Premeal injection of rapid-acting insulin reduces postprandial glycemic excursions in type 1 diabetes

Running Title: Premeal injection of rapid-acting insulin

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Objective: To assess the effect of three premeal timings of rapid-acting insulin on postprandial glucose excursions in type 1 diabetes.

Research design and methods: 10 subjects participated in a three-way randomized cross-over trial. Mean (± SD) age was 45.5 ± 12.09 years, HbA1c 8.55 ± 1.50%, duration of diabetes 23.8 ± 7.81 years and duration of CSII therapy 8.5 ± 6.10 years. Insulin aspart was administered at 30, 15 or 0 minutes before mealtime.

Results: Area under the curve was lower in the -15 stratum (0.41 ± 0.51 mmol/L/min) compared to the -30 stratum (1.89 ± 0.72 mmol/L/min, P = 0.029) and 0 stratum (2.11 ± 0.66 mmol/L/min, P = 0.030). Maximum glucose excursion was lower in the -15 stratum (4.77 ± 0.52 mmol/L) compared to the -30 (6.48 ± 0.76 mmol/L, P = 0.025) and 0 stratum (6.93 ± 0.76 mmol/L, P = 0.022). Peak glucose level was lower in the -15 stratum (9.26 ± 0.72 mmol/L) compared to the -30 stratum (11.74 ± 0.80 mmol/L, P = 0.007) and the 0 stratum (12.29 ± 0.93, P=0.009). Time spent in the 3.5-10 mmol/L range was higher in the -15 stratum (224.5 ± 25.0 min), compared to the 0 stratum (90.5 ± 23.2 min, P=0.001). There was no significant difference in occurrence of glucose levels <3.5mmol/L between strata (P=0.901).

Conclusions: Administration of rapid-acting insulin analogues 15 minutes before mealtime results in lower postprandial glucose excursions and more time spent in the 3.5-10.0 mmol/L range, without increased risk of hypoglycemia.

One of the most challenging aspects of attaining adequate glycemic control is limiting the postprandial rise of glucose. Current ADA guidelines recommend aiming for postprandial blood glucose levels below 10 mmol/L (1,2). With the advent of rapid-acting insulin analogues, insulin lispro, aspart and glulisine, people with diabetes can attain lower postprandial glucose excursions (3-5). Therefore and due to the possibility to dose insulin at mealtime rather than 15 to 30 minutes before the meal, as was recommended for human insulin (6), rapid acting insulin analogues have become the preferred mealtime insulin for people with type 1 diabetes (7,8).

After a meal, the postprandial glucose peak mostly occurs between one and two hours with a mean peak time of 75 minutes (9). Rapid-acting insulin analogues display a maximum effect at around 100 minutes after subcutaneous injection (10). Thus the question arises whether perhaps it would be better to inject the mealtime insulin 15 or even 30 minutes before the start of a meal. In this way the insulin peak action is better synchronized with the glycemic excursions after a meal, thereby potentially minimizing the height of the postprandial glucose excursions. Limited data addresses this topic. The aim of this study is to measure the effect of different premeal timing of rapid-acting insulin on postprandial excursions.

RESEARCH DESIGN AND METHODS
Subjects were recruited from a cohort of patients willing to participate in scientific research at the department of Internal Medicine at the Academic Medical Centre in Amsterdam, the Netherlands. The protocol was approved by the Medical Ethics Committee and all subjects signed a consent form. The study was carried out in concordance with the Declaration of Helsinki. The study was registered at the ISRCTN (ISRCTN13984129).
Ten people with type 1 diabetes were included in this study. All patients met the inclusion criteria, treatment with continuous subcutaneous insulin infusion (CSII) therapy for at least six months, duration of diabetes of at least 2 years and a body mass index $\leq 35$ kg/m$^2$. All patients were treated with insulin aspart, four patients who were treated with insulin lispro switched to insulin aspart for the duration of this trial.

The study consisted of three visits for each subject. On the day before the first study day, patients were started on a subcutaneous CGM sensor (SofSensor, Medtronic Diabetes, Northridge, CA) and were instructed to calibrate the sensor at home according to the manufacturer’s specifications. Patients received a telephone number with 24-hour availability for assistance on problems with the sensor (e.g. alarms, help with calibration) and returned home for the night.

At each visit, insulin to cover breakfast was administered using the patient’s insulin pump. The size of the insulin bolus was determined by the patient with their usual carbohydrate insulin ratio. Patients were randomized each day by means of sequentially numbered opaque, sealed envelopes to insulin bolus administration at 30, 15 or 0 minutes before the meal using a cross-over design. On each study day patients reported fasting to the clinical research unit and received an intravenous catheter in the antecubital vein for blood collection. Before the start of the study protocol blood glucose was measured by finger prick (OneTouch Ultra, Lifescan Inc, Milpitas, CA). If blood glucose was between 3.5 and 7.8 mmol/L, the study protocol would commence immediately. If blood glucose was too low, patients would not start the study protocol and were asked to return another day.

Each patient was provided with a breakfast comparable to their regular breakfast. The meal for an individual patient was identical for all study days. Blood was sampled every 15 minutes during one hour before the meal, every 10 minutes during the first two hours after the meal and every 20 minutes during the third and fourth hour after the meal. Blood samples were collected in 2cc sodium fluoride tubes for determination of blood glucose. Patients would go home four hours after the test meal while continuing to wear the CGM sensor and reported back to the clinical research unit the next days to complete the study. At the end of the third study day the CGM sensor was removed and the sensor data were plotted against the venous blood glucose. The area under the curve was calculated (trapezoid method) using as a baseline the mean values of the first three blood glucose values before insulin administration. Primary outcome measure was the area under the curve for the blood glucose values from the start of the meal until four hours afterwards. Secondary outcome measures were the area under the curve for the sensor glucose values, the maximum glucose excursion from baseline, the peak glucose value, the number of hypoglycemic episodes defined as glucose values below 3.5 mmol/L and total time spent in euglycemia, defined as the time spent in the glucose range between 3.5 and 10.0 mmol/L.

Outcome measures were analyzed for significance ($P<0.05$) using SPSS 17.0 (SPSS Inc, Chicago, IL). A repeated measures ANOVA was performed for all outcome measures. When the repeated measures ANOVA indicated an overall significant difference among treatment arms, a paired samples T-test was performed between treatment arms. Categorical variables were analyzed using the $\chi^2$ test or Fisher’s exact test.
test. Data are represented as mean ± SEM, mean ± SD, values and frequency.

**RESULTS**

All participants, 3 females and 7 males, completed the three study visits. Mean age was 45.5 ± 12.09 years. Mean HbA1c was 8.55 ± 1.50%, mean duration of diabetes was 23.8 ± 7.81 years and mean duration of CSII therapy was 8.5 ± 6.10 years. The mean carbohydrate content of the meal was 48.02 ± 6.23 grams. The mean size of the insulin bolus was 6.03 ± 0.60 IU. There was no significant difference in blood glucose levels at the start of the study between treatment arms (7.00 ± 0.55 mmol/L for the 0 treatment arm, 6.64 ± 0.41 mmol/L for the -15 treatment arm and 7.05 ± 0.59 mmol/L for the -30 treatment arm, P=0.749). Neither was there any difference between treatment arms for the need for IV insulin infusion to get glucose within the predefined range upon admittance (3 times in the 0 treatment arm, 3 times in the -15 treatment arm and 3 times in the -30 treatment arm, P= 1.000). Patients reported with a blood glucose value above 3.5 mmol/L on all study days. According to CGM values no patient experienced nocturnal hypoglycemia in the night before an experiment. Figure 1 shows the averaged blood glucose values from the start of the study protocol until the end of the study day per treatment arm. Primary and secondary outcome measures are summarized in table 1 for both blood glucose and CGM data. The -15 treatment arm had a significantly lower AUC of 0.41 ± 0.51 mmol/L/min compared to the 0 treatment arm at an AUC of 2.11 ± 0.66 mmol/L/min (P=0.030) and the -30 treatment arm which had a AUC of 1.89 ± 0.72 mmol/L/min (P=0.029). There was no significant difference in AUC between the -30 and 0 treatment arm (P=0.785). In a post-hoc analysis for differences in AUC between treatment arms in subgroups according to HbA1c level above or below the median and fasting blood glucose above or below the mean, no significant overall differences between treatment arms could be detected, however the AUC of the -15 treatment arm remained the smallest among the three treatment arms (data not shown).

The -15 treatment arm had a significantly lower glucose excursion (4.77 ± 0.52 mmol/L) compared to the 0 treatment arm (6.93 ± 0.76 mmol/L, P=0.022) and -30 treatment arms (6.48 ± 0.76 mmol/L, P=0.025). The -15 treatment arm had significantly lower maximum blood glucose values (9.26 ± 0.72 mmol/L) than the -30 treatment arm (11.74 ± 0.80 mmol/L, P=0.007) and the 0 treatment arm (12.29 ± 0.93 mmol/L, P=0.009). There was no significant difference between treatment arms 0 and -30 (P=0.456).

Time spent in euglycemia was highest in the -15 treatment arm (mean 224.5 ± 25.0 min), not significantly different from the -30 treatment arm (mean 182.5 ± 28.2 min, P=0.212) but significantly higher than the 0 treatment arm (mean 90.5 ± 23.2 min, P=0.000). Compared to the 0 treatment arm, the -15 treatment arm had a 80.6% lower AUC, 31.2% lower maximum blood glucose excursion, 24.7% lower maximum blood glucose and 148.1% more time spent in euglycemia. There was no significant difference between the occurrence of hypoglycemia defined as a blood glucose value lower than 3.5 mmol/L between treatment arms. All hypoglycemic values were noted afterwards in the lab report, not from the finger prick measurements during the study. None of the hypoglycemic values occurred before the start of the meal and no rescue carbohydrates were administered during the entire duration of the study.

When looking at the outcome measures using the data from the CGM device, no significant differences between treatment arms could be found in AUC (-30 treatment arm 2.32 ± 0.59 mmol/L/min, -15 treatment arm 1.10 ± 0.11 mmol/L/min, 0 treatment arm 1.89 ± 0.34 mmol/L/min).
(P=0.088)), maximum glucose values (P=0.174) and maximum blood glucose excursions (P=0.537). The overall mean absolute difference (MAD) from sensor values relative to the blood glucose values was 23.5 ± 1.0%. When divided into baseline MAD (the hour before administration of insulin) and postprandial MAD (the first four hours after the meal) there was a trend towards increased MAD postprandially, from 18.6 ± 1.6% in the baseline period to 22.8 ± 1.1% in the postprandial period (P=0.088). It should be noted that for this sub analysis data from study days on which patients had received an IV insulin correction bolus were discarded (9 out of 30 study days).

CONCLUSIONS
This study tested the hypothesis that earlier administration of a mealtime bolus of rapid-acting insulin would lower postprandial glucose excursions. We found administration of insulin 15 minutes before a meal to be optimal; it significantly lowered the area under the curve, the postprandial maximum blood glucose value and the maximal blood glucose excursion by 80.6, 24.7 and 31.2%. The administration of insulin 15 minutes before the meal led to significantly more time spent in euglycemia (3.5-10 mmol/L), when compared to administration at the start of a meal. In addition, these beneficial effects were not accompanied by an increase in the occurrence of hypoglycemia. As can be seen in figure 1 however, the blood glucose declines slightly before mealtime when administering insulin at -15 minutes. This implies that it might be prudent to administer insulin at this time only when pre-prandial glucose levels are above 5.0 mmol/L. This study did not show any significant difference in AUC, maximum blood glucose swing and postprandial maximum blood glucose between the -30 and 0 treatment arms, although an initial decline was noticeable in the -30 minute treatment arm.

An earlier study by Cobry et al. which tested the effect of insulin dosed 20 minutes before the meal, at the start of the meal and 20 minutes after the meal, also found significantly better postprandial glucose control with insulin injection 20 minutes before the meal (12). In addition, a study in a pediatric population by Scaramuzza et al. tested the effect of timing of mealtime insulin. The study was able to demonstrate a significant difference in 1 hour postprandial glucose levels, which were significantly higher when administering the insulin bolus after the meal, and lowest when insulin was administered 15 minutes prior to the meal. However, there was no significant difference in AUC between treatment arms (13). Thus, three studies argue for insulin injection 15 to 20 minutes before the meal, with our study arguing against even earlier administration at 30 minutes before the meal.

We can only speculate on why, in this study, the insulin administration at -30 minutes did not improve postprandial glycemic control as compared to the 0 treatment arm. One could argue that if insulin administration at 15 minutes before the meal is the optimum, than both -30 and 0 treatment arms had an equal 15 minute mismatch with the optimum, resulting in almost equal postprandial glycemic control. Further research is needed however to support this hypothesis.

During this study we fitted every patient with a CGM sensor. With use of sensor data alone we could not demonstrate any significant changes between insulin administration times. We hypothesize that this is due to the fact that sensor accuracy is worse with rapid increases and decreases in blood glucose and therefore tends to underreport the changes in glucose levels. This is supported by analyses of CGM accuracy by Breton et al. (15), who conclude that there is a correlation between rate of change and CGM accuracy. This study also found that at a positive rate of change of blood glucose, CGM tends to read lower
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glucose values. Reversely, at a negative rate of change, CGM tends to read higher glucose values. Thus CGM has a tendency to report flattened out postprandial excursions. The MAD of the sensor during our study was relatively high at 23.5 ± 1.0% when compared to other published MAD values, with a trend towards highest MAD’s in the postprandial period, confirming compromised sensor accuracy during the postprandial rapid rise and fall in glucose (16).

The data from this study could also prove valuable for use in closed-loop systems, in which dealing with the postprandial glucose excursions is one of the main challenges (17). According to our data regarding the effect of timing of insulin administration, an argument can be made for mealtime announcement by patients wearing future closed-loop devices, should these devices use current rapid-acting insulin analogs administered via CSII. Administration of rapid acting insulin analogues 15 to 20 minutes before the meal improves postprandial glucose control but will require added vigilance of patients. Thus, larger trials outside the clinical research center are needed before this recommendation is incorporated in clinical guidelines.

**Author Contributions.** YL researched data and wrote the manuscript. AvB wrote the study protocol and reviewed/edited the manuscript. JH reviewed/edited the manuscript and contributed to the discussions. JHDV supervised the protocol development as well as the research, contributed to the discussions, and reviewed/edited the manuscript.

**ACKNOWLEDGEMENTS**
Medtronic Netherlands B.V. provided the sensors for this study free of charge. Company representatives had no influence on study design or analysis and had no access to study data.

**Author Disclosure Statement.** No competing financial interests exist.

**REFERENCES**


**Figure legends**

**Figure 1:** Mean blood glucose during mealtime with different timing of insulin bolus.

The figure shows mean ± SEM blood glucose values before and after a meal (M). The timing of the insulin bolus was 30 minutes before the meal (black triangles), 15 minutes before the meal (black squares) and directly at the start of the meal (black circles). The area under the curve, maximum excursion and maximum blood glucose values are all significantly lower in the -15 treatment arm. The number of minutes in the 3.5-10 mmol/L glucose range is also significantly increased in the -15 treatment arm.
Table 1: Summary of results for blood glucose and CGM data.

<table>
<thead>
<tr>
<th></th>
<th>Treatment arm -30</th>
<th>Treatment arm -15</th>
<th>Treatment arm 0</th>
<th>Overall P-value (repeated measures ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose derived outcomes</td>
<td>Area under curve (mmol/L/min)</td>
<td>1.89 ± 0.72</td>
<td>0.41 ± 0.51</td>
<td>2.11 ± 0.66</td>
</tr>
<tr>
<td></td>
<td>Maximum glucose excursion (mmol/L)</td>
<td>6.48 ± 0.76</td>
<td>4.77 ± 0.52</td>
<td>6.93 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>Peak glucose level (mmol/L)</td>
<td>11.74 ± 0.80</td>
<td>9.26 ± 0.72</td>
<td>12.29 ± 0.93</td>
</tr>
<tr>
<td></td>
<td>Time spent in euglycemia (min)**</td>
<td>182.5 ± 28.2</td>
<td>224.5 ± 25.0</td>
<td>90.5 ± 23.2</td>
</tr>
<tr>
<td></td>
<td>Hypoglycemic events (nr of measurements)</td>
<td>6 out of 220</td>
<td>7 out of 220</td>
<td>4 out of 220</td>
</tr>
<tr>
<td>CGM derived outcomes</td>
<td>Area under curve (mmol/L/min)</td>
<td>2.32 ± 0.59</td>
<td>1.10 ± 0.11</td>
<td>1.89 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Maximum glucose (mmol/L)</td>
<td>11.48 ± 1.08</td>
<td>10.11 ± 0.59</td>
<td>11.31 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>Maximum glucose excursion (mmol/L)</td>
<td>5.24 ± 1.01</td>
<td>4.37 ± 0.64</td>
<td>5.41 ± 0.67</td>
</tr>
</tbody>
</table>

*results are significantly different among groups, **defined as blood glucose values between 3.5 and 10 mmol/L, ***defined as blood glucose values below 3.5 mmol/L. Significance between treatment arms when repeated measures ANOVA indicated an overall significant difference among treatment arms is given in the results section.

Figure 1.