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Effects of insulin and analogues on carcinogen-induced mammary tumours in high-fat-fed rats

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Abstract

It is not fully clarified whether insulin glargine, an analogue with a high affinity for insulin-like growth factor-1 receptor (IGF-1R), increases the risk for cancers that abundantly express IGF-1R such as breast cancer or some types of breast cancer. To gain insight into this issue, female Sprague–Dawley rats fed a high-fat diet were given the carcinogen N-methyl-N-nitrosourea and randomly assigned to vehicle (control), NPH (unmodified human insulin), glargine or detemir ($n=30$ per treatment). Insulins were given subcutaneously (15 U/kg/day) 5 days a week. Mammary tumours were counted twice weekly, and after 6 weeks of treatment, extracted for analysis. None of the insulin-treated groups had increased mammary tumour incidence at any time compared with control. At 6 weeks, tumour multiplicity was increased with NPH or glargine ($P<0.05$) and tended to be increased with detemir ($P=0.2$); however, there was no difference among insulins (number of tumours per rat: control = 0.8 ± 0.1 , NPH = 1.8 ± 0.3 , glargine = 1.5 ± 0.4 , detemir = 1.4 ± 0.4 ; number of tumours per tumour-bearing rat: control = 1.3 ± 0.1 , NPH = 2.2 ± 0.4 , glargine = 2.7 ± 0.5 , detemir = 2.3 ± 0.5). IGF-1R expression in tumours was lower than that in Michigan Cancer Foundation-7 (MCF-7) cells, a cell line that shows greater proliferation with glargine than unmodified insulin. In rats, glargine was rapidly metabolised to M1 that does not have greater affinity for IGF-1R. In conclusion, in this model of oestrogen-dependent breast cancer in insulin-resistant rats, insulin and insulin analogues increased tumour multiplicity with no difference between insulin types.

Key Words

- ▶ detemir
- ▶ glargine
- ▶ insulin
- ▶ mammary tumour
- ▶ rat

Endocrine Connections
(2018) 7, 739–748

Introduction

Insulin treatment is life saving for type 1 diabetes and essential for achieving glycaemic control and thus preventing complications in type 2 diabetes. However, insulin has

mitotic properties in addition to its well-known metabolic actions (1). Epidemiological studies showed that, in patients with type 2 diabetes and obese people with insulin resistance,

endogenous hyperinsulinaemia is associated with increased risk for several types of cancer including breast cancer (2, 3). Furthermore, although not consistently (4, 5), other studies have found that treatment with insulin increases the risk for breast cancer in type 2 diabetes (6, 7).

Insulin analogues are modified insulins with altered pharmacokinetics. Because of its pharmacokinetic properties, glargine exerts a sustained action up to 24 h after subcutaneous injection and is one of the most commonly used long-acting insulin analogues. Recently, two formulations of glargine have been approved for treatment of diabetes: biosimilar glargine that can be provided at a lower price, and concentrated glargine (300 IU/mL) that exerts a more prolonged action than standard glargine (100 IU/mL). Thus, the use of glargine is predicted to increase. In 2009, a large database study showed that glargine is associated with increased cancer risk compared with unmodified native insulin (8). Although subsequent studies did not confirm the risk for cancer of all types (9, 10), whether glargine increases the risk specifically for breast cancer or for some types of breast cancer is not fully clarified. Large cohort studies and meta-analyses found no association between glargine and breast cancer (11, 12, 13, 14). Also, a prospective trial found no increase in breast cancer incidence in glargine users; however, the trial was not designed to detect differences in cancer, the dose was low and the population at relatively low risk (15). In contrast, there are several studies that found increased breast cancer risk in glargine users (9, 16, 17, 18), suggesting the possibility that glargine might increase breast cancer risk in specific groups or in the long term (17, 18). Given the fact that breast cancer is the most common cancer in women and that new concentrated glargine and biosimilar glargine would contribute to increasing the number of patients treated with glargine, the potential cancer-promoting effect of glargine requires more detailed investigation.

Insulin receptor (IR) and insulin-like growth factor-1 receptor (IGF-1R) are highly expressed in most breast cancers (19). *In vitro* studies show that glargine, which has a higher affinity for the IGF-1R than human insulin, also has a greater mitotic effect in breast cancer cell lines (20, 21). However, *in vivo*, glargine undergoes rapid biotransformation after subcutaneous injection and its metabolites have similar affinity to IR and IGF-1R as unmodified human insulin (22). Thus, to take into account the metabolism of glargine, it is necessary to evaluate its effect on mammary tumour promotion in animal models. Unlike AspB10, an insulin analogue with proven tumorigenic potential (23), glargine did not increase the incidence of spontaneous mammary tumours in wild-type

(WT) mice and rats (24), which can be considered as low-risk groups for cancer. No insulin except AspB10 increased the growth of transplanted mouse mammary cancer cells in a model of extreme insulin resistance, which already displays accelerated tumour growth (25). In genetically modified mice prone to mammary tumour development, neither glargine nor unmodified insulin significantly altered mammary tumour latency (26). However, there was a trend ($P=0.07$) for glargine to shorten latency and glargine affected intracellular signalling in tumour tissue.

In the present study, we compared the mammary tumour-promoting effect of glargine and detemir, a different long-acting modified insulin analogue, to that of unmodified insulin in the commonest condition of insulin resistance, that is, after high-fat diet. We used a model of oestrogen-dependent carcinoma (commonest form of breast cancer), the N-methyl-N-nitrosourea (MNU)-induced mammary tumour model (27).

Materials and methods

Chemicals

NPH insulin and Humulin R were purchased from Eli Lilly, Canada, glargine from Sanofi, Canada and detemir from Novo Nordisk, Canada. MNU was from MRI Global (Kansas City, MO, USA).

Animals

All procedures were conducted according to the Guide for the Care and Use of Laboratory Animals, Eighth edition (2011), Office of Laboratory Animal Welfare, National Institutes of Health; Chapter XXI-Laboratory Rats ver. 2 and the Canadian Council on Animal Care guidelines. The study was approved by the Animal Care Committee of the University of Toronto. Details are described in [Supplementary methods](#) (see section on [supplementary data](#) given at the end of this article). In brief, 4-week-old female Sprague-Dawley rats were put on a high-fat diet (40% of total calories), which is an established model of insulin resistance, as shown in [Supplementary Fig. 1](#). The diet was continued throughout the study. One week later, the rats were injected subcutaneously with MNU (97 mmol/L in saline) at 485 $\mu\text{mol/kg}$ (28). Thereafter, mammary glands were palpated twice per week throughout the study. Three weeks after MNU injection and 1 week before the onset of insulin treatment, the light–darkness cycle was shifted from 07:00–19:00 h to 13:00–01:00 h to accustom the rats to eat at the time of peak insulin action, once insulin injection was given at 11:00–12:00 h.

When the rats were 9 weeks old, they were randomly assigned to four treatment groups ($n=30$ each): saline, NPH, glargine or detemir. All insulins were injected subcutaneously 5 consecutive days per week between 11:00 and 12:00h (29). To avoid hypoglycaemia, insulin was started at 5U/kg for the first 2 days, and then raised to 10U/kg for the following 2 days while blood glucose was monitored. Finally, the target dose of 15U/kg was given. During the treatment period, blood glucose levels before and 4h after the injection were measured three times per week. No rat experienced severe hypoglycaemia defined as blood glucose level <2 mmol/L. Following 6 weeks of treatment and 4h after the last injection, blood samples and palpable mammary tumours were collected under general anaesthesia with i.p. injection of a ketamine/xylazine/acepromazine cocktail (555, 12 and $2\mu\text{mol/kg}$, respectively). The rats were then euthanized with a cocktail overdose. After measuring the weight and size, the tumours were cut in half. One half was fixed in 10% neutral buffered formalin for histological analysis, and the other half was snap-frozen in liquid nitrogen for Western blot analysis. Tumour volume was calculated with the following formula: $4/3*\pi*r1*r2*r3$ (r =radius).

Glucose and insulin measurements

Blood glucose levels were measured using OneTouch Glucose Metre (LifeScan Canada Ltd., Burnaby, British Columbia, Canada). Blood samples for glucose measurement were obtained from a nick in the tail. Glycated haemoglobin (HbA1c) levels were measured as a National Glycohemoglobin Standardization Program value (%) during euthanasia using A1C NOW (Bayer Inc.). Plasma samples obtained 4h after the last injections were used for measurement of insulin levels by radioimmunoassay (SRI-13K-Sensitive Rat Insulin RIA; Millipore Canada Ltd). The cross-reactivity of the insulin kit antibody, which was raised against rat insulin, is 100% against human insulin.

Histology

The fixed tumours were embedded in paraffin, and the sections were stained with haematoxylin and eosin. Slides were examined by one of us (A. M.), a pathologist, according to the histological classification of Russo and Russo (30).

Western blots

Western blots were performed to evaluate IR, IGF-1R, phosphorylated and total Akt, phosphorylated and total ERK 1/2. Details are described in [Supplementary methods](#).

Cell culture

Michigan Cancer Foundation (MCF)-7 cells (American Type Culture Collection, Manassas, VA, USA) were cultured as described in [Supplementary methods](#).

Kinetics study

A separate group of 9-week-old female Sprague–Dawley rats fed the high-fat diet for 5 weeks received cannulation of the carotid artery and, following 3 days of recovery, underwent injection of analogues with timed blood sampling as described in [Supplementary methods](#) and [Supplementary Fig. 2](#) for the analysis of insulin analogues via liquid chromatography coupled to ion mobility mass spectrometry (31).

Statistics

Data are expressed as mean \pm s.e.m. Sample size was calculated to give a power of 80% to detect a twofold difference in tumour multiplicity based on our preliminary data. Statistics were conducted with Fisher's exact test for categorical data, and one-way or two-way ANOVA, as appropriate, followed by Waller–Duncan K-ratio t test for continuous data. Calculations were conducted using R software (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria).

Results

Metabolic parameters

[Table 1](#) shows the metabolic parameters. Initial body weight was similar in all groups. Although there was no difference in final body weight or food intake among the groups, rats treated with NPH showed a greater weight gain than those treated with detemir.

Blood glucose levels

[Figure 1A](#) and [B](#) show blood glucose levels before and 4h after the treatment injection along the experimental timeline, respectively. As expected, all insulins reduced glucose levels after injection compared with control. Glargine and NPH groups showed slightly higher levels of blood glucose before the injection than the control or detemir groups. Although blood glucose levels after the injection were decreased by all insulins, the decrease was slightly greater with NPH and glargine than detemir. Plasma insulin levels

Table 1 Metabolic parameters.

	Control	NPH	Glargine	Detemir
Number	30	30	30	30
Initial body weight (g)	247±4	247±4	249±3	257±4
Final body weight (g)	333±6	346±7	343±5	338±6
Body weight increase (g)	86.5±4.4	98.7±4.5 ^a	93.8±4.4	80.7±3.9
Initial fasting blood glucose levels (mmol/L)	7.1±0.1	7.1±0.1	7.2±0.1	7.2±0.1
Food intake (g/day)	18.3±0.6	21.1±1.4	21.1±1.2	19.7±0.8

Initial body weight and blood glucose levels were measured before the onset of treatment. Food intake shows the average over the treatment period. ^a*P*<0.05 vs Detemir.

and HbA1c levels were evaluated at the end of treatments. The rats treated with NPH insulin showed >3-fold higher levels of plasma insulin than the controls (control (*n*=7), 425±90 pmol/L; NPH (*n*=6), 1472±221 pmol/L, *P*<0.001). Due to unknown cross-reactivity of the kit with analogues, samples of the glargine and detemir groups were not assayed. HbA1c levels were slightly lower in the NPH group (4.40±0.05%) (*P*<0.05) and tended to be lower in glargine and detemir groups (4.46±0.04% and 4.45±0.04%, *P*=0.06 and 0.08, respectively) than the control group (4.61±0.05%). The fact that significance was reached for

NPH but not glargine despite similar glucose levels at 4h after injection presumably indicates that NPH was more effective at other time points.

Mammary tumour incidence and multiplicity

All groups developed the first mammary tumour between 4 and 5 weeks after MNU injection (Fig. 2). Although the NPH group appeared to show a greater incidence than the other groups starting at 8 weeks after MNU injection, there was no statistical difference

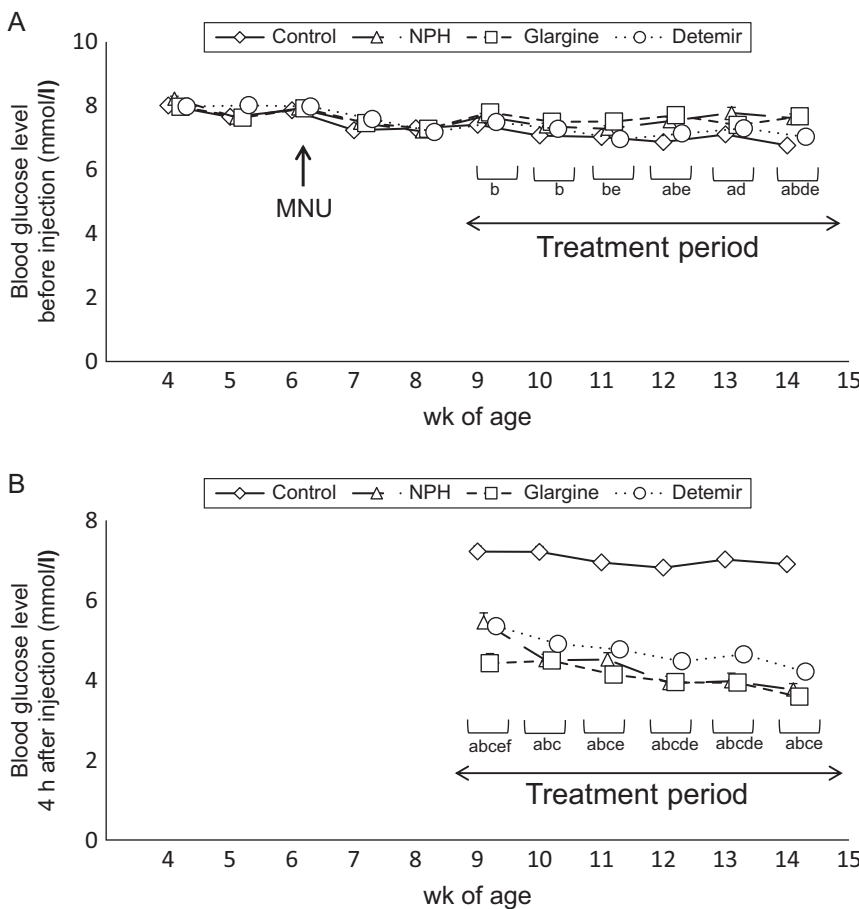


Figure 1 Blood glucose levels taken over the treatment period. (A and B) Blood glucose levels taken over the treatment period before and 4h after treatment injection, respectively. *n*=30 per group. *P*<0.05: (a) Control vs Glargine; (b) Control vs NPH; (c) Control vs Detemir; (d) Detemir vs NPH; (e) Detemir vs Glargine; (f) Glargine vs NPH. MNU, N-methyl-N-nitrosourea.

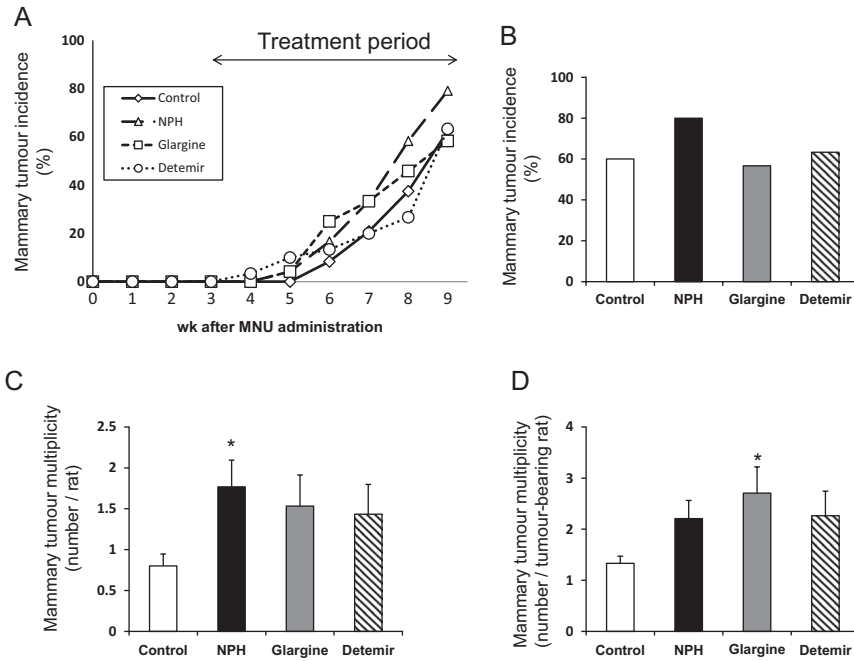


Figure 2 Mammary tumour incidence and tumour multiplicity. (A) Mammary tumour incidence overtime. (B) Mammary tumour incidence at the end of the study. There is no significant difference between the groups with Fisher's exact test. (C and D) Mammary tumour multiplicity expressed as number of mammary tumours per rat in all rats (C) and in tumour-bearing rats (D), respectively. (a) Control, $n=24$; NPH, $n=24$; Glargine, $n=24$; Detemir, $n=30$. (b and c) $n=30$ per group. (d) Control, $n=18$; NPH, $n=24$; Glargine, $n=17$; Detemir, $n=19$. The number of animals in (A) is less than that in (B) and (C) because the first few animals were not monitored overtime. * $P<0.05$ vs Control. MNU, N-methyl-N-nitrosourea.

in mammary tumour incidence at any time ($P \geq 0.1$). Tumour incidence at the end of the study (6 weeks after insulin treatment) is shown in Fig. 2B. Figure 2C shows tumour multiplicity expressed as number of mammary tumours per rat at the end of the study, including the rats bearing no tumour. The NPH group showed the highest tumour number with significance vs control. Although the glargine and detemir groups also showed an increased tumour number by approximately 1.5-fold compared with controls, these differences were not significant ($P=0.1-0.2$). Figure 2D shows tumour multiplicity expressed as number of mammary tumours in the tumour-bearing rats. In the glargine group the mammary tumour number was significantly elevated by 2-fold compared with controls. NPH and detemir groups showed approximately doubled mammary tumour number; however, these changes did not reach significance ($P=0.1-0.2$). There were no differences in tumour multiplicity expressed in either way between NPH, glargine and detemir. When all insulin-treated rats were grouped together, we found a significant difference in tumour multiplicity, which was higher than that in controls (Supplementary Fig. 3).

Mammary tumour growth

The total volume or weight of the tumour tissue per tumour-bearing rat tended to be increased with all insulins corresponding to increased tumour multiplicity; however, these changes were not significant

(Fig. 3A and B). The average volume or weight of each tumour, which is a better surrogate marker of tumour growth than total tumour volume, was similar between control and insulin-treated groups (Fig. 3C and D).

Histology

One-third of the tumours in each group were randomly selected for histological analysis (Fig. 4). According to the previously reported classification (30), all the tumours were grade 2 papillary carcinomas (subtype of invasive ductal carcinomas) with focal comedo and cribriform components (not shown).

IR and IGF-1R expression

Although IR and IGF-1R are highly expressed in most human breast cancers, their expression in MNU-induced mammary tumours has not been determined. MNU-induced mammary tumours had significantly higher IR expression and >10-fold higher IGF-1R expression than non-tumourous mammary glands although the difference in IGF-1R expression did not reach significance ($P=0.2$) (Supplementary Fig. 4). In MCF-7 cells, a cell line where glargine induces greater proliferation compared to unmodified insulin (20, 21), there was a much greater expression of IGF-1R than in MNU-induced tumours, while IR expression was not different. Insulin treatment did not significantly affect IR and IGF-1R expression in mammary tumours (Supplementary Fig. 5).

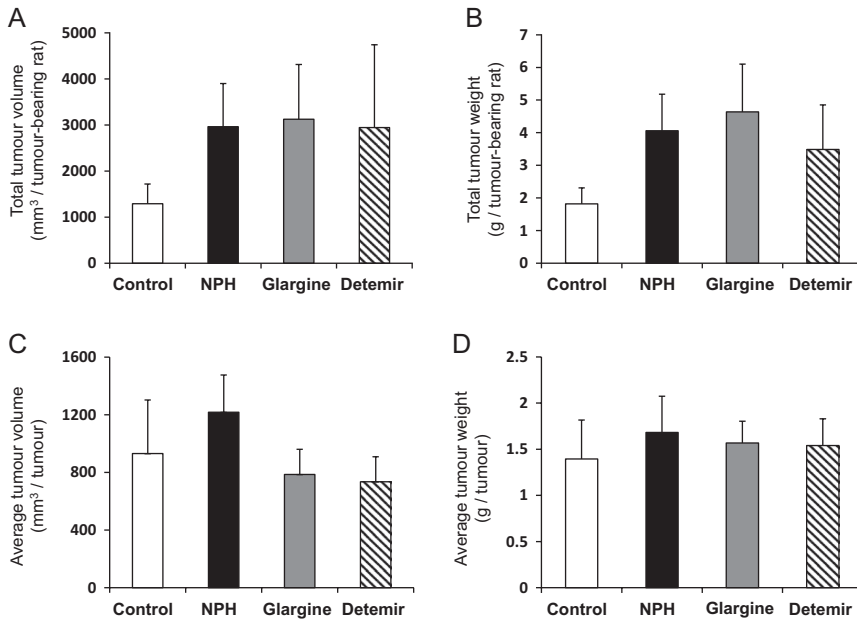


Figure 3 Volume and weight of mammary tumour. (A and B) Total volume and weight of mammary tumour tissue per tumour-bearing rat. (C and D) average volume and weight per tumour. (A and B) Control, $n=12$; NPH, $n=19$; Glargine, $n=11$; Detemir, $n=13$. (C and D) Control, $n=19$; NPH, $n=44$; Glargine, $n=41$; Detemir, $n=43$.

Insulin signalling in tumours

To understand the molecular mechanisms underlying the increased tumour multiplicity in insulin-treated groups, we evaluated the activation of the two major insulin signalling pathways in mammary tumours: phosphoinositide 3-kinase (PI3K)/Akt and MAPK/ERK pathways. The ratio of phosphorylated (active) Akt to total Akt was higher in the glargine and detemir groups than the control group (Supplementary Fig. 6A and B). In contrast, insulin treatment at this dose (15 U/kg) did

not change phosphorylation of ERK 1 or 2 compared with control (Supplementary Fig. 6C and D).

Plasma concentrations of NPH, glargine and its metabolites and detemir after subcutaneous injection

In a separate group of high-fat-fed rats, we examined the time-course of glucose and insulin analogue concentrations after injection. At 4 h after the injection, all insulins similarly reduced plasma glucose levels (Fig. 5A).

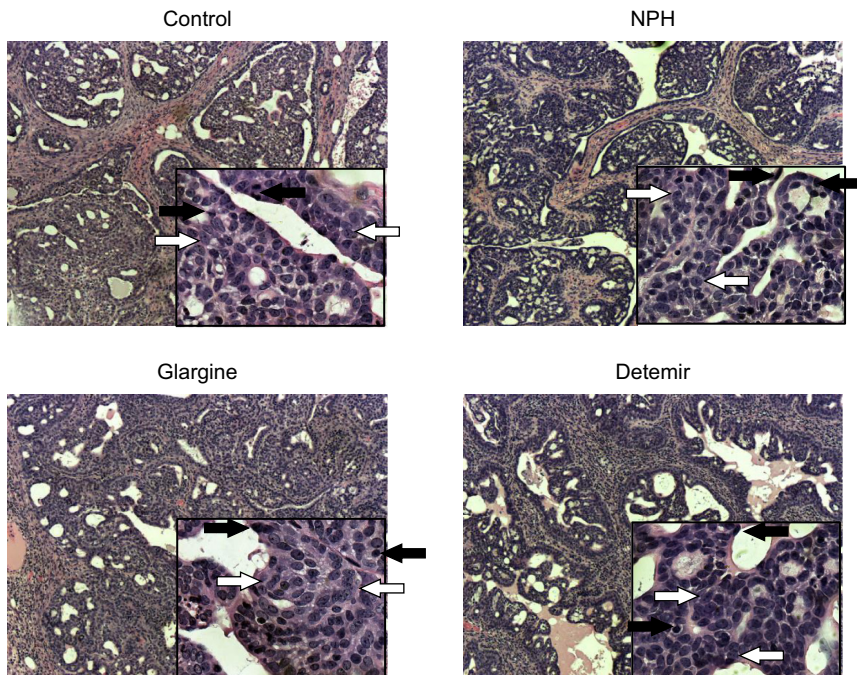


Figure 4 Representative cross-sections of mammary tumours stained with haematoxylin and eosin from each group. Large images, $\times 100$; Small images, $\times 400$. White and black arrows show prominent nucleoli and hyperchromatic nuclei, respectively, which are commonly seen in malignancy and did not differ among groups.

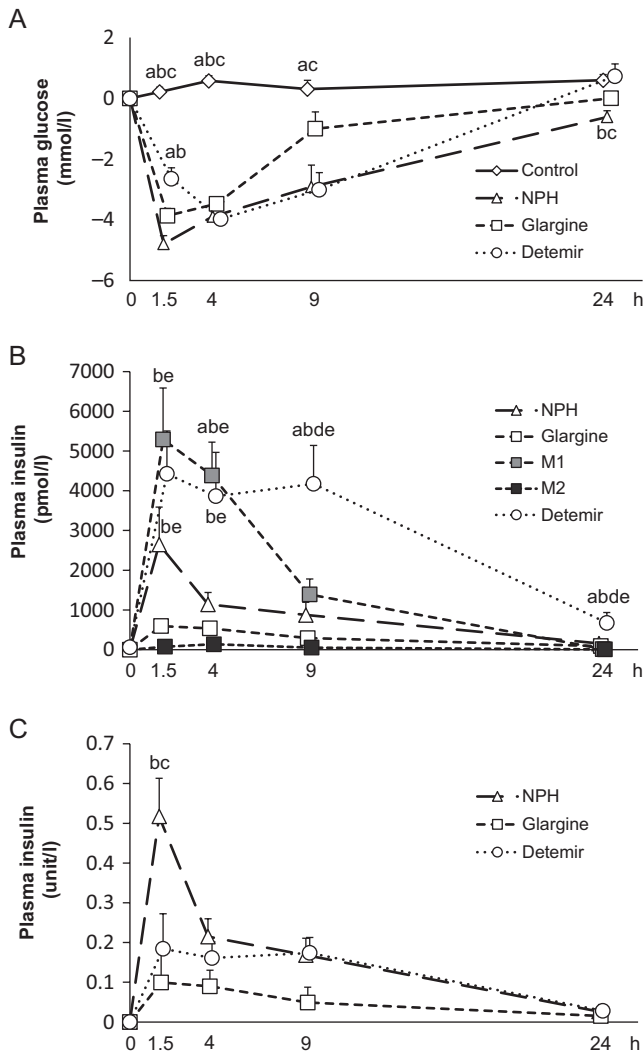


Figure 5 Changes in plasma levels of glucose and insulin after treatment injection at different time points. (A) Plasma glucose levels. (B) Plasma levels of NPH, glargine and its metabolites (M1 and M2), and detemir expressed as pmol/L. (C) Plasma levels of NPH, glargine and detemir expressed as unit/L. $n=6$ per group. $P<0.05$: (a) vs NPH; (b) vs Glargine; (c) vs Detemir; (d) vs M1; (e) vs M2.

However, plasma glucose reduction was greater with NPH than detemir at 1.5 h and with NPH and detemir than glargine at 9 h. NPH reached its peak concentration at 1.5 h and rapidly decreased thereafter (Fig. 5B). After glargine injection, M1 concentrations were rapidly elevated, while M2 concentrations were very low (Fig. 5B). Detemir showed sustained concentrations between 1.5 and 9 h (Fig. 5B). Because insulin concentration per unit is different between unmodified human insulin and modified insulins, we also compared insulin concentrations by unit/L as shown in Fig. 5C. Unmetabolised glargine and detemir showed steady concentrations up to 9 h, whereas NPH showed a

sharp peak at 1.5 h; thereafter, the concentrations of NPH and those of detemir were approximately twice as high as those of unmetabolised glargine, although the difference did not reach significance ($P=0.1-0.3$).

Discussion

We here demonstrated that, compared with NPH (unmodified human insulin), glargine and detemir did not increase incidence or multiplicity of mammary tumours in a carcinogen-induced rat model of breast cancer in the presence of insulin resistance. However, tumour multiplicity was increased by all insulins, significantly with NPH and glargine. With detemir, the increase in multiplicity failed to reach statistical significance; however, detemir was also slightly less potent than the other insulins on plasma glucose at 4 h.

Several animal studies have been conducted to determine whether insulin can increase mammary tumours. AspB10, an insulin analogue that was never used clinically, increased the incidence of spontaneous mammary tumours in rodents (23). Gammeltoft *et al.* also reported spontaneous mammary tumours with human insulin and insulin aspart after 52 weeks in rats (32). Insulin treatment increased growth of carcinogen (7,12-dimethylbenzanthracene)-induced mammary tumours in rats (33, 34) and human breast cancer cell orthografts in nude mice (35). There is a study showing that insulin did not increase mammary tumour incidence or multiplicity in MNU-administered rats (29); however, the rats were fed normal chow, unlike our rats that were fed a high-fat diet to make them insulin resistant. Models of insulin resistance and endogenous hyperinsulinaemia also show accelerated chemically and genetically induced mammary tumour growth (36).

To date, the effect of insulin analogues in animal models of breast cancer has only been addressed by two publications (25, 26). One of these reported that 2-week treatment with long-acting insulin analogues, including glargine and non-metabolisable glargine, did not increase mammary tumour growth vs control in mice with extreme insulin resistance grafted with two types of mouse mammary tumour cells (25). The other paper (26) investigated the effect of glargine in mice with a mammary-specific mutation of p53. Unmodified insulin or glargine treatment did not affect mammary tumour latency time or multiplicity. However, as noted earlier, there was a trend ($P=0.07$) for glargine to shorten latency and glargine-affected intracellular signalling in tumour

tissue. Unlike our study and the majority of studies in animals (32, 33, 34, 35), there was no effect of unmodified insulin in either of these papers. This could be explained by the short duration of treatment in the Gallagher *et al.* paper (25). In the mouse model of the ter Braak *et al.* paper, loss of p53 function by mutation could have prevented the effect of insulin to inhibit apoptosis via PI3K/Akt-mediated suppression of p53 (26, 37). Importantly, in the latter study, carcinosarcoma, which is an uncommon mammary tumour in humans, accounted for over 80% of tumours, while ductal carcinoma, as in the present study, is the predominant mammary tumour in humans.

The effect on tumour multiplicity found in this study suggests that insulin, a growth factor but not a mutagen itself, may increase the susceptibility to mutagens perhaps because it increases proliferation (proliferating cells are more susceptible to mutations) and/or allows mutated cells to survive because of decreased apoptosis. This assumption remains to be verified. Indeed, further study is required to evaluate the effects of insulin and analogues on cell proliferation and apoptosis of mammary tumours, especially early at the time of initiation and also on factors involved in initiation such as p53. A recent study has demonstrated that a decreased mammary gland tumour latency time caused by chronic IGF-1R activation is related to modulation of tumour progression rather than increased tumour initiation (38). Unlike some other studies (33, 34, 35), we did not find any effect of insulin on parameters of tumour growth, such as tumour volume or weight. However, our study was a short-term treatment not designed to evaluate tumour growth, and we cannot exclude that insulin might have promoted the growth of small undetectable tumours to become palpable.

In the present study, we focused on the MAPK/ERK and PI3K/Akt signalling cascades because they are the major insulin signalling pathways. ERK is a mediator of insulin's mitotic effect. We found that phosphorylation of ERK1/2 was not increased by insulins compared with control in mammary tumours evaluated 4 h after s.c. injection. Akt, which is involved in cell proliferation as well as inhibition of apoptosis, was significantly phosphorylated by insulin analogues (the non-significant effect of NPH may be due to the decrease in plasma levels from 1.5 to 4 h after injection). The involvement of Akt rather than ERK in the effect of insulin is in accordance with results in multiple models of tumours of insulin-resistant mice with endogenous hyperinsulinaemia (36). It is possible that ERK is already maximally activated in tumours; however, lack of effect of endogenous insulin on ERK was also reported in nontumorous mammary

gland (36). It is also possible that ERK1/2 was increased by insulin at earlier time points than 4 h after injection.

In the present study, we conducted the kinetic study of glargine to obtain a better understanding of glargine's action and metabolism in insulin-resistant rats. In humans, glargine exerts a sustained action over 20 h after subcutaneous injection. On the other hand, the action profile of glargine has been found to be shorter in rodents than humans (39). Our data show the peak of glargine's action at 1.5–4 h and a partial recovery of plasma glucose reduction at 9 h, indicating that the daily glucose-lowering effect of glargine may be less than that of other insulins in rats. Plasma levels of glargine's main metabolite M1, which has a similar binding affinity to human insulin, showed opposite increases to plasma glucose levels. Also in humans, M1 was elevated after subcutaneous injections of glargine, while glargine and M2 were very low or undetectable (40). These findings indicate that the lack of greater tumour-promoting effect by glargine than unmodified insulin might have been due to rapid glargine metabolism *in vivo*.

Glargine shows a greater proliferative effect than unmodified insulin in MCF-7 cells, which have abundant expression of hormone receptors including IR and IGF-1R (20, 21). Furthermore, although glargine was barely detected in the human kinetics studies, it has been reported that plasma obtained from patients with type 1 diabetes treated with glargine induced greater proliferation of MCF-7 cells than unmodified human insulin or detemir (41). This evidence is quite indirect; however, MCF-7 cells are used to test tumour growth effects of insulin not only *in vitro* but also *in vivo* as a xenograft model (35); therefore, it is possible that *in vivo* glargine has a greater cancer-promoting effect than unmodified insulin only in tumours with similar IGF-1R and IR profile to that of MCF-7 cells. To obtain insight into this issue and thus justify further studies in the xenograft model, we compared IR and IGF-1R expression of MNU-induced mammary tumour and MCF-7 cells. We found that IGF-1R expression was considerably higher in MCF-7 cells than in MNU-induced mammary tumour, while IR expression was similar. Thus, it is possible that a greater cancer-promoting effect of glargine than unmodified insulin is restricted to cancers with very high IGF-1R expression as that of MCF-7 cells. However, attention needs to be paid in the translation of receptor expression of MCF-7 cells, which was assessed *in vitro*, to an *in vivo* setting. In addition, we did not evaluate the activation of IR and IGF-1R or expression of IR subtype. Further studies are required to clarify these issues.

One limitation of the study is that we did not treat a group of rats with IGF-1 or AspB10, which could have put

into perspective the tumour-promoting effect of insulin. Also, ours was not a model of diabetes. However, the insulin dose was close to that required to normalise fasting glucose in diabetic rats (42). Further studies in streptozotocin-induced diabetic rats will be required to extend our findings in a setting of diabetes and its insulin treatment.

In conclusion, although insulin increased tumour multiplicity, we found no greater effect of glargine compared with native or detemir insulin in insulin-resistant rats. Our findings are in accordance with the majority of epidemiological studies indicating that, while glargine does not increase breast cancer hazard in women with prediabetes or type 2 diabetes, all hyperinsulinaemia, whether endogenous or exogenous, is a risk factor and of great concern in this population.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EC-17-0358>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

The present study was carried out without any support or gift from industry. Y M was funded by Showa University Research Grant for Young Researchers.

Author contribution statement

Y M and E K researched and analysed data and drafted the manuscript. R F, L C Q and S W researched and analysed data and contributed to revising the manuscript. M T and A M researched and analysed data, contributed to interpretation and reviewed and edited the manuscript. I G F contributed to data analysis and interpretation and reviewed and edited the manuscript. A G designed the study, contributed to the discussion and reviewed and finalised the manuscript. All the authors gave final approval to the submission of the manuscript. A G is the guarantor of this work and is responsible for the integrity of the work as a whole.

Acknowledgements

The authors wish to thank Loretta Lam, Department of Physiology, University of Toronto, for technical support and Yuri Crispim de Moraes, exchange student from Brazil in Dr Giacca's laboratory, for help in experiments.

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Received in final form 18 April 2018

Accepted 24 April 2018

Accepted Preprint published online 24 April 2018

