Metabolic effects of pulsatile insulin infusion in the elderly

Giuseppe Paolisso¹, Teresa Salvatore², Saverio Gsambato², Roberto Torella², Michele Varricchio¹ and Felice D’Onofrio²

Istituto di Gerontologia e Geriatria¹, Istituto di Medicina Generale, Terapia Medica e Malattie del Metabolismo²,
1st Medical School, University of Naples, Naples, Italy

Abstract. The present study investigated the metabolic effects of pulsatile insulin delivery at a pulse rate of 2+11 and 2+18 min in 7 healthy, elderly subjects (71.4±2.1 years), submitted to 260 min controlled iv glucose infusion via the Biostator. The endogenous secretion of pancreatic hormones was inhibited by somatostatin (3 u.g/min) and glucagon was replaced (67 ng/min) to basal levels. The same total insulin dose was delivered on both occasions. Insulin infusion rate was 1.3 and 2.0 mIU · kg⁻¹ · min⁻¹ during switching on/off of 2+11 and 2+18 min, respectively. Blood glucose levels and glucose infusion rate were monitored continuously by the Biostator; [D-3-H]glucose infusion allowed determination of glucose turnover. During the last 60 min of the experiment, pulsatile insulin at a pulse rate of 2+11 vs 2+18 min produced a stronger inhibition of endogenous glucose production, whereas glucose disappearance rate and glucose metabolic clearance rate were similarly affected. Plasma triglycerides, apolipoprotein B, and free fatty acids levels were also more suppressed during insulin delivery at pulse rate of 2+11 than at 2+18 min.

Previous reports in animals and man have clearly demonstrated the existence of oscillations in peripheral plasma insulin and glucagon levels (1-3). It has been suggested that those oscillations depend on a pacemaker present in the gland itself. Moreover, it has been shown that in normal healthy man, insulin oscillations have a periodicity of 13 min and a mean amplitude of 11.5 pmol/l (1-3). In non-insulin-dependent diabetic patients the oscillations of plasma insulin are more rapid and generally less regular than in normal subjects (4).

More recently, Matthews et al. (5) have shown that increasing age is associated with a longer pulse interval (20 min) of B-cell secretion. However, no data on the metabolic consequences of this prolonged pulsatile insulin secretory pattern are available.

The aim of the present study was to compare the changes in glucose turnover parameters and in plasma lipid levels in response to pulsatile insulin infusion, 2+11 and 2+18 minutes, in healthy men aged 68 to 79 years.

Subjects and Methods

Seven healthy men aged 71.4±2.1 years (range 68-79) volunteered for the study. The mean Body Mass Index was 21±1.3 kg · m⁻². None had a family history of diabetes mellitus or was taking any drug during at least three weeks before the experiments. All subjects had normal oral glucose tolerance test and were consuming a regular, weight-maintaining diet. All gave their informed consent to participate in the study, which was approved by the Ethical Committee of our institution.

Experimental design

The subjects were studied in the morning, starting at 8.00-9.00 h after a 12-h overnight fast. Each subject was tested on two occasions, in random order, separated by at least 1 week. Total insulin dose delivered on both occasions was identical. In one experiment insulin (Actrapid HM®) Novo Industri A/S, Denmark) was delivered at a pulse rate of 2+18 (2 mIU · kg⁻¹ · min⁻¹ during 2 min followed by 18 min with no insulin infused). In the other experiment, insulin pulse rate was 2+11 (1.3 mIU · kg⁻¹ · min⁻¹ during 2 min followed by 11 min with no insulin
infused). Glucose clamp was performed as previously described (6). In all experiments, the saline channel of the Biostator was used to infuse from 0 to 260 min somatostatin (Modulstatin®, Midy, Italy; 3 μg/min) and glucagon (Novo Industri A/S, Denmark; 67 ng/min).

All three hormones, insulin, glucagon and somatostatin, were dissolved in saline containing 3 g/l human albumin (ISI, Italy).

To quantify the rate of glucose appearance and the rate of overall glucose disappearance in the basal state and during the experiment, we used a primed (20 μCi) continuous (0.2 μCi/min) infusion of [D-3-3H]glucose (New England Nuclear, Boston, MA; specific activity 11.5 Ci/mmol) dissolved in saline. At least 2 h were allowed for isotopic equilibration. An additional high precision pump (Hoechst pump, Frankfurt, FRG) was used to infuse the [D-3-3H]glucose solution.

Blood sampling

For glucose turnover measurements, samples of blood were collected at −30, −20, −15, −10, −5 and 0 min and then every 20 min during the 60 min study period. Samples for determination of plasma C-peptide and glucagon were collected at −20 and 0 and then every 60 min. Blood samples for plasma insulin determination were collected at the times indicated in the legends to the figures. Blood samples for hormones and glucose kinetic parameters were collected and treated as previously reported (6).

Analytical technique

Except for plasma glucose which was determined immediately after the experiment by Auto-Analyzer (Beckman Instruments, USA), all other blood samples were centrifuged after each experiment; plasma was stored at −20°C until assay.

The specific activity of plasma [D-3-3H]glucose was determined as follows: 1-ml aliquots of plasma were deproteinized according to Somogyi (7). The resultant filtrate was aliquoted in two 0.5-ml samples that were lyophilized to remove the tritiated water resulting from metabolism of [D-3-3H]glucose during the experiment. The dry residue was resuspended with 0.5 ml of distilled water and its radioactivity counted in a refrigerated liquid scintillation counter after addition of 5 ml of Aquasol (New England Nuclear). The average glucose radioactivity of each plasma sample was divided by its glucose concentration to obtain the glucose specific activity. Similarly, four aliquots of the infused solution of [D-3-3H]glucose were counted and the average radioactivity, as well as the infusion rate, was used in subsequent calculations. Plasma insulin, glucagon, C-peptide and lipid levels were all determined as reported elsewhere (8).

Calculations and statistical analysis

Rates of glucose turnover parameters were calculated from isotopic dilution data on 20-min integrated values using the classic monocompartmental model of Steele (9). All other calculations were performed as previously reported (6). All statistical comparisons between each modality of hormone administration were performed by two-tailed t-test analysis. A p-value of 0.05 or less was considered of statistical significance. Sequential paired t-tests were validated by Bonferroni's test. All data are expressed as mean±SEM.

Results

Pancreatic hormone data

As indicated in Fig. 1, plasma C-peptide levels decreased progressively to reach very low values at the end of the experiment in both conditions tested, thus indicating an almost complete inhibition of endogenous insulin secretion. Intermittent insulin administration at the pulse rate of 2+11 min resulted in regular oscillations of plasma insulin levels, which varied between 16 and 148 pmol/l at time 0 and 3 min respectively, in all cycles analysed. The mean amplitude of insulin oscillations averaged 139 pmol/l. The pulse rate of 2+18 min resulted in regular oscillations of plasma insu-

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Fig. 1. Plasma insulin, glucagon and C-peptide levels during pulsatile insulin delivery at a pulse rate of 2±11 (•) and 2+18 (○) min. Multiple collections of blood permitting demonstration of oscillations in plasma insulin were performed in the last 65 min of the experiment. Results are expressed as mean±sem (N=7).
plasm levels between 19 and 227 pmol/l at 0 and 3 min, respectively, in all cycles analysed. The mean amplitude of insulin oscillations averaged 206 pmol/l. No statistically significant difference was observed in plasma insulin area calculated in the last 60 min of the experiment (4778 ± 551 vs 5171 ± 669 pmol l⁻¹ min⁻¹). Basal plasma glucagon levels were similar in the two experimental conditions and averaged 113 ± 33 vs 129 ± 41 ng/l during the pulse rate of 2+11 and 2+18 min, respectively. Replacement of glucagon during somatostatin infusion resulted in similar peripheral plasma glucagon levels in both conditions: 118±31 vs 131±38 ng/l (NS) at the end of the experiment during insulin pulse rate 2+11 and 2+18 min, respectively.

**Plasma glucose and glucose kinetic parameters**

As illustrated in Fig. 2 and detailed in Table 1, plasma glucose levels were significantly different in the first part of the study. In fact, plasma glucose, in both basal conditions about 4.7 mmol/l, rose to almost 6 and 5 mmol/l during insulin delivery at pulse rates of 2+18 and 2+11 min, respectively. In contrast, in the last 65 min (Fig. 2 and Table 2), no differences were observed in plasma glucose whatever the pulse rate of insulin delivery. In each subject, the coefficients of variation of plasma glucose values during the last 65 min of the test were 2.6±0.9 and 3.0±0.5% (NS) for pulse rates 2+11 and 2+18 min, respectively.

The glucose infusion rate was markedly inhibited, reaching values close to zero in the first 65 min of the experiment (pulse rate of 2+18 min), whereas it averaged 2.5±0.6 μmol · kg⁻¹ · min⁻¹ during intermittent hormone administration (pulse rate 2+11 min). Similarly, in the last hour (Table 2) insulin delivery at a pulse rate of 2+11 vs 2+18 min produced a greater increase in this parameter.

Glucose appearance rate slightly increased during the first 65 min and was statistically higher during the insulin pulse rate 2+18. At the end of the experiments the appearance rate averaged 12.8±0.3 and 10.3±0.2 μmol · kg⁻¹ · min⁻¹ (p<0.05) during pulse rates 2+18 and 2+11 min, respectively. Endogenous glucose production, cal-

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**Table 1.**

Glucose turnover parameters in the first 65 min of the experiment. Pulsatile insulin delivery with 2+11 min (A) and 2+18 min (B) periodicity.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
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<tbody>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>4.8±0.3</td>
<td>5.8±0.2</td>
</tr>
<tr>
<td>Glucose infusion rate</td>
<td>2.5±0.6</td>
<td>1.3±0.5</td>
</tr>
<tr>
<td>(μmol · kg⁻¹ · min⁻¹)</td>
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<tr>
<td>Glucose appearance rate</td>
<td>13.1±0.1</td>
<td>14.8±0.1</td>
</tr>
<tr>
<td>(μmol · kg⁻¹ · min⁻¹)</td>
<td></td>
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<tr>
<td>Endogenous glucose</td>
<td>10.6±0.1</td>
<td>13.6±0.2</td>
</tr>
<tr>
<td>production (μmol · kg⁻¹ · min⁻¹)</td>
<td></td>
<td></td>
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<tr>
<td>Glucose disappearance</td>
<td>9.6±0.2</td>
<td>8.7±0.3</td>
</tr>
<tr>
<td>rate (μmol · kg⁻¹ · min⁻¹)</td>
<td></td>
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<tr>
<td>Glucose metabolic</td>
<td>2.31±0.37</td>
<td>2.59±0.31</td>
</tr>
<tr>
<td>clearance rate (ml · kg⁻¹ · min⁻¹)</td>
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Results are mean ± SEM.
Table 2.
Glucose turnover parameters in the last 65 min of the experiment. Pulsatile insulin delivery with 2+11 min (A) and 2+18 min (B) periodicity.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A</th>
<th>B</th>
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<tr>
<td>Plasma glucose (mmol/l)</td>
<td>5.2±0.2</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td>Glucose infusion rate (μmol·kg⁻¹·min⁻¹)</td>
<td>5.1±0.2</td>
<td>3.1±0.1</td>
</tr>
<tr>
<td>Glucose appearance rate (μmol·kg⁻¹·min⁻¹)</td>
<td>10.3±0.2</td>
<td>12.8±0.3</td>
</tr>
<tr>
<td>Endogenous glucose production (μmol·kg⁻¹·min⁻¹)</td>
<td>5.2±0.3</td>
<td>9.7±0.2</td>
</tr>
<tr>
<td>Glucose disappearance rate (μmol·kg⁻¹·min⁻¹)</td>
<td>9.9±0.1</td>
<td>10.5±0.2</td>
</tr>
<tr>
<td>Glucose metabolic clearance rate (ml·kg⁻¹·min⁻¹)</td>
<td>2.28±0.25</td>
<td>2.31±0.27</td>
</tr>
</tbody>
</table>

Results are mean ± SEM.

culated from the difference between glucose appearance rate and glucose infusion rate, was significantly less for insulin pulse rate 2+11 than for 2+18 min. These lower values were found both during the first (Table 1) and the last (Table 2) 65 min of the experiment. In both modes of insulin delivery, glucose disappearance rate and glucose metabolic clearance rate did not differ significantly.

**Plasma lipid variations**
The mean basal plasma lipid levels were similar on both days. During insulin pulse rate 2+11 min, but not 2+18 min, hormone administration induced a significant decline in plasma triglycerides and apolipoprotein B levels, whereas it increased plasma HDL-cholesterol (Fig. 3). Plasma total cholesterol and LDL-cholesterol levels did not change during either mode of insulin administration (Fig. 3). Plasma free fatty acids, similar in basal conditions (841±79 vs 869±93 μmol/l, p=NS), were also significantly more suppressed (518±136 vs 666±149 μmol/l, p<0.005) during insulin administration with pulse rate 2+11 than with 2+18 min.

**Discussion**
In normal man, basal plasma insulin levels oscillate regularly, with a mean periodicity of 13 min and a mean amplitude of 11.5 pmol/l (10-12). In patients with non-insulin-dependent diabetes mellitus, the oscillations of plasma insulin are more rapid and generally less regular than in normal subjects (4). These brief irregular oscillations, with a mean periodicity of 8.8 min in are superimposed on slower 30-min oscillations (4). More recently, Matthews et al. (5) provided evidence that old age is also a condition associated with a longer pulse interval of B-cell secretion. In particular, these authors showed that in a group of 6 elderly subjects,
matched for Body Mass Index and fasting plasma glucose concentration, oscillatory patterns of B-cell secretion had a peak shift to 20 min, as examined by Fourier transformation.

In light of this knowledge, we have investigated the metabolic consequences of this abnormal length of pulsatile B-cell secretion in the elderly. Our results demonstrate that a shift from a shorter to a longer periodicity of pulsatile B-cell secretion might greatly contribute to impair glucose handling in old age. In fact, endogenous glucose production was significantly less inhibited when an identical dose of insulin was delivered with a 2+18 min than a 2+11 min rate.

Our results are in agreement with those reported in normal man by Matthews et al. (13) and ourselves (6), showing pulsatile (13 min periodicity) vs continuous (no periodicity) insulin delivery to have greater metabolic effects.

The recent in vitro data of Goodner et al. (14) may explain the stronger metabolic effects of pulsatile insulin delivery with a pulse rate of 2+11 min. These authors demonstrated that a B-cell pulse length of 2+11 min is absolutely necessary for allowing a rapid reduction and return of surface receptor at the membrane level in the liver. Through the longer stay of insulin in the plasma compartment, a pulse rate of 2+18 min may down-regulate the insulin receptor and impair insulin action.

As far as lipid metabolism is concerned, a longer pulse interval (2+18 min) of B-cell secretion may also derange lipid metabolism. In particular, inappropriate plasma insulin oscillations may increase the production or decrease the clearance of very low-density lipoprotein. This hypothesis seems also supported by the observation that VLDL overproduction is linked to hyperinsulinism and that the correction of glucose homeostasis is associated with changes in plasma FFA, triglycerides, and VLDL triglycerides levels (15).

In conclusion, the present study shows that a pulsatile insulin delivery rate of 2+18 min similar to the length of pulsatile B-cell secretion in the elderly (20 min), can contribute to impair glucose and lipid handling in the elderly.

References

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Dr Giuseppe Paolisso, 
Instituto di Gerontologia e Geriatria, 
1st Medical School, Piazza Miraglia 2, 
I-80138 Napoli, Italy.