Reduced gastric inhibitory polypeptide but normal glucagon-like peptide 1 response to oral glucose in postmenopausal women with impaired glucose tolerance

Bo Ahrén, Hillevi Larsson and Jens J Holst

Department of Medicine, Lund University, Malmö, Sweden and Department of Medical Physiology, Panum Institute, Copenhagen University, Copenhagen, Denmark

(Correspondence should be addressed to B Ahrén, Department of Medicine, Malmö University Hospital, S-205 02 Malmö, Sweden)

Abstract

Objective: The gastrointestinal hormones, gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), are both released from the gut after oral glucose ingestion and stimulate insulin secretion. This study examined the release of these hormones in subjects with impaired glucose tolerance (IGT), which precedes the development of non-insulin-dependent diabetes.

Design and methods: Six postmenopausal women with IGT, aged 59 years, underwent a 75 g oral glucose tolerance test and plasma levels of GIP and GLP-1 were determined regularly during the following 2 h. The results were compared with those in seven age- and weight-matched women with normal glucose tolerance (NGT).

Results: Basal plasma levels of GIP and GLP-1 were not different between the groups. In response to the oral glucose ingestion, plasma levels of both GIP and GLP-1 increased in both groups. The plasma GIP increase after glucose ingestion was, however, reduced in women with IGT. Thus, the GIP response as determined as the area under the curve for the 60 min after oral glucose was 34.8 ± 3.2 pmol/l per min in women with IGT versus 56.4 ± 7.8 pmol/l per min in those with NGT (P = 0.021). In contrast, the GLP-1 response to oral glucose was not different between the groups. By definition, the glucose response to oral glucose was markedly increased in women with IGT, and the insulin response during the second hour after glucose ingestion was exaggerated.

Conclusions: The GIP response to oral glucose is impaired in postmenopausal women with IGT, whereas the plasma GLP-1 response is not affected.

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Introduction

The plasma insulin response following ingestion of glucose is higher when compared with that obtained by intravenous infusion of glucose eliciting the same blood glucose concentrations (1). This potentiation of insulin secretion is due to the action of gut hormones, so called incretins, released during the oral glucose ingestion (2). Several previous studies have shown that gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), which are released from the gut during meal intake and stimulate insulin secretion at concentrations which circulate postprandially, are the major incretin hormones (2–6). GIP is produced in the K cells in the duodenum whereas GLP-1 is produced in the L cells in the distal portion of the small intestine (2, 5).

It has been demonstrated that impaired glucose tolerance (IGT) is a condition associated with an increased risk for developing non-insulin-dependent diabetes mellitus (NIDDM) (7–10). Several recent studies have shown that subjects with IGT exhibit islet dysfunction (11–13). Based on studies in a large number of individuals, we recently suggested that insulin secretion is inadequately increased to compensate for the accompanying insulin resistance in IGT (14). One tentative mechanism underlying this impaired insulin secretion in IGT is impaired release or action of the gut hormones which participate in the regulation of insulin secretion, i.e. GIP and GLP-1. In NIDDM, basal plasma GIP levels have been shown to be unaltered when compared with healthy controls (15). In contrast, whether stimulated GIP release is affected in NIDDM is controversial, since some studies have demonstrated impaired response to meal stimulus or oral glucose (16–18), whereas other studies have reported exaggerated responses (15). Similarly, the GLP-1 response to meal ingestion or oral glucose in NIDDM is controversial, since both impaired (18) and potentiated (19) responses have been reported. However, whether abnormal GIP or GLP-1 secretion exists in IGT is not known. We have therefore, in this study, examined the GIP and GLP-1 plasma responses to an
oral glucose load in a homogenous group of postmeno-
 pausal women with IGT in comparison with an age- and
 weight-matched control group with normal glucose
tolerance (NGT). The study was undertaken in subjects
of the same sex, to avoid the previously described sex-
dependent difference in gastric emptying (20–22), and
we studied women of an age group with a high
preponderance of IGT and thereby an increased risk
for the development of NIDDM (23).

**Subjects and methods**

**Subjects**

We studied 13 postmenopausal women aged 59 years
(mean ± s.d. 59 years 2 months±6 months). According
to a 75 g oral glucose tolerance test using WHO criteria
(24) seven of the subjects had NGT, whereas six subjects
had IGT. All subjects had normal liver and thyroid
function tests and none was taking any medication
known to affect glucose tolerance. All subjects received
oral and written information concerning the aims and
methods of the study, and signed a consent declaration
before the start of the study. The study protocol was
approved by the Ethics Committee of Lund University.

**Study protocol**

Following an overnight fast, an intravenous cannula
was inserted into one antecubital vein. Two baseline
samples were then taken, whereafter 75 g glucose
dissolved in 250 ml water was ingested. Thereafter,
blood samples were taken every 15 min for 120 min.
The subjects spent the 2 h in a semi-recumbent position,
and were not allowed to smoke during the test.

**Analyses**

Blood samples were immediately centrifuged at 5 °C and
serum or plasma frozen at −20 °C until analysis. Serum
insulin concentrations were analysed with a double-
antibody RIA technique. Guinea-pig anti-human
insulin antibodies, human insulin standard and mono-
125I-tyr-labelled human insulin tracer (Linco Res. Inc., St Charles, MO, USA) were used. Samples for
analysis of GIP and GLP-1 were obtained in prechilled
test tubes containing EDTA (2.8 mmol/l blood; Sigma
Chemical Co., St Louis, MO, USA) and aprotinin
(250 KIU/ml blood; Bayer AG, Leverkusen, Germany). Analyses of GIP concentrations were performed with a
double-antibody RIA technique using rabbit anti-human
GIP antibodies (R65). 125I-labelled human GIP and
human GIP standard (25). The antibody employed
cross-reacts fully with human GIP, but not with so-
called 8 kDa GIP, the nature and relationship of which to
the synthesis or secretion of GIP is still unclear (26, 27).
Plasma concentrations of GLP-1 were measured with an
RIA after extraction with ethanol as previously described
(28). The antiserum (code no. 89390) is directed against
the amidated C-terminus of GLP-1 and therefore mainly
measures GLP-1 of intestinal origin. Plasma glucose
concentrations were determined using the glucose-
oxidase method. All samples of glucose, insulin, GIP and
GLP-1 were analysed in duplicate, and the mean values
for each time-point are given in the Results.

**Calculations and statistics**

Data are presented as mean ± s.e. unless otherwise noted.
Statistical analyses were performed with the SPSS for
Windows system (29). The areas under the curve for the
supra-basal GIP and GLP-1 levels were calculated by the
trapezoid rule. Differences between subjects with NGT
versus those with IGT were evaluated with the Mann–
Whitney U test for unrelated samples.

**Results**

Table 1 shows that subjects with IGT had higher fasting
insulin levels than those with NGT (P=0.015),
whereas the fasting glucose, GIP and GLP-1 levels did
not differ significantly between the two groups. Figure 1
shows that following the oral ingestion of 75 g glucose,
glucose levels rose to a peak level of 7.2 ± 0.4 mmol/l at
30 min in subjects with NGT, whereafter they gradually
returned to fasting levels. In contrast, in subjects with
IGT, glucose levels increased markedly to the peak of
10.1 ± 0.5 mmol/l, which was reached at 90 min.
Serum insulin increased by the same pattern and
degree in the two groups until 75 min, whereafter it
deprecated in subjects with NGT, but continued to increase
in subjects with IGT. At 120 min, serum insulin was
270 ± 43 pmol/l in NGT versus 535 ± 125 pmol/l in
IGT (P=0.015).

Plasma GIP increased sharply in subjects with
NGT and had already reached its peak level of
80.1 ± 12.2 pmol/l at 15 min after ingestion of glucose.
Plasma GIP was thereafter stable at this elevated level,
until 90 min, whereafter it fell slowly. Also in subjects
with IGT, plasma GIP increased markedly and had
already reached a level of 64.8 ± 7.7 pmol/l at 15 min.
The area under the curve for the 60 min after the
glucose challenge of the supra-basal GIP levels was
lower in the subjects with IGT (34.8 ± 3.2 pmol/l per
min) than in the women with NGT (56.4 ± 7.8 pmol/l per
min; P=0.021). The absolute GIP levels were
significantly lower in women with IGT than in women
with NGT at 15, 30 and 60 min after oral glucose
ingestion (P<0.05). Also plasma GLP-1 increased after
oral glucose ingestion, with a peak level being seen after
30 min. After 30 min, plasma GLP-1 slowly decreased
with no difference between the two groups. The increase
in plasma GLP-1 was not different between the groups
with IGT and NGT, since the area under the curve for
the 60 min after the oral glucose was 10.7 ± 0.6 pmol/l
Discussion
The subjects with IGT in the present study had slightly increased basal serum insulin levels as a sign of reduced insulin sensitivity. They had also, by definition, a markedly elevated plasma glucose response to the glucose ingestion and this was accompanied by continuously increasing serum insulin levels during the second hour after glucose ingestion. It has previously been shown that IGT is accompanied by islet dysfunction, manifested as an inadequate insulin secretion for the prevailing glucose level (11–14). The mechanism for this inadequate insulin response has not been established. One tentative explanation is impaired release or action of gut hormones with incretin activity, mainly GIP and GLP-1 (2–6). Both impaired and potentiated GIP and GLP-1 responses to meal ingestion or oral glucose in NIDDM have been reported (15–19). In IGT accompanying liver cirrhosis and Turner syndrome, GIP responses to oral glucose have been shown to be impaired (30, 31), whereas a normal GIP response has been found in subjects with IGT and cystic fibrosis (32). However, no earlier study has examined the GIP and GLP-1 responses to oral glucose in otherwise healthy postmenopausal women with IGT.

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Per min in women with IGT versus 11.5 ± 0.8 pmol/l per min in those with NGT (not significant).

Table 1 Characteristics of the two study groups. Means ± s.d. are given.

<table>
<thead>
<tr>
<th>NGT (n = 7)</th>
<th>IGT (n = 6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.7 ± 0.3</td>
<td>58.5 ± 0.3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.1 ± 1.6</td>
<td>25.1 ± 1.8</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.8 ± 0.3</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>Fasting GIP (pmol/l)</td>
<td>53.4 ± 14.8</td>
<td>95.3 ± 47.3</td>
</tr>
<tr>
<td>Fasting GLP-1 (pmol/l)</td>
<td>18.7 ± 6.0</td>
<td>16.4 ± 6.9</td>
</tr>
</tbody>
</table>

P indicates the probability level of random difference between the groups. NS = not significant.

Figure 1 Plasma levels of glucose, GIP and GLP-1 and serum levels of insulin before and after oral ingestion of 75 g glucose in seven postmenopausal women with NGT (W) and in six postmenopausal women with IGT (X). Means ± S.E.M. are shown.
has been suggested to be one such mechanism, since GIP has been demonstrated to stimulate GLP-1 release in rats (38), although such an effect has not been observed in humans (18). However, we found no correlation between plasma GIP and plasma GLP-1 in our study, suggesting that other mechanisms are of greater importance for GLP-1 secretion in humans. We found that plasma GLP-1 did not differ between subjects with NGT versus those with IGT, neither in the basal state nor after glucose ingestion. Therefore, it is unlikely that altered GLP-1 secretion contributes to impaired insulin secretion in IGT.

In conclusion, we have shown that during an oral glucose tolerance test, the plasma GIP response is reduced in postmenopausal women with IGT, whereas the plasma GLP-1 response is not different from controls with NGT.

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