The relationship between first-phase insulin secretion and glucose metabolism

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A possible pathogenetic link between absence of first-phase insulin secretion and development of impaired glucose metabolism has been suggested by the results of several cross-sectional studies. First-phase insulin secretion measured during a +7 mmol/l hyperglycemic glucose clamp correlated with total glucose disposal during the clamp (r = 0.65, p < 0.001, N = 59). To examine whether restoration of first-phase insulin secretion improves peripheral glucose uptake in subjects with impaired glucose utilization, seven insulin-resistant subjects (age 54 (38–62) years; BMI 29.3 (21.7–35.8); fasting plasma glucose 5.5 (4.8–7.2) mmol/l; fasting insulin 57 (37–105) pmol/l with impaired first-phase (148 (29–587) vs controls 485 (326–1086) pmol/l × 10 min; p < 0.05) and normal second-phase (1604 (777–4480) vs controls (1799 (763–2771) pmol/l × 110 min) insulin secretion were restudied. The impaired first-phase insulin secretion was restored by an iv insulin bolus at the start of the hyperglycemic clamp. Substrate oxidation rates and hepatic glucose production were determined by indirect calorimetry and [3-¹H]glucose infusion. Total glucose uptake was impaired in the insulin-resistant subjects with impaired first-phase insulin secretion compared to controls (18.8 (13.2–22.2) vs 34.8 (24.3–62.1) µmol·kg⁻¹·min⁻¹; p < 0.01). Restoration of first-phase insulin secretion (1467 (746–2440) pmol/l × 10 min) did not affect glucose uptake (20.2 (9.9–23.8) µmol·kg⁻¹·min⁻¹), with no difference in oxidative and non-oxidative glucose metabolism between the experiments. Second-phase insulin secretion was similar during both experiments. We conclude that although first-phase insulin secretion correlates with total glucose uptake, replacement of impaired first-phase insulin secretion does not improve glucose uptake in subjects with impaired glucose disposal and normal second-phase insulin secretion. The data dispute a causal relationship between first-phase insulin secretion and impaired glucose uptake in these subjects.

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The normal insulin response to intravenous glucose is biphasic, consisting of an initial burst (first-phase) of insulin followed by a slowly increasing phase (second-phase) of insulin secretion (1–3). A lacking first-phase as well as a diminished second-phase insulin secretion is a consistent finding in patients with type 2 diabetes (4, 5).

The physiological implication of the early peak of insulin secretion on glucose homeostasis is unclear. Previous cross-sectional studies suggest that the absence of the early peak of insulin secretion is pathogenetically involved in the development of glucose intolerance (3, 5–8). Early insulin secretion could act on either the liver or the peripheral muscle tissue, since elevated postprandial blood glucose could be due to impaired suppression of hepatic glucose output or diminished peripheral glucose uptake. A recent study addressing this question was carried out in healthy insulin-sensitive subjects in whom alterations in insulin secretion were caused by infusion of somatostatin (9). Absence of first-phase insulin secretion did not affect glucose uptake but led to a delayed suppression of hepatic glucose production. The authors concluded that this might contribute to the glucose intolerance seen at the early stages of diabetes. It is not known to what extent somatostatin can have influenced the results, however, since somatostatin is known to reduce splanchnic blood flow (10). Thus, another approach to studying the impact of first-phase insulin secretion on glucose uptake, avoiding the use of somatostatin, would be to try to restore the impaired first-phase insulin secretion in subjects with impaired glucose uptake.

This study was therefore undertaken to evaluate the acute effect of replacing the diminished first-phase insulin burst on glucose utilization and hepatic glucose production in subjects with impaired glucose uptake and normal second-phase insulin secretion.

Subjects and methods

Insulin secretion was measured using a +7 mmol/l hyperglycemic glucose clamp in 59 (28F, 31M; age 50 (26–71) years, fasting plasma glucose 5.8 (4.0–17.0) mmol/l, fasting insulin 51 (15–203) pmol/l) subjects (18 patients with type 2 diabetes, 31 first-degree rela-
tives of patients with type 2 diabetes and 10 healthy controls). The subjects came in part from our previous study (7). From the subjects studied initially, seven insulin-resistant subjects with impaired first-phase and normal second-phase insulin response were selected for further study (Table 1). The diminished first-phase insulin secretion was replaced by an iv insulin bolus (120 (80–133) pmol) (Actrapid Human, Novo Nordisk, Copenhagen, Denmark) given at the same time as the glucose infusion was initiated. Hepatic glucose production was measured with a primed constant infusion of [3-3H]glucose. Substrate oxidation rates and energy production were measured using indirect calorimetry. Seven healthy control subjects from the 10 control subjects studied initially were selected to serve as age and BMI matched controls to the seven subjects participating in the replacement protocol. Informed consent was obtained from all the subjects and the study was approved by the ethics committee at the Helsinki University Hospital.

Hyperglycemic glucose clamp

After three baseline samples for plasma glucose and serum insulin determinations were drawn, the plasma glucose concentration was rapidly raised by 7 mmol/l above the baseline value with a priming infusion of 20% glucose. The desired plasma glucose level was maintained for 120 min by adjusting the glucose infusion (2). Blood samples were drawn at 2-min intervals for determining plasma glucose and serum insulin concentrations during the first 10 min of the clamp. Thereafter, plasma glucose was determined every 5 min and serum insulin every 10 min throughout the clamp.

Hepatic glucose production was measured with the isotope dilution technique using a primed (25 μCi) constant (0.25 μCi/min) infusion of [3-3H]glucose (99.6% purity) (Amersham Inc., Amersham, UK) for 150 min and throughout the clamp. The priming dose was adjusted for the degree of glycemia. Blood samples for determining [3-3H]glucose specific activity were obtained at baseline and at 10-min intervals during clamping.

Indirect calorimetry was applied for 60 min in the basal state and during the last 30 min of the clamp to estimate substrate oxidation rates and energy production rates (11). A computerized open-circuit system was used to measure gas exchange through a transparent plastic canopy (Deltatrac, Datex, Helsinki, Finland). Protein oxidation was calculated from the urinary urea nitrogen excretion obtained before and during clamping and corrected for changes in urea pool (12).

Analytical determinations

Plasma glucose was measured using a glucose oxidase method (Beckman Glucose Analyzer II, Beckman Instruments, Fullerton, CA). The serum insulin concentrations were measured using a double-antibody radioimmunoassay (interassay CV 5%) (Pharmacia, Uppsala, Sweden). [3-3H]glucose specific activity was measured in duplicate on supernatants of 0.5 mol/l perchloric acid extracts of plasma samples after evaporation of tritiated water. The plasma extracts were suspended in xylene (Lumagel®, Lumac LSC, Olen, Belgium) and counted in a liquid scintillation counter (Wallac, Turku, Finland) for 5 min.

Calculations

Incremental insulin areas were calculated with the trapezoidal rule. The first- and second-phase insulin secretory responses were estimated by calculating the incremental insulin area during the first 10 min and between 10 and 120 min of the hyperglycemic clamp.

Basal hepatic glucose production was calculated by dividing the [3-3H]glucose infusion rate by the steady-state plateau of [3-3H]glucose specific activity achieved during the last 30 min of the basal tracer infusion period. After administration of glucose and stimulation of endogenous insulin secretion a non-steady-state in

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Table 1. Characteristics of subjects.

<table>
<thead>
<tr>
<th></th>
<th>Insulin-resistant subjects with impaired first-phase insulin secretion</th>
<th>Controls</th>
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<tbody>
<tr>
<td>N (F/M)</td>
<td>7 (4/3)</td>
<td>7 (2/5)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 (38–62)</td>
<td>51 (27–66)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.3 (21.7–35.8)</td>
<td>25.1 (24.6–28.5)</td>
</tr>
<tr>
<td>f-plasma glucose (mmol/l)</td>
<td>5.5 (4.8–7.2)</td>
<td>5.5 (4.2–6.2)</td>
</tr>
<tr>
<td>2-h postchallenge plasma glucose</td>
<td>8.8 (7.6–11)**</td>
<td>4.9 (3.5–6.6)</td>
</tr>
<tr>
<td>f-insulin (pmol/l)</td>
<td>57 (37–105)**</td>
<td>30 (15–53)</td>
</tr>
<tr>
<td>First-phase insulin secretion (pmol/l x 10 min)</td>
<td>148 (29–587)*</td>
<td>485 (326–1086)</td>
</tr>
<tr>
<td>Second-phase insulin secretion (pmol/l x 110 min)</td>
<td>1604 (777–4480)</td>
<td>1799 (763–2771)</td>
</tr>
<tr>
<td>Glucose uptake (μmol/kg⁻¹·min⁻¹)</td>
<td>18.8 (13.2–22.2)**</td>
<td>34.8 (24.3–62.1)</td>
</tr>
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</table>

BMI = body mass index.

*p < 0.05, **p < 0.01 vs control subjects.

# 75 g oral glucose load.
glucose turnover rate exists and the rate of glucose appearance was calculated using a two-compartment model (13). The infusion rate of cold glucose was integrated over 10 min and subtracted from the total rate of glucose appearance to obtain hepatic glucose production rate. However, under conditions of rapid expansion of the glucose pool and rapidly changing plasma glucose concentrations the two-compartment model will not estimate the glucose turnover adequately. The result will be an underestimation of glucose appearance and disappearance rates. As a consequence, physiologically impossible negative values for hepatic glucose production will be obtained. Accordingly, in the present study we observed negative hepatic glucose production rates during the first 30 min of the hyperglycemic clamp. Negative values for hepatic glucose production occurred occasionally during the second hour of clamping and these values were taken to indicate that endogenous glucose production was completely suppressed. Total body glucose metabolism was calculated by adding the rate of hepatic glucose production rate during the last 30 min to the glucose infusion rate and subtracting urinary glucose loss during the same period.

Net glucose and lipid oxidation rates were calculated from indirect calorimetric measurements in the basal state and during the second hour of clamping. The constants to calculate glucose, lipid and protein oxidation from gas exchange data are given in reference 11. Non-oxidative glucose metabolism was calculated as the difference between the total body glucose metabolism and glucose oxidation as determined by indirect calorimetry.

Statistical analysis

All data are expressed as median and range, unless otherwise stated. A BMDP statistical package was used for the statistical analysis. Differences from baseline were tested using the Wilcoxon rank sum test. Differences between group means were tested using the Mann-Whitney-U test. Correlations were tested by linear regression analysis. A p value of less than 0.05 was considered statistically significant.

Results

Total glucose uptake during the hyperglycemic clamp correlated with first-phase insulin secretion in the whole study population (r = 0.65, p < 0.001) (Fig. 1). This was due to the correlation in the relatives to type 2 diabetic patients (r = 0.48, p < 0.01) and the type 2 diabetic patients (r = 0.47, p < 0.05). Furthermore, there was a positive correlation between second-phase insulin secretion and total glucose uptake in all subjects (r = 0.49, p < 0.01). The seven restudied insulin-resistant subjects with impaired first-phase and normal second-phase insulin secretion showed an impaired rate of glucose utilization compared to the controls (Table 1).

Steady-state plasma glucose during the last 30 min of clamping did not differ between experiments (12.8 (11.9–14.6) vs 12.8 (11.7–16.2) mmol/l) in the subjects with impaired glucose uptake; this was no different from the controls (12.4 (10.5–13) mmol/l). Second-phase insulin secretion was similar during both experiments and did not differ from the controls (Fig. 2). Replacement of first-phase insulin secretion did not affect total glucose uptake (20.2 (9.9–23.8) μmol·kg⁻¹·min⁻¹). The contribution of glucose oxidation (8.3 (5.4–11.9) vs 7.6 (6.3–12.6) μmol·kg⁻¹·min⁻¹) and non-oxidative glucose metabolism (9.5 (7.8–14.1) vs 10.7 (2.3–14.4) μmol·kg⁻¹·min⁻¹) to total body glucose uptake remained unchanged during both experiments. Suppression of hepatic glucose production by insulin was unaffected by the replacement of first-phase insulin secretion (Table 2).
Discussion

In the present study, first-phase insulin secretion correlated with total glucose uptake measured during a hyperglycemic clamp in individuals with different degrees of insulin resistance and insulin deficiency. We therefore anticipated that if first-phase insulin secretion is an important determinant of glucose homeostasis, restoration of first-phase insulin secretion should improve glucose uptake in individuals with impaired glucose uptake but normal second-phase insulin secretion. This was not the case, since replacement of first-phase insulin secretion did not affect total glucose uptake in the subjects with impaired glucose utilization participating in the replacement protocol. This finding is in accordance with a recent study by Luzi and DeFronzo where the role of absent first-phase insulin secretion was studied in healthy control subjects using the hyperglycemic clamp technique in combination with somatostatin (9). In that study, loss of first-phase insulin secretion did not cause impairment in total glucose disposal during the clamp. It may be argued that the hyperglycemic glucose clamp is not an appropriate measure of glucose metabolism because of low insulin levels during the clamp. However, neither did mimicking the first-phase insulin secretion at the beginning of an 0.75 mU/kg⁻¹·min⁻¹ hyperinsulinemic euglycemic clamp, resulting in insulin levels of 85 mU/l influence insulin sensitivity during the clamp (14). From these studies, no conclusions can be drawn on the impact of chronic replacement of early insulin secretion on glucose uptake. Anyhow, the findings from the acute studies are supported by a more chronic study by Hosker et al. (15). In the study, the hyperglycemic clamp technique was used to assess insulin secretion and glucose uptake in subjects with mild type 2 diabetes. It was found that six weeks of treatment with glicazide nearly normalized both the first- and second-phase insulin secretion but did not affect glucose uptake in these subjects.

First-phase insulin secretion has also been ascribed a role in the regulation of hepatic glucose output. Absence of first-phase insulin secretion enhanced the stimulatory effect of glucagon on hepatic glucose output in dogs (16, 17). Accordingly, Luzi and DeFronzo showed that abolishing first-phase insulin secretion by infusion of somatostatin resulted in delayed suppression of hepatic glucose output by insulin (9). In the present study, replacement of the early insulin secretion did not significantly affect suppression of hepatic glucose production by insulin. The use of somatostatin infusion in the study by Luzi and DeFronzo could at least partially explain the different results, since somatostatin has been shown to influence splanchnic blood flow (10).

In a recent study Elahi et al. proposed that in healthy subjects regulation of hepatic glucose production by insulin is dependent not just on the ambient insulin concentration but also on the temporal pattern of insulin secretion (18). The authors observed an escape of hepatic glucose production during the hyperglycemic clamp which was not overcome until systematic insulin concentrations surpassed the peak of first-phase insulin release after 120 min of clamping. In keeping with the findings of Luzi and DeFronzo, we could not demonstrate any escape of hepatic glucose production during the hyperglycemic clamp. At the end of the 120 min clamp, hepatic glucose production was almost completely suppressed. Caution is still warranted when viewing the effect of first-phase insulin secretion on hepatic glucose production.

Since attempts to modulate first-phase insulin secretion have failed to influence glucose uptake, the question remains whether glucose uptake directly influences insulin secretion, i.e. whether first-phase insulin secretion could be the consequence rather than the cause of impaired glucose uptake. One way of addressing this question would be to assess insulin secretion in subjects with experimentally induced insulin resistance. We have recently studied the effect of steroid-induced insulin resistance on insulin secretion in subjects receiving steroid therapy following kidney transplantation (19). Patients developing steroid diabetes were compared with steroid-treated patients with normal glucose tolerance. Both groups showed a similar degree of insulin resistance. Those who developed diabetes showed impaired first- and second-phase insulin secretion, whereas those with normal glucose tolerance showed increased first- and second-phase insulin responses. The insulin response was, thus, either reduced or enhanced in the individuals with steroid induced insulin resistance. Only insulin resistant individuals with an additional β-cell defect developed diabetes. Impaired first-phase insulin secretion would then reflect a concomitant β-cell defect which has developed independently of any changes in insulin action.

We conclude that first-phase insulin secretion correlates with glucose uptake measured during a hyperglycemic clamp. Acute replacement of the early insulin burst does not affect glucose homeostasis in subjects characterized by impaired glucose uptake and normal glucose production.

Table 2. Hepatic glucose production (μmol/kg⁻¹·min⁻¹) during the hyperglycemic clamp.

<table>
<thead>
<tr>
<th>Min</th>
<th>basal</th>
<th>0-30</th>
<th>30-60</th>
<th>60-80</th>
<th>80-100</th>
<th>100-120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>8.4 (7.7-14.3)</td>
<td>-5.1 (-15.2-1.11)</td>
<td>5.4 (2.4-10.5)</td>
<td>4.8 (-2.3-8.9)</td>
<td>-2.25 (-3.6-9.3)</td>
<td>1.2 (-1.6-3.2)</td>
</tr>
<tr>
<td>After insulin bolus</td>
<td>8.7 (7.4-13.5)</td>
<td>-9.0 (-14.1-6.1)</td>
<td>1.7 (-10.3-11.1)</td>
<td>3.7 (-8.2-15.6)</td>
<td>-2.0 (-0.9-7.3)</td>
<td>1.5 (-1.6-2.6)</td>
</tr>
</tbody>
</table>
second-phase insulin secretion. The data suggest that even though present at the same time, impaired glucose uptake and impaired first-phase insulin secretion are separate defects without a causal relationship in these subjects. The effect of first-phase insulin secretion on hepatic glucose production remains to be elucidated.

Acknowledgments. This study was supported by grants from Finska Läkaresällskapet, the Sigrid Juselius Foundation, the Nordisk Insulin Foundation and the Perkin Foundation.

References


Received November 20th, 1991
Accepted June 3rd, 1992