with other long-acting insulins whose duration of action is dependent on the rate of dissociation of various sized crystals at the subcutaneous site. The mechanism of an insulin analogue binding to albumin provides a depot of insulin in the blood rather than the subcutaneous tissue.

The use of long-acting insulin analogues in gestational diabetes was started only recently. So far the studies are of short duration. The authors report included also a few reports on the use of insulin analogues in the pediatric age group. Judging in terms of glycated haemoglobin (HbA1C) and incidence of hypoglycemia, the authors concluded that rapid and long-acting insulin analogues offer little benefit relative to conventional insulins.

7. Basic aspects of insulin analogues

The question whether insulin is capable of inducing or promoting mitogenic effects through its cognate receptor or via IGF-IR has been a controversial issue for many years [26-28]. In fact, a number of studies revealed that some of the newly developed insulin analogues exhibit an increased affinity for the IGF-IR and display atypical activities, such as inhibition of apoptosis and abnormal post-receptor signaling compared with native insulin [29-31]. We describe below recent *in vitro* and *in vivo* studies analyzing some of the biological actions of insulin analogues, as well as the signaling pathways activated by the analogues.

7.1 *In vitro* studies

In a recent study we investigated the proliferative activities of long-acting insulin analogues glargine and detemir and short-acting analogue lispro in cultured colorectal (HCT116), prostate (PC3), and breast (MCF7) cancer cell lines [32]. Results of cell counting and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays revealed that the proliferative effects of the analogues in the above cell lines significantly exceeded the effect of regular human insulin (**Figure 3A**). The analogues, however, were usually less potent than IGF-I. In addition to their proliferative activity, our

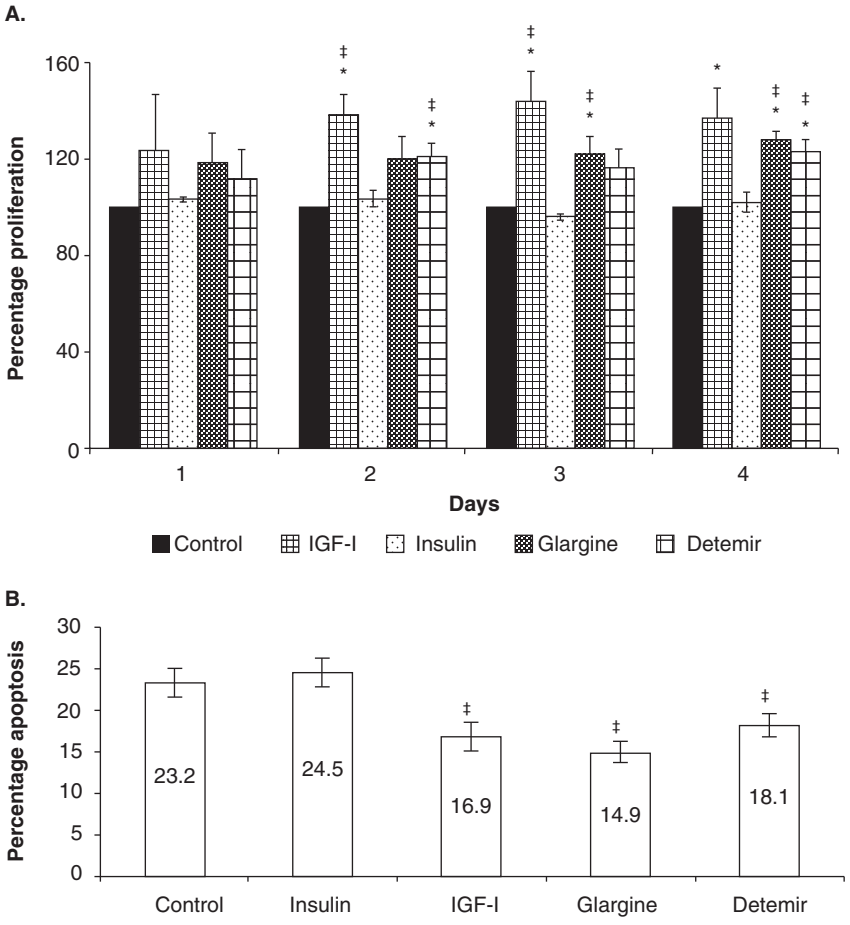

Figure 3. A. Proliferative effects of insulin analogues in the colorectal cancer cell line HCT116. HCT116 cells were plated in 6-well plates in complete medium. After 24 h cells were transferred to serum-reduced medium and incubated for 4 days in the presence of 100 nM of IGF-I, regular insulin, glargine, or detemir. Hormones were replenished on a daily basis. Cells were tripsinized every 24 h, stained with Trypan blue, and counted using a hemocytometer. The number of cells in diluent-treated wells (controls) each day was assigned a value of 100%. This type of graphic representation allows for a comparison between the effect of the various analogues at any time point, however, it doesn't allow visualization of the growth progression during the four days of the experiment. Bars are mean ± sem (n = 3). **B**. Analysis of the effect of glargine and detemir on apoptosis. Serum-starved HCT116 cells were treated with 100 nM of IGF-I, regular insulin, glargine, or detemir for 12 h and apoptosis was evaluated using an Annexin-fluorescein isothiocyanate (FITC) kit. Quantitative analysis of the flow cytometry data was performed using the WinMDI 2.8 software.

*Significantly different versus controls ($p < 0.05$)

‡significantly different versus insulin-treated cells ($p < 0.05$).

study indicates that insulin glargine and detemir elicited an antiapoptotic action in HCT116 cells (**Figure 3B**) [32]. The effect of these analogues resembled the typical prosurvival activity of IGF-I. Taken together, our results indicate that pharmacological doses of long-acting insulin analogues can potentiate the intrinsic mitogenic capabilities of cancer cells *in vitro*. It is of interest that a recent study showed that both human insulin and glargine significantly enhanced ^{3}[H]-thymidine uptake and MTT-assayed proliferation of normal human breast epithelial cells (MCF-10) and MCF7 breast cancer cells at doses of 50 – 100 nM. No differences, however, were seen in this study between regular insulin and the analogue [33].

In terms of the signaling pathways elicited by long-acting analogues we have recently performed a study aimed at identifying the receptor/s and cytoplasmic mediators responsible for the biological actions of insulin glargine and detemir [34]. Using co-immunoprecipitation assays and confocal microscopy we provided empirical evidence for dual activation of both the insulin receptor (IR) and IGF-IR by the analogues. **Figure 4** shows results of experiments obtained with insulin glargine. Dose-dependency assays showed that

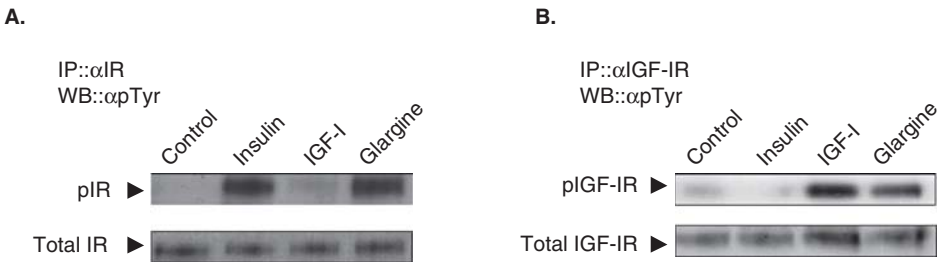

Figure 4. Activation of the insulin receptor (IR) and IGF-IR by glargine. To compare the activating potential of glargine with that of regular insulin or IGF-I, starved HCT116 cells were treated with the ligands (50 ng/ml) for 10 min, after which cells were collected, immunoprecipitated (IP) with anti-IR (**A**) or anti-IGF-IR (**B**) for 24 h, electrophoresed through 10% SDS-PAGE, and immunoblotted with anti-phosphotyrosine. Membranes were then incubated with antibodies against total IR or IGF-IR to confirm equal loading of samples. Results indicate that glargine was able to activate both the IR and IGF-IR.
Adapted from [34].

glargine phosphorylated the IGF-IR at fivefold lower doses than those required to activate the IR. Furthermore, our data indicate that the analogue can lead to prolonged activation of the receptors and, therefore, promote abnormal signaling. In terms of downstream signaling activation, the picture was very complex and we observed that glargine activated Akt in an insulin-like manner, whereas it activated Erk similarly to IGF-I. Finally, using a green-fluorescent-tagged IGF-IR we showed using confocal microscopy that glargine, similarly to IGF-I, induced IGF-IR internalization and redistribution (**Figure 5**). Regular insulin was unable to induce IGF-IR internalization. The biological implications of the atypical signaling patterns of glargine and detemir need to be further investigated.

A recent study by Shukla *et al.* [35] provided a comparative analysis of the proliferative potency of regular insulin and four analogs that are presently approved for clinical use, as well as the signaling pathways activated by them in seven human mammary epithelial cell lines expressing different levels of IR, IGF-IR, and the IR substrate-1 (IRS-1). Only insulin glargine in comparison with regular insulin had significantly stronger mitogenic properties in MCF7 breast cancer cells characterized by a high IGF-IR:IR ratio. This effect was mainly due to the activation of the IGF-IR and MAPK pathway. In MCF10A cells characterized by a low IGF-IR:IR ratio, glargine-induced proliferation was not different from that induced by regular insulin.

In a recent study by Sciacca *et al.* [31], short-acting (insulin lispro, aspart, and glulisine) and long-acting (insulin glargine and detemir) analogues were studied in three engineered cell models, IGF-IR-deprived mouse fibroblasts transfected with either human IR-A, IR-B or IGF-IR. Receptor binding and phosphorylation, Akt and Erk activation, cell proliferation and colony formation were evaluated after exposing the cells to each analogue and were compared with insulin, IGF-I and the carcinogenic analogue B10Asp. Results showed that all short-acting analogues produced molecular and biological effects similar, but not identical, to those of regular insulin. Relative to insulin, long-acting analogues more strongly activated the Erk pathway via both IR-A and IGF-IR. At the concentration tested, no analogue (except B10Asp via IR-A) had increased transforming activity.

Consistent with the potential mitogenic action of insulin glargine, a recent paper by Mayer and Chantelau [36] examined the proliferative potency of serum of patients with type 1 diabetes treated with glargine. Pairs of serum samples from 31 C-peptide-negative patients were investigated. In cross-over fashion, 23 patients were treated with glargine plus rapid-acting insulin analogues, and similar doses of NPH insulin and rapid-acting insulin. For comparison, eight patients were treated with insulin detemir and NPH insulin. Proliferation was assessed by incubating MCF-7 cells with 10% serum for 72 h. Results showed that serum containing insulin glargine was 1.11 (95% CI 1.05 – 1.18) fold more mitogenic than human-insulin-containing serum ($p < 0.005$); mitogenicity of serum containing detemir was 0.99 (95% CI 0.98 – 1.02) fold that of human-insulin-containing serum. The clinical implication of the slightly enhanced mitogenic potency of glargine-containing serum needs to be further investigated.

7.2 Animal studies

In contradistinction to the *in vitro* studies reported above and those of others [30,35,37,38], life-long insulin glargine treatment of rats and mice receiving daily injections of increasing doses of the analogue had no carcinogenic effect [39]. Although an increase in mortality rate was observed in male rats at every glargine dose and in female rats in the high-dose-glargine group, these results are consistent with the prevailing view that regular insulin as well as insulin analogues by themselves do not induce malignant transformation. It should be mentioned, however, that in this experiment a large proportion of the animals died from hypoglycaemia before the end of the 12-month observation period. Therefore, the number of animals that could be observed in relation to malignancies was rather small. Furthermore, neither regular insulin nor

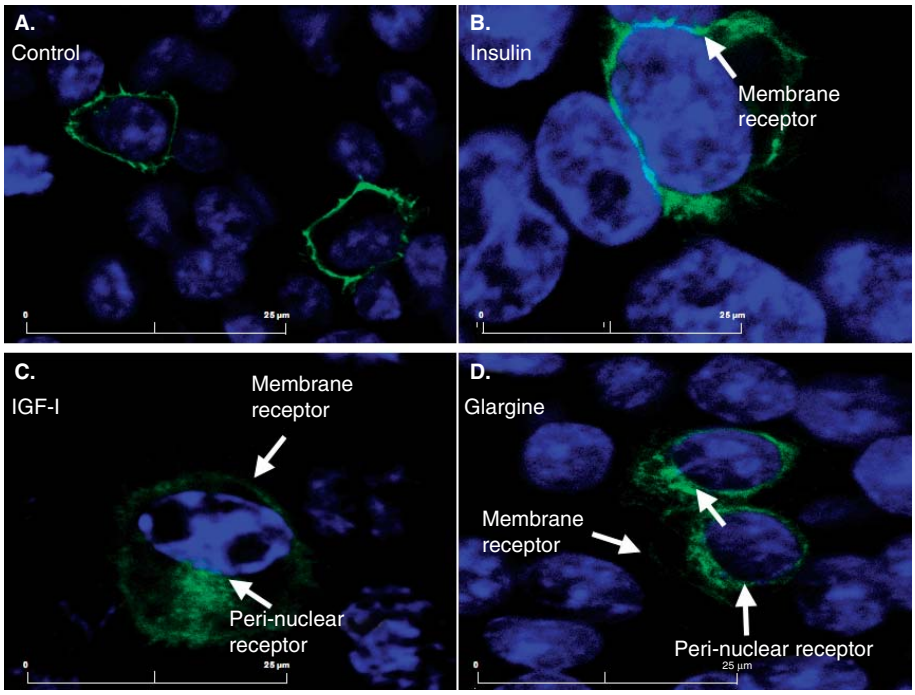

Figure 5. Glargine-induced IGF-IR internalization. HCT116 cells were plated on cover slips in 6-well plates for 24 h. Cells were then transfected with a plasmid containing an IGF-IR cDNA fused to a green fluorescent protein (GFP) marker (1 µg). After 48 h, the cells were treated with 50 ng/ml of insulin (**B**), IGF-I (**C**), or glargine (**D**) for 20, 40, or 60 min, or left untreated (**A**), and fixed for confocal microscopy. Shown are results obtained after 40 min. Glargine, similarly to IGF-I, led to internalization and redistribution of the IGF-IR.
Adapted from [34].

glargine were shown to affect viability and proliferation of non-transformed human coronary endothelial and smooth muscle cells [33]. Studies on the effect of insulin analogues on the growth of existing or transplanted tumors as has been shown for IGF-I are needed.

8. Expert opinion

The experimental data published on the strongly suggestive evidence for the mitogenic activity of some of the long-acting insulin analogues led to hasty analysis of existing clinical data. At stake are large amounts of money as the analogues are more expensive than the regular insulin preparations. Holleman and Gale [40] presented an objective and unbiased analysis of the 'insulin wars' and of the many forces involved in this 'battle', including patients, practitioners, pharmaceutical companies and public/private regulatory and consultative bodies. The authors strongly advise on the use of evidence-based medicine to help establish the best deal for the patient. Furthermore, Holleman and Gale conclude that 'If Europe unites behind the resolution that drug prices should be linked to evidence of benefit, manufacturers would be obliged to provide better evidence or adjust their prices downwards. Otherwise said, if analogues were to cost the same as human insulin, and we could choose freely between them, the controversy concerning their use would soon become irrelevant'.

As mentioned above, controversial opinions resulted in a series of articles and symposia at major meetings. A selection of opinion papers is presented here. Garg *et al.* [41] criticized the data analysis by Hemkens *et al.* [14] for not dividing the group by dose administered at start and excluding those patients who changed insulins during therapy. Their main critique of the Swedish study [16] was that they mixed different types of databases, and that the data by Rosenstock *et al.* [18] comprised only a small number of subjects. Their summary was that if one discards the dose effect shown in the German study, the allegation that glargine is related to cancer is unsubstantiated. On the other hand, Mannucci *et al.* [19] found a dose effect difference in the mitogenic effect of glargine.

Pocock and Smeeth [15], referring to the same publications as Garg *et al.* [41], wrote that the data presented is not conclusive evidence that insulin glargine carries an increased risk for malignancy. Smith and Gale [42] reviewed the relation between insulin and cancer, both in experimental studies as well as the first clinical data published in Diabetologia. In referring to the clinical data, including the possible link between glargine and retinopathy [43], they conclude that medicine has entered an

area of great complexity and demonstrate the problems and pitfalls of observational studies. Nevertheless, the possibly not ideal data are of potential importance and high relevance.

Pollak and Russell-Jones [44], in a review article entitled 'Insulin analogues and cancer risk: cause for concern or cause célèbre?', emphasize the protective actions of metformin, as opposed to the growth promoting activities of insulin, insulin analogues, and IGF-I. Importantly, the authors point out 'even if future research were to document an increase in cancer among insulin users, this would be unlikely to significantly diminish the favourable benefit:risk ratio for patients requiring insulin therapy'. We agree with these authors that special consideration of the treatment options for patients with high risk of cancer due to family history, as well as patients with both cancer and diabetes, might be wise.

In response to the criticism published by Pocock and Smeeth [15] in The Lancet, Edwin Gale [45], Editor-in-Chief of Diabetologia replied 'We believe that people have every right to be informed of possible danger. Imperfect information is better than uninformed ignorance'. Alarmed by the public, their millions of customers and by the pharmaceutical companies involved, the European Association for the Study of Diabetes (EASD), the American Diabetes Association (ADA), and the International Society for Pediatric and Adolescent Diabetes (ISPAD) have issued statements that present data on the link between insulin analogues and cancer are not conclusive and further studies should be conducted.

The National Institute for Health and Clinical Excellence (NICE), U.K., has recently issued the following statement: 'The report advises that any decision to start a patient on an insulin analogue to treat diabetes should be balanced carefully against the lack of long-term safety data and increased prescribing costs. NICE recommends that long-acting insulin analogues have a specific but limited place in therapy. They are substantially more expensive than conventional insulins, but their use has increased enormously over the past few years'.

Finally, a recent Consensus Statement Report of a meeting held by experts assembled by the ADA and the American Cancer Society (ACS) concluded that [46]:

- The association between diabetes and some cancers may be due to shared risk factors between the two diseases, including age, obesity, diet, and (lack of) physical activity.
- Possible mechanisms for a direct link between diabetes and cancer include hyperinsulinemia, hyperglycemia and inflammation.
- Patients with diabetes should be strongly encouraged by their health care professionals to undergo appropriate cancer screenings.
- The evidence for specific drugs affecting cancer risk is limited, and observed associations may have been confounded by indications for specific drugs, effects on other cancer risk factors such as body weight and hyperinsulinemia, and the complex progressive nature of hyperglycemia and pharmacotherapy in type 2 diabetes.
- Cancer risk should not be a major factor in choosing between available therapies for the average patient. For selected patients with high risk for cancer occurrence, selection of appropriate therapy may require more careful consideration.
- Further research is needed to clarify whether all currently marketed insulin analogues and, in particular, insulin glargine are more strongly associated with cancer risk compared with other insulins.

In summary, the concepts expressed above are shared by the authors of this Expert Opinion review. While we agree that the introduction of most insulin analogues had a visible effect in diabetes care, we feel that the benefit of some of these new insulins for the patient has yet to be demonstrated. Of utmost importance, further clinical and basic research is needed to clarify whether insulin analogues and, particularly, insulin glargine are more strongly associated with cancer risk compared with native insulin.

Declaration of interest

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