Pancreatic glucagon response to glucose in hyperlipoproteinaemia with and without abnormalities in carbohydrate metabolism

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Abstract. The pancreatic glucagon (IRG) and insulin (IRI) secretion patterns were studied in 19 normal weight healthy male subjects without a family history of diabetes, and in 24 male patients with endogenous hyperlipoproteinaemia, by means of a 2-h glucose infusion (12 mg/kg/min) primed by an initial injection of 0.33 g/kg. The hyperlipoproteinaemic group consisted of 8 non-obese subjects with normal carbohydrate tolerance (group A), 8 non-obese (group B) and 8 obese (group C) subjects with maturity onset type diabetes mellitus controlled diet alone.

Group B was characterized by a lack of IRI-response to glucose whereas a hyperinsulinaemia was found both in group A and C. The fasting IRG levels increased from controls (29.7 ± 2.3 pmol/l) to group A (36.7 ± 6.5 pmol/l; n.s.), group B 47.3 ± 8.8 pmol/l; P < 0.05) and group C (57.7 ± 8.2 pmol/l; P < 0.01). In group A the IRG concentration pattern was comparable with that of the controls. On the contrary, in groups B and C the IRG levels remained significantly higher inspite of the excessive hyperglycaemia achieved by the glucose infusion, suggesting a reduced suppressibility of the alpha cell function to glucose. In addition, the latter groups were characterized by a lack of an IRG rebound after termination of the glucose infusion. No correlation was found between hyperglycaemia and the degree of IRG rebound in all groups so far studied. A diminished molar IRI:IRG-ratio was found in group B whereas an increase ratio was recorded in groups A and C during glucose infusion. There were no correlation between IRG secretion and relative body weight, carbohydrate tolerance or IRI-response to glucose.

Our findings suggest a normal alpha cell response to glucose in hyperlipoproteinaemia without obesity and carbohydrate intolerance. The abnormal alpha cell function in non-obese and obese hyperlipoproteinaemics with maturity onset type diabetes mellitus is a feature characteristic of diabetes than of hyperlipoproteinaemia.

Numerous abnormalities of glucagon secretion have been described in both juvenile-onset and adult-onset diabetes mellitus (Gerich 1976; Unger et al. 1972; Unger 1974). In addition, a basal and arginin-induced hyperglucagonaemia was observed in hyperlipoproteinaemic patients (Eaton 1973b; Tiengo et al. 1977). Variations in insulin and glucagon concentrations and their molar ratio may play an important role in the pathogenesis of abnormal triglyceride metabolism by the liver (Eaton 1976). Insulin and glucagon have a direct antagonistic effect upon hepatic lipoprotein production (Eaton 1977). An alteration in glucagon secretion or a decreased glucagon activity may be responsible for the development and/or maintenance of the hyperlipoproteinaemic states (Eaton et al. 1974).

In order to investigate possible abnormalities in the alpha cell function we have characterized the pancreatic glucagon response to constant glucose infusion, in hyperlipoproteinaemic patients with and without abnormalities in carbohydrate metabolism.
Table 1.
Clinical characteristics and metabolic data (mean ± SEM) of all groups studied.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Age (years)</th>
<th>Relative body (%)</th>
<th>Triglyceride (mmol/l)</th>
<th>Cholesterol (mmol/l)</th>
<th>Parameters of carbohydrate tolerance and insulin secretion</th>
<th>IRI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fasting-PG (mmol/l)</td>
<td>PG-area 60–120 min (mmol/l · min)</td>
</tr>
<tr>
<td>Controls</td>
<td>19</td>
<td>22.1</td>
<td>102.4</td>
<td>1.31</td>
<td>4.75</td>
<td>4.65</td>
<td>626.4</td>
</tr>
<tr>
<td></td>
<td>± 0.3</td>
<td>± 1.4</td>
<td>± 0.21</td>
<td>± 0.23</td>
<td>± 0.11</td>
<td>± 44.5</td>
<td>± 0.20</td>
</tr>
<tr>
<td>Group A</td>
<td>8</td>
<td>39.0</td>
<td>108.6</td>
<td>3.05</td>
<td>7.19</td>
<td>4.83</td>
<td>863.1</td>
</tr>
<tr>
<td></td>
<td>± 3.6</td>
<td>± 5.0</td>
<td>± 0.40**</td>
<td>± 0.71**</td>
<td>± 0.34</td>
<td>± 66.4</td>
<td>± 0.44</td>
</tr>
<tr>
<td>Group B</td>
<td>8</td>
<td>39.0</td>
<td>106.7</td>
<td>14.31</td>
<td>9.21</td>
<td>8.03</td>
<td>1561.7</td>
</tr>
<tr>
<td></td>
<td>± 5.6</td>
<td>± 2.2</td>
<td>± 0.11**</td>
<td>± 2.84**</td>
<td>± 0.65</td>
<td>± 57.2</td>
<td>± 1.76</td>
</tr>
<tr>
<td>Group C</td>
<td>8</td>
<td>38.0</td>
<td>147.0</td>
<td>3.71</td>
<td>6.56</td>
<td>8.66</td>
<td>1585.9</td>
</tr>
<tr>
<td></td>
<td>± 2.0</td>
<td>± 9.4</td>
<td>± 0.59**</td>
<td>± 0.58**</td>
<td>± 1.60</td>
<td>± 190.1</td>
<td>± 3.97</td>
</tr>
</tbody>
</table>

Group A (non-obese hyperlipoproteinaemics with normal carbohydrate tolerance).
Group B (non-obese hyperlipoproteinaemics with mild overt diabetes mellitus).
Group C (obese hyperlipoproteinaemics with mild overt diabetes mellitus).

* P < 0.05 compared with controls.
** P < 0.01 compared with controls.
Material and Methods

We studied 19 normal weight healthy male subjects without a family history of diabetes or hyperlipoproteinaemia and 24 male patients with endogenous hyperlipoproteinaemia by means of a 2-h glucose infusion test (GIT; 12 mg/kg/min), primed by an initial injection of 0.33 g/kg glucose. The hyperlipoproteinaemic group (type IIb and IV according to Fredrickson) consisted of 8 normal weight (group B) as well as 8 obese subjects with maturity onset type diabetes mellitus (group C). The study was performed under clinical conditions. All hyperlipoproteinaemic patients were under treatment with a diet consisting of 49% carbohydrate, 19% fat and 32% protein for at least one year. Diabetics with hyperlipoproteinaemia (groups B and C) were hospitalized for two weeks. Carbohydrate metabolism of both groups B and C was very well compensated by diet alone. Plasma glucose (9 measurements in the course of 24 h: 7.00, 9.15, 12.00, 14.15, 17.30, 22.00, 24.00, 3.30 and 7.00 h) ranged from 7.22 to 8.33 mmol/l during the course of day and night. No hyperlipoproteinaemic patient was under treatment with hypolipaemic drugs prior to the test. The control subjects consumed the diet mentioned above for at least three days before testing. Table 1 summarized some relevant data regarding all subjects including age and body weight as well as parameters of carbohydrate tolerance, insulin secretion and serum lipids. The body weight of all obese subjects was more than 20% in excess of the optimal body weight as given in the tables of Mohr & Johnsen (1972). The age difference in the range of 20–40 years between controls and hyperlipoproteinaemic patients is of no importance with regard to glucagon and insulin secretion. Dudl & Ensinck (1977) did not find any correlation between increasing age and basal and stimulated glucagon or insulin secretion in a selected population of healthy men. This is consistent with our preliminary studies concerning the basal and glucose stimulated insulin response during GIT. In 36 normal weight healthy subjects without a history of diabetes or hyperlipoproteinaemia and having normal carbohydrate tolerance, and who were 20–40 years of age, we failed to demonstrate a correlation of basal and glucose stimulated insulin response (ΔIRI-area 0–5 min and ΔIRI-area 30–120 min) with advancing age (unpublished results).

The GIT was performed under standard conditions with a dietary preparation containing 250 g carbohydrates during bed rest following overnight fast in all subjects. Informed consent was obtained from all subjects after a full explanation of the nature and purpose of the study. The criteria for interpretation of the carbohydrate tolerance have been published in detail (Ratzmann et al. 1979; Schulz et al. 1978) and are as follows: The area under the plasma glucose curve 60–120 min (PG-area 60–120 min) and the plasma glucose concentration at 150 min. The early and late phases of insulin response to glucose were expressed as incremental IRI-area above 0-min level from 0–5 min (ΔIRI-area 0–5 min) and from 30–120 min (ΔIRI-area 30–120 min), respectively. The plasma glucose concentration was determined by an Beckman-Autoanalyzer and the plasma insulin (IRI) concentration was measured radioimmunologically by the back titration method (Ziegler et al. 1971). Pancreatic glucagon (IRG) was measured by radioimmunoassay using specific antiserum R4 obtained from a rabbit by immunization with cross-linked glucagon (Ziegler et al. 1975). This antiserum R4 did not cross-react with porcine gut-GLI in a physiological range. The glucagon standard used was a gift of the WHO International Laboratory for Biological Standards, London. The method for pancreatic glucagon determination has already been described in detail (Schulz et al. 1978). Mean value and standard errors of the mean were calculated and statistical analysis was performed by using Student’s t-test.

Results

The patterns of plasma glucose and IRI-concentration during GIT are shown in Fig. 1. The mean fasting IRI-levels were not significantly different among all other groups studied. The early insulin response to glucose was not different between controls and group A. However, an attenuated early insulin response was found in group B (Table 1). In addition, marked differences from controls with respect to the late insulin response were exhibited by all three hyperlipoproteinaemic groups (Fig. 1 and Table 1). Both in groups A and C the late insulin response to glucose was significantly increased as demonstrated by higher absolute IRI-concentrations at few points (Fig. 1) and by the significantly greater ΔIRI-area 30–120 min (Table 1). On the contrary, group B is characterized by a significantly reduced early and late insulin secretion phase (Fig. 1 and Table 1).

In groups B and C the IRG-levels in the fasting stage and during glucose infusion were significantly higher in comparison to controls (Fig. 2 and Table 2). In group A, however, the mean fasting IRG concentration was found to be slightly but not significantly higher whereas the IRG levels during glucose infusion were comparable with those of the controls (Fig. 2).

The artificial hyperglycaemia was associated with a tendency to suppress the IRG levels during the first 60 min of the GIT in all groups (Table 2). A rebound phenomenon of the IRG response after termination of the glucose infusion was observed in
Fig. 1.
Means (± SEM) of plasma glucose (PG) and insulin concentrations (IRI) during glucose infusion (12 mg/kg/min), primed by an initial injection of 0.33 g glucose/kg body weight in healthy controls and hyperlipoproteinaemics (group A: normal weight and normal carbohydrate tolerance; group B: normal weight and maturity onset type diabetes; group C: obesity and maturity onset type diabetes). * P < 0.05 vs. controls. ** P < 0.01 vs. controls.

Fig. 2.
Means (± SEM) of pancreatic glucagon (IRG) concentrations during a glucose infusion test in healthy controls and hyperlipoproteinaemic subjects (group A, B and C). * P < 0.05 vs. controls. ** P < 0.01 vs. controls.
Table 2.
Fasting IRG as well as changes of IRG response (mean ± SEM) during the glucose infusion test in all groups studied.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Fasting IRG-concentration (pmol/l)</th>
<th>ΔIRG 0−60 min (pmol/l)</th>
<th>ΔIRG 120−180 min (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>19</td>
<td>29.7 ± 2.3</td>
<td>−5.0 ± 2.3</td>
<td>+12.3 ± 3.1</td>
</tr>
<tr>
<td>Group A</td>
<td>8</td>
<td>36.7 ± 6.5</td>
<td>−15.0 ± 2.8</td>
<td>+20.1 ± 7.7</td>
</tr>
<tr>
<td>Group B</td>
<td>8</td>
<td>47.3 ± 8.8*</td>
<td>−9.3 ± 2.7</td>
<td>+1.8 ± 3.2</td>
</tr>
<tr>
<td>Group C</td>
<td>8</td>
<td>57.7 ± 8.2**</td>
<td>−10.5 ± 5.0</td>
<td>+2.4 ± 3.7</td>
</tr>
</tbody>
</table>

* P < 0.05 compared with controls.
** P < 0.01 compared with controls.

Fig. 3 illustrates clear differences with respect to the molar IRI-IRG ratio among all groups studied. In group A, the molar IRI-IRG ratio was significantly higher in comparison to controls during the glucose infusion. The diminished molar IRI-IRG ratio observed in group B (Fig. 3) reflects a lack of insulin response (Fig. 1) and significantly increased IRG levels (Fig. 2) in these subjects. In comparison to controls the molar insulin-glucagon ratio in group C was found to be significantly reduced during the first 15 min of the glucose infusion,
whereas an increase was recorded until the termination of the glucose infusion at 120 min.

There was no correlation between parameters of pancreatic glucagon secretion (fasting IRG level, ΔIRG 0–60 min, ΔIRG 120–180 min), and relative body weight, carbohydrate tolerance (fasting PG-level, PG-area 60–120 min) or insulin response (ΔIRI-area 0–5 min, ΔIRI-area 30–120 min) if all subjects were regarded as one population. This was also relevant when all groups were analyzed separately. With one exception, in neither group did the fasting IRG level or fasting molar IRI-IRG ratio correlate with serum lipids. In group B however, a slight correlation was found between fasting IRG concentration and triglyceride (r = 0.8174; P < 0.05) and cholesterol levels (r = 0.8217; P < 0.05). It should be mentioned in this connection, that cholesterol levels were comparable in all hyperlipoproteinaemic groups whereas the triglyceride concentration of group B was significantly higher in comparison to groups A and C (P < 0.05; Table 1).

Discussion

When studying the glucagon and insulin secretion in this selected population of healthy controls and hyperlipoproteinaemias of 20–40 years of age, the age difference between the groups studied should be taken into consideration. However, glucagon and insulin secretion of healthy subjects in the age range of 20–40 years are not appreciably altered with advancing age (Dudl & Ensink 1977). This is in agreement with our preliminary findings concerning the basal and glucose stimulated insulin response during a glucose infusion test. For this reason, the age difference in the range of 20–40 years between controls and hyperlipoproteinaemics patients is of no importance with regard to the glucagon and insulin secretion in this selected population studied.

Insulin and glucagon have a direct and antagonistic effect upon hepatic lipoprotein production. In fact, whereas glucagon decreases the triglyceride lipoprotein production in the perfused rat liver (Eaton 1973a; Tiengo et al. 1977) insulin has the opposite effect (Topping & Mayes 1971).

Eaton (1977) proposed the hypothesis that a reduction in net glucagon activity may be of significance for mediating or participating in the development and/or maintenance of endogenous hyperlipoproteinaemia in man. It was suggested that this hormonal mechanism may be initiated by diet, drugs or genetic influences. In contrast to this hypothesis Tiengo et al. (1977) could not demonstrate a reduced hypotriglyceridaemic effect of glucagon in subjects with endogenous hypertriglyceridaemia. On the other hand, these investigators have drawn attention to the abnormal alpha cell function demonstrated in hyperlipoproteinaemia. It is, however, difficult to determine the significance of pancreatic glucagon secretion in the pathogenesis of hyperlipoproteinaemia when hyperlipoproteinaemia is associated with disturbances in the carbohydrate metabolism. The overt diabetes, early stages of diabetes and already the potential diabetic stage are characterized by abnormalities of the alpha cell function (Ratzmann & Ratzmann 1978; Schulz et al. 1978; Tiengo et al. 1978). Therefore, we have studied hyperlipoproteinaemic subjects with normal glucose tolerance and with maturity onset type diabetes mellitus.

With regard to the late insulin secretion phase in hyperlipoproteinaemias a hyperinsulinaemia was observed in non-obese subjects with normal carbohydrate tolerance (group A) as well as in obese patients with diabetes (group C), whereas non-obese diabetics (group B) were characterized by a lack of insulin response to glucose. In addition, the early insulin secretion phase was significantly reduced in hyperlipoproteinaemic subjects with diabetes mellitus independent of the body weight. This is consistent with findings in subjects with different degrees of carbohydrate intolerance without hyperlipoproteinaemia (Ratzmann et al. 1978; Schulz et al. 1978).

Normally a rise in glucose concentration is accompanied by a decline in plasma IRG levels, and a decline in glycaemia is associated with a rise in IRG (Unger & Orci 1977). Our study demonstrates a similar IRG secretion pattern during glucose infusion both in healthy controls and hyperlipoproteinaemic subjects with normal carbohydrate tolerance. These results are in agreement with observations of Tiengo et al. (1977) under conditions of an oral glucose load whereas an arginine-induced hyperglucagonaemia was apparent. Thus, our findings provide no evidence of an abnormal alpha cell sensitivity to glucose in hyperlipoproteinaemia in the absence of diabetes. This is consistent with investigations in non-diabetic hypertriglyceridaemics who exhibited a normal glucagon suppression.
during combined glucose and insulin infusion (Tiengo et al. 1977).

In contrast to findings in hyperlipoproteinaemics without carbohydrate intolerance IRG levels in hyperlipoproteinaemic subjects with diet-controlled diabetes (groups B and C) were significantly increased in the basal state and during the glucose infusion. Patients exhibiting hyperlipoproteinaemia, obesity and diabetes were characterized by the highest IRG concentrations. In spite of the excessive hyperglycaemia achieved by glucose infusion the IRG levels remained significantly higher in hyperlipoproteinaemics with diabetes suggesting a reduced suppressibility of the alpha cells to glucose in these patients.

The attenuated IRG rebound after termination of the glucose infusion observed in hyperlipoproteinaemic subjects with diabetes cannot be attributed to higher hyperglycaemia in this subjects. In no group the blood glucose concentration at 150 min correlated with IRG rebound at 120–180 min, suggesting a lack of a direct relationship between changes of glycaemia and pancreatic glucagon secretion under this condition. The abnormal alpha cell function in non-obese and obese hyperlipoproteinaemics with diabetes is probably not caused by a relative endogenous insulin deficiency, but rather represents a primary inability of the alpha cells to respond to changes of glucose. This is strongly suggested by the fact that an abnormal alpha cell function was apparent in obese hyperlipoproteinaemics with diabetes in spite of the hyperinsulinemia observed. This observation is consistent with the lack of a decrease in glucagon secretion during glucose plus insulin infusion in diabetic hypertriglyceridaemics despite the presence of supraphysiological insulin levels (Tiengo et al. 1977). Summing up our results we are tempted to hypothesize that the reduced sensitivity of alpha cells to glucose demonstrated here is a characteristic feature of diabetes rather than of hyperlipoproteinaemia.

The molar IRI-IRG ratio plays an important role in the regulation of various hepatic functions such as the production of proteins, lipids and glucose (Eaton et al. 1974; Eaton 1977). Variations in the relationship of insulin and glucagon levels are probably of significance in determination of the abnormal triglyceride production by the liver (Eaton 1977). Both in normal weight non-diabetic and obese diabetic hyperlipoproteinaemics the molar IRI-IRG ratio during glucose infusion was markedly increased reflecting a disturbed bihoronal regulation in these subjects. However, the ratio was found to be diminished in non-obese diabetic hyperlipoproteinaemics. This is in agreement with findings in maturity onset type diabetics without hyperlipoproteinaemia (Schulz et al. 1978).

In accordance with findings in non-hyperlipoproteinaemic subjects with different degrees of carbohydrate intolerance (Schulz et al. 1978) we could not find any correlations between insulin and pancreatic glucagon response under the condition of a glucose infusion. In addition no correlations were observed between basal glucagon levels and serum lipids whereas a weak correlation of this type existed in non-obese hyperlipoproteinaemics with diabetes.

References

Ratzmann K P, Knospe S, Heinke P & Schulz B (1979): Relationship between body fat mass, carbohydrate to-


Received on July 20th, 1979.


