Non-glycemic effects of insulin therapy: a comparison between insulin aspart and regular human insulin during two consecutive meals in patients with type 2 diabetes

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Abstract

Objective: To control postprandial hyperglycemia in insulin-treated type 2 diabetic patients, prandial therapy with regular human insulin (HI) or fast acting insulin analogs is used. Postprandial hyperglycemia seems to be reduced more effectively with insulin analogs than with normal insulin, but there are no data concerning the effect on lipolysis or pancreatic insulin and proinsulin secretion of normal insulin in comparison to insulin analogs.

Design and methods: We included 13 patients with type 2 diabetes mellitus (age 62.2 ± 10.3 years) with preexisting insulin therapy in this crossover, prospective, open-labeled, randomized trial comparing regular HI with insulin aspart (IA) in the setting of a standardized breakfast and a standardized lunch 4 h later. Blood samples for determination of glucose, free fatty acids (FFA), triglycerides, C-peptide, and intact proinsulin were drawn during fasting and every 30 min until 4 h after the second test meal. Statistical analysis was performed with ANOVA for repeated measurements and paired Student’s t-test.

Results: The mean increase in blood glucose was significantly lower after IA (24.18 ± 16.33 vs 34.92 ± 29.07 mg/dl, P < 0.02) compared with HI. Both therapies reduced FFA; however, the mean reduction was significantly higher after IA than after HI (−0.47 ± 0.16 vs −0.35 ± 0.15 μmol/l, P < 0.001). The mean increase in intact proinsulin was significantly lower after IA than after HI (10.53 ± 5 vs 15.20 ± 6.83 pmol/l, P < 0.001). No differences were observed in the C-peptide levels between the two groups.

Conclusion: In the setting of two consecutive meals, IA reduces lipolysis and proinsulin secretion more effectively than HI.

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Introduction

Postprandial hyperglycemia and hyperlipidemia have been established as risk factors for cardiovascular complications in type 2 diabetic or prediabetic subjects with impaired glucose tolerance (1, 2). Postprandial metabolic disturbances occur when a number of homeostatic mechanisms following a meal intake are blunted. In particular, insulin-mediated increase in glucose uptake, suppression of hepatic gluconeogenesis, or postprandial reduction of free fatty acids (FFA) represents important mechanisms that are impaired in the diabetic state after the ingestion of a meal (3–5).

Currently, HbA1c is regarded as the gold standard for measuring glycemic control in diabetic patients but HbA1c does not accurately account for postprandial glucose fluctuations. However, as humans spend half their lives in the postprandial state (6), it is not surprising that a number of studies found an association of postprandial hyperglycemia and microvascular as well as macrovascular complications (1, 2, 7).

Insulin resistance and β-cell dysfunction, both present from the very beginning of type 2 diabetes, are not only associated with hyperglycemia but also associated with fasting and postprandial hyperlipidemia (8, 9). Postprandial hypertriglyceridemia, for example, has been shown to be the strongest independent predictor of carotid intima media thickness in type 2 diabetic patients (10). In addition, increased exposure to elevated levels of FFA is associated with detrimental
effects on β-cell function and insulin sensitivity (11), and the amount of postprandial reduction on FFA levels is a reasonable measure for lipolysis (12). Therefore both postprandial hyperglycemia and hyperlipidemia should be considered in the therapy of type 2 diabetic patients.

Owing to the progressive nature of the disease caused by the ongoing decline in pancreatic β-cell function, insulin therapy becomes mandatory in many patients with longer duration of disease to achieve glycemic goals (1, 6, 13). With respect to the reduction of postprandial glucose excursions in type 2 as well as type 1 diabetic patients, recent data favor the use of rapid acting insulin analogs over human regular insulin (14–16).

However, until now, there are no studies comparing both types of insulin with respect to postprandial hyperlipidemia. The aim of our study was to investigate the impact of regular human insulin (HI) and the short acting analog insulin aspart (IA) in a ‘real-life setting’ of two consecutive meals (breakfast and lunch) on postprandial glucose, triglycerides, FFA, C-peptide, as well as proinsulin levels.

Methods

The study was performed as a crossover, prospective, open-labeled, randomized trial comparing regular HI (Insulin Actrapid, NovoNordisk A/S, Denmark) and IA (Insulin NovoRapid, NovoNordisk A/S).

We recruited 13 patients with type 2 diabetes (six females and seven males) from the diabetes outpatient clinic of the Department of Internal Medicine, Medical University of Graz. The enrolled subjects had to be either on a preexisting insulin therapy or on a combination therapy of insulin with metformin. Patients with severe renal or hepatic failure and subjects on sulfonylurea therapy were not eligible for the study. The study was performed according to the Declaration of Helsinki and approved by the local ethics committee. Written informed consent was obtained from all subjects.

Study procedures were performed on 2 days separated by at least 3 days. Subjects were randomized to receive either regular HI or IA on the first day and consequently the alternative insulin on the second day.

Examinations were performed after an overnight fast of at least 12 h. Patients received a standardized breakfast with 1.8 IU (in total 15 IU) regular HI or IA per 12 g carbohydrates. The standard breakfast was the same for all study days and had to be consumed within 15 min. The meal contained 773 kcal (100 g carbohydrates, 35 g fat, and 13.6 g proteins). Patients were permitted to consume only water throughout the study procedures. After 4 h, participants received a standardized lunch (611 kcal; 84 g carbohydrates, 21 g fat and 21.5 g protein) with ~1.5 IU (in total 11 IU) of the same insulin (regular HI or IA) per 12 g carbohydrates.

Venous blood samples for determination of FFA, C-peptide, and intact proinsulin were drawn before breakfast as well as after 30, 60, 90, 120, 180, 240, 270, 300, 330, 360, 420, and 480 min. Therefore, the sampling at 240 min served as preprandial reference for the second meal.

Glucose, C-peptide, and HbA1c were measured by routine methods using commercially available kits.

Triglyceride concentrations were determined enzymatically with the GPO-PAP method (Roche Diagnostics) on a Modular analyzer (Roche Diagnostics). FFA was measured enzymatically (Wako Chemical, Neuss, Germany) on an Olympus AU640 (Olympus Diagnostics, Hamburg, Germany). Lipid measurements had coefficients of variation <5% throughout.

Intact proinsulin was measured using an immunochromoluminometric assay for the quantitative measurement of intact proinsulin (MLT Research Limited, Cardiff, UK). The sensitivity of the test was estimated as two standard deviations from the mean of 20 replications of the zero standard with 0.02 pmol/125 mU per ml.

Sample size estimation was calculated with GPower on an Apple Macintosh. Assuming a P<0.05, a power of 0.80, and double-sided paired testing, a sample size of 13 was calculated to detect a reduction of 20% in glucose area under curve (AUC) after a single meal only (4 h period). SD assumed for power calculation was 130 mg×4 h/dl.

Total AUC of glucose, proinsulin, C-peptide, triglycerides, and FFA were calculated according to the trapezoid rule. The ‘mean changes’ were calculated as followed: for each time point, the difference to baseline was calculated and finally a mean of all these differences (over both meals) was calculated.

All analyses were performed using SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). Data are expressed as mean±S.D. unless stated. Statistical analysis was performed with ANOVA for repeated measurements and paired Student’s t-test. Differences were considered statistically significant when P<0.05.

Results

The patients included in this study represent a common patient group with type 2 diabetes with a mean age of 62 years, a body mass index (BMI) of 30.6 kg/m², and a mean HbA1c of 7.7%. All baseline characteristics of the population are shown in Table 1.

Table 2 summarizes all the parameters investigated throughout the study.

Postprandial hyperglycemia

The mean increase in blood glucose (Fig. 1) was significantly lower after IA compared with regular HI. Blood glucose values from the first measurement after the breakfast up to 7 h postprandially were lower after administration of IA than after regular HI, although no
Table 1 Baseline characteristics of the study population (mean ± s.d.).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (m/f)</td>
<td>7/6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.2 ± 10.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.6 ± 4.3</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>12.8 ± 9.2</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.97 ± 0.06</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>137 ± 13/75 ± 6</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.7 ± 0.7</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>184 ± 34</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>93 ± 22</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>58 ± 15</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>176 ± 114</td>
</tr>
<tr>
<td>Insulin therapy</td>
<td></td>
</tr>
<tr>
<td>Premixed insulin (twice daily)</td>
<td>9 (69%)</td>
</tr>
<tr>
<td>Premixed insulin (thrice daily)</td>
<td>3 (23%)</td>
</tr>
<tr>
<td>Prandial insulin</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Metformin</td>
<td>12 (92%)</td>
</tr>
</tbody>
</table>

statistical significance could be reached for single time points. After IA treatment, blood glucose reached peak values after 1.5 and 5 h, and after HI treatment, peak values were seen after 2 and 5 h. The AUC for glucose was not significantly lower after IA than after HI.

Postprandial hyperlipidemia

During the two consecutive test meals, FFA (Fig. 1) were reduced under either insulin treatment. The mean reduction, however, was significantly greater after IA than after HI. This reduction was observed after breakfast (P = 0.004) as well as after lunch (P = 0.001).

After treatment with regular HI, the mean increase in triglycerides was lower than that after IA but without statistical significance. The curve progression was quite similar for the two different treatment regimens.

C-peptide and intact proinsulin

The mean increase in C-peptide, reflecting pancreatic insulin secretion, was slightly but not significantly higher after HI compared with IA.

Postprandial intact proinsulin (Table 2) at baseline was not different between both regimens and levels continuously increased after the breakfast reaching peak values within 1 h after lunch in both treatment regimens. The mean increase in intact proinsulin was significantly lower after IA injections than after HI application.

Discussion

Our study compares for the first time postprandial glucose, lipid, and intact proinsulin excursions over two consecutive meals after the injection of either human regular insulin or IA.

We confirm and extend previously published data (17, 18) showing that postprandial glucose excursions are lower over 2 consecutive meals when IA is injected preprandially in comparison to HI. High amplitudes of glucose oscillation have been shown to activate the protein kinase C pathway (19), inducible nitric oxide synthase (20), and to induce inflammatory markers (21) much more than elevated but stable glucose levels. Furthermore Monnier et al. (22) clearly showed that acute glucose fluctuations cause considerably more oxidative stress than continuous hyperglycemia. Concordantly, recently published data demonstrated that oscillating glucose levels have a significantly more deteriorating effect on endothelial function than elevated but stable glucose levels. Also, platelets were shown to be activated by postprandial hyperglycemia (23).

Table 2 Comparison of baseline and outcome measurements between treatment groups. Measurements are given as mean ± s.d.

<table>
<thead>
<tr>
<th></th>
<th>Regular human insulin</th>
<th>Insulin aspart</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>180 ± 73</td>
<td>197 ± 78</td>
<td>0.26</td>
</tr>
<tr>
<td>C-peptide (mg/dl)</td>
<td>3.79 ± 1.81</td>
<td>4.17 ± 2.24</td>
<td>0.32</td>
</tr>
<tr>
<td>Free fatty acids (µmol/l)</td>
<td>0.66 ± 0.42</td>
<td>0.82 ± 0.28</td>
<td>0.32</td>
</tr>
<tr>
<td>Proinsulin (pmol/l)</td>
<td>16.07 ± 13.25</td>
<td>14.64 ± 14.33</td>
<td>0.26</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>191 ± 143</td>
<td>165 ± 101</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Mean change from Baseline (Δ)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>34.92 ± 29.07</td>
<td>24.18 ± 16.33</td>
<td>0.02</td>
</tr>
<tr>
<td>C-peptide (mg/dl)</td>
<td>2.2 ± 1.03</td>
<td>1.96 ± 0.94</td>
<td>0.41</td>
</tr>
<tr>
<td>Free fatty acids (µmol/l)</td>
<td>−0.35 ± 0.15</td>
<td>−0.47 ± 0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proinsulin (pmol/l)</td>
<td>15.2 ± 6.8</td>
<td>10.5 ± 5.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>44.3 ± 30.3</td>
<td>47.2 ± 35.3</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>Area under curve (AUC)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose (mg×8 h/dl)</td>
<td>656 ± 200</td>
<td>669 ± 182</td>
<td>0.72</td>
</tr>
<tr>
<td>C-peptide (mg×8 h/dl)</td>
<td>18.48 ± 9.11</td>
<td>18.32 ± 7.41</td>
<td>0.90</td>
</tr>
<tr>
<td>Free fatty acids (µmol×8 h/l)</td>
<td>0.88 ± 0.54</td>
<td>0.97 ± 0.34</td>
<td>0.23</td>
</tr>
<tr>
<td>Proinsulin (pmol×8 h/l)</td>
<td>90.07 ± 64.09</td>
<td>87.97 ± 68.16</td>
<td>0.56</td>
</tr>
<tr>
<td>Triglycerides (mg×8 h/dl)</td>
<td>711 ± 408</td>
<td>646 ± 334</td>
<td>0.07</td>
</tr>
</tbody>
</table>

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Although the glucose excursions were decreased, the AUC for glucose was not improved. The lack of reduction of overall glucose exposure noted with IA despite the apparent flattening of the glucose excursions after the meal may be explained by the high fasting glucose levels prior to the meal tests.

Extending these known effects and advantages of a rapid acting insulin analog on postprandial hyperglycemia, we could furthermore demonstrate differences in the impact of the insulins tested on FFA levels. Reductions in mean FFA levels were significantly more pronounced when using IA in comparison to HI.

This effect could be observed both after breakfast and lunch. Such data suggest that in the setting of two consecutive meals, IA reduces lipolysis more effectively than human regular insulin.

Relevance of proinsulin

By splitting off the connecting peptide (C-peptide) from the precursor molecule proinsulin (24), insulin is produced in the pancreatic β-cells. With increasing insulin resistance and hyperglycemia, processing of proinsulin becomes incomplete and the β-cells start to secrete proinsulin and as diabetes duration rises, the percentage of proinsulin further increases (25). Elevated levels of proinsulin are indicators of impaired function as well as chronic over-stimulation of the β-cells caused by insulin resistance or β-cell stimulating drugs such as sulfonylureas (26). Furthermore proinsulin levels are associated with atherosclerosis or future cardiovascular events (27–29). Schneider et al. (30) showed that increased proinsulin levels are associated with an enhanced secretion of plasminogen activator inhibitor I that in turn inhibits the fibrinolytic cascade and consequently proinsulin was proven to be an independent cardiovascular risk factor (31) and external administration of proinsulin in a phase II clinical trial was associated with excess cardiovascular events and therefore the trial was early terminated (32). Therefore one could speculate that the between-group difference in the mean increase in proinsulin levels observed in our study might have an impact on cardiovascular outcome.

An important strength of our study is that in contrast to many other studies investigating postprandial effects of short acting insulins only after one single meal or a glucose tolerance test, we investigated cumulative effects over two consecutive meals closer mimicking ‘real-life’ conditions. Even if a return to baseline levels could be observed for glucose after 4 h, the levels of FFA or proinsulin do not return to baseline levels until the next meal and a pronounced carryover effect could be observed.

A limitation of our study is that, in retrospect, it was underpowered to detect a difference on the primary outcome parameter, the AUC for glucose. However, our data do not even show a trend in favor of IA with regard to the AUC of glucose. Another limitation in this regard is that fasting glucose levels of participants were not well controlled. It could be speculated that a better control of fasting glucose levels might have made a difference in the AUC of glucose between the two insulins investigated more likely.

We demonstrated that IA exhibits beneficial effects on postprandial glucose levels that persist during two consecutive meals in comparison to regular HI. Besides these known effects on glucose levels, we provide evidence that IA reduces postprandial lipolysis as well as proinsulin secretion more effectively than HI does. We believe that our findings urge a need for further...
studies investigating potential differences of short acting analogs and regular HI with regard to insulin resistance, β-cell function, or cardiovascular endpoints.

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.

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References
25 Blickle JF, Sapin R & Andres E. Contribution of total and intact proinsulins to hyperinsulinism in subjects with obesity, impaired glucose tolerance or type 2 diabetes. Diabetes and Metabolism 2000 26 274–280.


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