ORIGINAL ARTICLE

MINIMAL INFLUENCE OF THE TIME INTERVAL BETWEEN INJECTION OF REGULAR INSULIN AND FOOD INTAKE ON BLOOD GLUCOSE CONTROL OF TYPE 1 DIABETIC PATIENTS ON A BASAL-BOLUS INSULIN SCHEME

A.J. SCHEEN, M.R. LETIEXHE, P.J. LEFÈBVRE

SUMMARY - The present study aimed at investigating the influence of the time interval between injection of regular insulin and meal ingestion on postprandial glucose changes and overall blood glucose control in patients with type 1 diabetes on intensive insulin therapy. Fifteen C-peptide negative subjects were submitted, in a randomized order, to two 6-week treatment periods in which regular insulin was injected either 5 minutes or 30 minutes before each of the three main meals, in combination with a bedtime NPH insulin injection. The changes in plasma glucose excursions following a breakfast test (Cmax, Tmax, Cmin, Tmin, AUC0-240 min) were similar in the two experimental protocols. Furthermore, no significant changes were observed in daily insulin dosages nor in glucose profiles obtained using home blood glucose monitoring. Only a tendency to a greater 90-minutes postprandial increase in blood glucose levels was observed when regular insulin was injected 5 minutes rather than 30 minutes before meal. Glycated haemoglobin levels were similar after each treatment period (7.6 ± 0.2 % versus 7.5 ± 0.2 %; NS) and no differences in the incidence or severity of hypoglycaemic episodes were noticed between the two insulin schemes. In conclusion, in type 1 diabetic patients who are rather well controlled with a basal-bolus insulin scheme, the injection of regular insulin 30 minutes before each main meal provides no significant advantage as compared to the injection of regular insulin 5 minutes before meal.

Key-words: type 1 diabetes, insulin injection, pharmacokinetics, postprandial glucose control, meal test.

RÉSUMÉ - Influence modeste de l’intervalle entre l’injection d’insuline rapide et la prise alimentaire sur le contrôle glycémique de patients diabétiques type 1 sous régime insulinique basal-bolus. Cette étude a pour but d’étudier l’influence du moment de l’injection d’insuline ordinaire par rapport au repas sur les modifications glycémiques post-prandiales et le contrôle glycémique global chez les patients avec diabète de type 1 sous insulinothérapie intensive. Quinze sujets, peptide-C négatifs, ont été soumis, dans un ordre aléatoire, à deux périodes de traitement successives de 6 semaines durant lesquelles l’insuline ordinaire a été injectée soit 5 minutes, soit 30 minutes avant chacun des trois repas principaux, en plus d’une injection d’insuline NPH au coucher. Les variations glycémiques lors d’un test “petit déjeuner” (Cmax, Tmax, Cmin, Tmin, AUC0-240 min) ont été similaires dans les deux protocoles expérimentaux. Il n’y a pas eu de différences significatives dans les dosages d’insuline ni dans les profils glycémiques obtenus par l’autosurveillance à domicile. Seule une tendance à des augmentations glycémiques plus marquées 90 minutes après les repas a été mise en évidence lorsque l’insuline ordinaire était injectée 5 minutes plutôt que 30 minutes avant les repas. Les taux d’hémoglobine glyquée ont été comparables après chaque période de traitement (7.6 ± 0.2 % versus 7.5 ± 0.2 %; NS) et aucune différence n’a été notée dans l’incidence ni dans la sévérité des malaises hypoglycémiques entre les deux protocoles d’administration de l’insuline. En conclusion, chez des patients diabétiques de type 1 relativement bien équilibrés par un schéma insulinique basal-bolus, l’injection de l’insuline ordinaire 30 minutes avant chaque repas n’entraîne pas d’avantage significatif par comparaison à une injection réalisée 5 minutes avant les repas.

Mots-clés : Diabète de type 1, Injection d’insuline, Pharmacocinétique, Glycémie post-prandiale, Test repas.

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The Diabetes Control and Complications Trial (DCCT) definitely demonstrated the positive effects of intensified insulin therapy in patients with type 1 diabetes mellitus [1]. The development and progression of microangiopathic complications were markedly and significantly reduced in a large cohort of patients in whom intensified insulin therapy using multiple daily insulin injections succeeded in reducing glycated haemoglobin levels from 9% to 7.2%. However, the DCCT also pointed out the well-known difficulties in normalizing blood glucose control in this population [2, 3], despite a large utilization of resources, and emphasized the potential risk of severe hypoglycaemia with intensified insulin therapy [1].

In order to better control early postprandial hyperglycaemia and to reduce the risk of late hypoglycaemia, the use of a rapid-acting insulin analogue (lispro) has been recently recommended [4, 5]. Because such an insulin analogue is more rapidly absorbed after subcutaneous administration, it offers the practical advantage to be injected immediately rather than 30 minutes before meal as recommended for regular insulin whose hypoglycaemic activity is somewhat delayed. However, the influence of the time interval between the injection of regular insulin and food intake on blood glucose control has not been adequately addressed in the literature. Indeed, all studies concerned insulin schemes with two injections of regular + intermediate-acting insulin per day [6-12], except one recent study which reported only a minimal influence of timing of preprandial subcutaneous regular insulin administration in diabetic patients treated with a basal-bolus insulin scheme [13].

Thus, the present study aimed at comparing postprandial glucose changes and overall glycaemic control in adult type 1 diabetic patients who were submitted, in a randomized order, to two 6-week periods during which regular insulin was injected either 5 minutes or 30 minutes before each main meal.

**METHODS**

**Subjects** – Fifteen patients with type 1 diabetes mellitus participated in the study. Their main characteristics were as follows (mean ± SE): 8 males and 7 females; age: 40 ± 2 years; duration of diabetes: 14 ± 3 years; body mass index: 24.7 ± 0.6 kg/m². Basal and stimulated plasma C-peptide levels were below 0.150 pmol/ml in all patients, demonstrating the absence of residual insulin secretion. All individuals were treated with four insulin injections per day, comprising a regular insulin injection (Actrapid™, Novo Nordisk) before each of the three main meals and a NPH insulin injection (Insulatard™, Novo Nordisk) at bedtime. All insulin injections were performed using a Novopen® device and a 8 mm-length 30-gauge needle. They were performed in the abdominal wall, into a lifted skin flap, with the needle angled perpendicularly insuring deep subcutaneous injection of insulin. Insulin dosages were adjusted upon the basis of regular home blood glucose monitoring, aiming at target postabsorptive plasma glucose levels below 7.5 mmol/l and postprandial plasma glucose concentrations below 10 mmol/l, without severe hypoglycaemia. Baseline glycated haemoglobin levels averaged 7.5 ± 0.3% (normal values: 4-6%). All subjects gave informed written consent.

**Study design** – The protocol of the study was approved by the Ethical Committee of the Faculty of Medicine of the University of Liège. Each patient was submitted, in a randomized order, to two consecutive 6-week treatment periods during which regular insulin was injected either 5 minutes or 30 minutes before each of the three main meals. In addition NPH insulin was injected at bedtime in both experimental periods. The scheme of insulin regular and NPH dosages remained almost stable throughout the whole study period.

A 7-point daily glucose profile was requested at the end of each 6-week study period: it comprised pre-meal and 90 minutes postmeal glucose measurements three times a day (before and after breakfast, lunch and dinner), and a bedtime glucose analysis, all performed by home blood glucose monitoring. The patients were encouraged to maintain their normal life style as regards diet and physical activities, particularly on the days of self-monitoring.

In addition, the diabetic patients were submitted to a breakfast test after each period of treatment and meal ingestion (between 0 and 15 min) was preceded by the injection of regular insulin in the abdominal wall either 5 minutes or 30 minutes before food intake depending on the study period considered. In order to mimic usual daily life conditions, breakfast was not standardized from patient to patient [14] but each patient was requested to eat the same breakfast, both quantitatively and qualitatively, in the two experimental conditions. An intravenous catheter was inserted into an antecubital vein 45 minutes before breakfast and blood was drawn at regular intervals (-30, -15, 0, 30, 60, 90, 120, 150, 180, 210 and 240 min) in order to measure plasma glucose concentrations before and after breakfast. In both experimental conditions, subjects remained seated in a comfortable armchair during the 4 hours following breakfast.

**Measurements** – Plasma glucose was measured by a glucose oxidase method using an Autoanalyzer (ESAT 6660, Eppendorf, Germany) and glycated haemoglobin concentration was determined using a automated boronate affinity chromatography method [15]. Home blood glucose monitoring was performed using a One Touch device (Life Scan).

Patients were asked to record all measured and/or symptomatic hypoglycaemic episodes and adjustments of insulin dosage throughout the trial period. Hypoglycaemic episodes were classified as mild to moderate when only mild well-recognized symptoms or asymptomatic blood glucose levels below 2.5 mmol/l were present, and as severe if a third-party assistance was required.

**Statistical analysis** – Results were expressed as mean ± SE. Kinetics analysis used standardized parameters and areas under the curve (AUC) were calculated using the trapezoidal method using the mean of – 30 min, – 5 minutes and 0 minutes values as baseline levels. Significance of differences between the two experimental conditions was tested by Wilcoxon’s rank-sum test. Pos-
sible interactions between treatment and period were also assessed as previously reported [16].

\section*{RESULTS}

Changes in plasma glucose levels following breakfast are illustrated in Figure 1. A similar dose of 12 ± 1.5 U of Actrapid™ insulin was injected before breakfast in the two meal tests. Baseline premeal values (0 min) were similar (NS) in both experimental conditions (Table I). A tendency to a modest decline in plasma glucose concentrations between - 30 minutes and 0 minutes was observed when insulin was injected 30 minutes before meal (− 0.64 ± 0.33 mmol/l, \(p = 0.07\)) and not when insulin was injected 5 minutes before breakfast (+ 0.23 ± 0.17 mmol/l, NS). Individual maximum plasma glucose concentrations were reached after about 90 minutes in both conditions (Table I). Post-meal glucose increases (Table I) and maximum glucose values (12.67 ± 0.85 mmol/l versus 12.56 ± 1.00 mmol/l, NS) were also similar in the “− 5 minutes” and “− 30 minutes” protocols, respectively. A tendency to higher late nadir plasma glucose levels was noticed when insulin was injected 5 minutes before meal (7.51 ± 0.84 mmol/l after 226 ± 8 min) than when it was injected 30 minutes before breakfast (6.11 ± 0.89 mmol/l after 234 ± 4 min), but this difference was not statistically significant. Finally, the areas under the curve of plasma glucose levels between 0 and 240 minutes were similar in the “− 5 minutes” protocol (2465 ± 179 mmol.min.l \(^{-1}\)) and in the “− 30 minutes” protocol (2353 ± 214 mmol.min.l \(^{-1}\); NS).

Daily profiles of capillary glucose levels as determined by home blood glucose monitoring in the two conditions are illustrated in Figure 2. Insulin doses of Actrapid™ were similar in the two experimental periods: 10.3 ± 1.2 U versus 11.8 ± 1.3 U before breakfast, 10.1 ± 1.2 U versus 10.2 ± 1.4 U before lunch, 12.5 ± 1.4 U versus 12.1 ± 1.2 U before dinner, when insulin was injected 5 minutes versus 30 minutes before meal, respectively. Similarly, no significant difference was observed in the dose of Insulatard™ insulin injected at bedtime (21.9 ± 2.8 U versus 21.5 ± 2.5 U).

No significant differences between the two experimental conditions were observed in blood glucose concentrations at each timepoint of the daily profile, both after an overnight fast (NS) and after each of the three

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Changes in plasma glucose concentrations following breakfast (ingested between 0 and 15 minutes) when regular insulin was injected either 5 minutes (open circles) or 30 minutes (full circles) before meal in 15 type 1 diabetic patients. None of the differences was statistically significant.}
\end{figure}

\begin{table}[h]
\centering
\caption{Parameters of blood glucose control at the end of two 6-week study periods when regular insulin was injected either 5 minutes or 30 minutes before each meal in 15 type 1 diabetic patients.}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Parameters} & \textbf{- 5 min} & \textbf{- 30 min} & \textbf{p} \\
\hline
Daily insulin dose (IU) & 54.8 ± 4.0 & 55.7 ± 4.2 & NS \\
Glycated haemoglobin (%) & 7.6 ± 0.2 & 7.5 ± 0.2 & NS \\
Basal plasma glucose (mmol/l) & 9.65 ± 0.83 & 9.94 ± 0.96 & NS \\
Post-breakfast maximum glucose increase (mmol/l) & 3.02 ± 0.61 & 2.61 ± 0.90 & NS \\
Time of post-breakfast maximum plasma glucose level (min) & 92 ± 9 & 88 ± 9 & NS \\
Post-breakfast glucose AUC\(_{0-240 \text{ min}}\) (mmol.min.l \(^{-1}\)) & 2465 ± 179 & 2353 ± 214 & NS \\
Mean daily glucose profile (mmol/l) & 10.0 ± 0.7 & 9.8 ± 0.8 & NS \\
Average 0-90 min post-meal glucose rise (mmol/l) & +2.6 ± 0.7 & +0.9 ± 0.9 & 0.07 \\
Total number of hypoglycaemic episodes (n/6 weeks) & 1.7 ± 0.6 & 2.0 ± 0.9 & NS \\
\hline
\end{tabular}
\end{table}
Daytime meals (NS). The highest blood glucose values were recorded 90 minutes after breakfast and the lowest before lunch in both conditions. However, a tendency to higher increment between premeal and postmeal values was observed when regular insulin was injected 5 minutes rather than 30 minutes before food intake: +3.7 ± 1.4 mmol/l versus +0.7 ± 1.6 mmol/l after breakfast (NS), +3.0 ± 1.1 mmol/l versus +2.4 ± 1.3 mmol/l after lunch (NS), and +0.7 ± 0.9 mmol/l versus −0.2 ± 1.9 mmol/l after dinner (NS). When all these time values were considered together, the difference tended to reach the level of statistical significance: the 90 minutes postprandial rise averaged 2.6 ± 0.7 mmol/l when regular insulin was injected 5 minutes before meal whereas it only averaged 0.9 ± 0.9 mmol/l when it was injected 30 minutes before meal (p < 0.07). That the level of statistical significance was not attained because of the rather low number of subjects (type B error) can not be excluded. Mean glucose values during the daily profiles were not significantly different between the two experimental conditions (Table 1).

Body weight (76.3 ± 3.3 kg versus 76.2 ± 3.3 kg, NS) and glycated haemoglobin levels (7.6 ± 0.2 % versus 7.5 ± 0.2 %, NS) remained unchanged after the two 6-week periods when regular insulin was injected 5 minutes and 30 minutes before meal, respectively. No significant difference was also noticed in the frequency and severity of hypoglycaemic episodes (Table 1). Seven patients had no hypoglycaemic episodes in any of the two trial periods while 5 patients had hypoglycaemic events in both periods (1 patient had hypoglycaemic episodes in the “−5 minutes” period and 2 patients in the “−30 minutes” period only). During all the study period, severe hypoglycaemic episodes were recorded in three patients, one during both periods and two when insulin was injected 30 minutes prior to the meal.

**DISCUSSION**

The results of the present study demonstrate that the time elapsed between injection of regular insulin and food intake exerts only a marginal influence in the postprandial glucose changes and in the overall glucose control of individuals with type 1 diabetes. In contrast to those of most previous studies [6-12], these results were obtained in adult patients treated with intensified insulin therapy combining an injection of regular insulin before each of the three main meals and an injection of NPH insulin at bedtime. Furthermore, all patients performed regular home blood glucose monitoring allowing adjustments of insulin doses and succeeded in obtaining a rather good glucose control. Glycated haemoglobin levels were indeed similar to those obtained in the group receiving intensified insulin therapy in the DCCT [1] and better than the mean levels measured in a large cohort of diabetic patients followed in Belgium [17].

Several studies investigated the influence of the time elapsed between regular insulin injection and food intake [6-12]. However, almost all these studies were published more than 10 years ago and concerned insulin schemes with two injections of regular + intermediate-acting insulin per day. As the combination of long-acting insulin with regular insulin may interfere with the kinetics of short-acting insulin [18, 19], the precise influence of the time factor on the action of regular insulin alone (as widely used in more modern basal-bolus insulin schemes) may be difficult to assess in these conditions. Most of these studies investigated acute meal (usually breakfast) tests and demonstrated that early postprandial hyperglycaemia was significantly decreased when the insulin mixture was injected 20-60 minutes rather than 0-5 minutes before breakfast [7, 8, 10]. Those results were confirmed in two studies using an artificial pancreas (Biostator®), one in healthy volunteers [20] and one in type 1 diabetic patients [21]; however, both studies emphasised the risk of hypoglycaemic episodes occurring between insulin injection and meal ingestion when insulin was administered too early in normoglycaemic individuals. Finally, one trial suggested that a greater time interval between regular + zinc insulin injection and breakfast may be more important with human insulin than with porcine insulin [10]. It is noteworthy, however, that none of these studies investigated whether such a reduction in early postprandial glucose excursions when insulin was injected on an average 30 minutes before rather than just before meals could have a positive impact on glycated haemoglobin levels, and our study failed to detect any significant influence of time interval on this index of
blood glucose control after a 6-week time-interval. In order to minimize the risk of premeal hypoglycaemia and of postmeal early hyperglycaemia, the best solution would probably be to select the time interval between insulin injection and meal ingestion depending upon the preprandial blood glucose concentration, the highest the latter, the longest the former and vice versa [20, 21].

The results of the present study confirm the recently published observations of Christensen et al. [13] in adult type 1 diabetic patients submitted to a quite similar protocol as that used in our study. Several differences should, however, be pointed out between the two studies as the Danish patients had initial poorer metabolic control (mean glycated haemoglobin levels of 8.7 ± 0.9 %), injected regular insulin 0 minutes instead of 5 minutes prior to the meal (compared to 30 minutes pre-meal), were submitted to a standardized rather than an individually-adjusted breakfast and, most importantly, were hospitalized the evening before the breakfast test in order to receive a continuous intravenous insulin infusion maintaining blood glucose between 4 and 7 mmol/l throughout the night. Such particular experimental (and quite different from the everyday life) conditions may explain why significantly smaller blood glucose excursions were observed 20-70 minutes after meal when insulin was injected 30 minutes rather than just before breakfast. Nevertheless, the insulin injection at meal time instead of 30 minutes before had no significant influence on blood glucose control throughout the rest of the day, glycated haemoglobin levels after a 6-week period or the total number of hypoglycaemic episodes [13], thus in agreement with the results of the present trial which better reproduces the conditions of everyday clinical practice.

Recent studies compared the postprandial glucose excursions after regular insulin and a rapid-acting insulin analogue (lispro) [22-25]. They demonstrated that early postprandial glucose peaks were significantly reduced after lispro when compared to regular insulin, because of an earlier insulin absorption from the subcutaneous depot and a more rapid elevation in plasma insulin levels [22-25]. However, almost all clinical studies failed to evidence a significant reduction in glycated haemoglobin levels with lispro as compared to regular insulin, demonstrating that such an improvement of insulin pharmacokinetics is insufficient to improve overall glucose control, an objective which probably requires additional adjustment of long-acting insulin injections [4, 5, 25].

Numerous factors may indeed influence the pharmacokinetics of regular insulin after subcutaneous injection [26-28]. Furthermore these insulin-related factors are superimposed to a variety of other potential interferences (gastric emptying, gastrointestinal hormones, counterregulatory hormones, physical exercise, ...) which are difficult to standardize and are all able to influence the degree and duration of postprandial hyperglycaemia [29]. Consequently, it is not surprising that modifying only one of these factors, i.e. the time interval between injection of regular insulin and meal or the use of a rapid-acting insulin, has only minimal influence on overall blood glucose control in most patients.

In conclusion, in adult type 1 diabetic patients who are rather well-controlled with a basal-bolus insulin scheme, the injection of regular insulin 30 minutes before each main meal provides only marginal advantages as compared to the injection of regular insulin 5 minutes before meal. The minimal differences in glycaemic control between the two insulin schemes may result from the numerous factors which could influence glycaemic excursions in type 1 diabetes in daily life. These observations emphasize the limits of acting on only one precise factor, such as the time elapsed between insulin injection and food or the use of a rapid-acting insulin analogue, to obtain improved glycaemic control in patients with type 1 diabetes.

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