Evaluation of Insulin Resistance During Inhibition of Endogenous Insulin and Glucagon Secretion by Somatostatin in Non-Obese Subjects with Impaired Glucose Tolerance

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Summary. Insulin resistance was studied in seven non-obese male subjects with impaired glucose tolerance and four healthy, age and body-weight matched male control subjects by means of a continuous intravenous infusion of somatostatin, glucose and insulin over 150 min. Glucose tolerance was evaluated by means of a 2-h glucose infusion test. Endogenous insulin (C-peptide), growth hormone, and glucagon secretion were suppressed by somatostatin in both groups. Steady-state plasma insulin and glucose levels were achieved between 90–135 min. Since similar steady-state levels of exogenous insulin were achieved, the resulting steady-state plasma glucose level provided a direct estimate of the ability of insulin to dispose of the infused glucose. The glucose levels were higher in subjects with impaired glucose tolerance with values of 14.6 ± 1.8 mmol/l compared with 5.1 ± 1.2 mmol/l in control subjects ($p < 0.01$), thus indicating insulin resistance. There was a direct correlation between the steady-state plasma glucose level and glucose tolerance suggesting that the degree of glucose intolerance is proportional to the degree of insulin resistance. These results revealed that decreased insulin sensitivity is found in non-obese subjects with impaired glucose tolerance.

Key words: Insulin resistance, somatostatin infusion, C-peptide, insulin, pancreatic glucagon, growth hormone, non-esterified fatty acids, impaired glucose tolerance.

Abnormalities in carbohydrate metabolism can result from either decreased insulin secretion or increased resistance in insulin responsive tissues or both. Insulin resistance is well established in Type 2 (insulin independent) diabetes and in patients with impaired glucose tolerance [10, 11, 14, 22]. To determine the degree of insulin resistance, Shen et al. [25] have described an infusion technique in which patients receive a constant intravenous infusion of adrenaline, propranolol, glucose and insulin. Under these experimental conditions endogenous insulin secretion is inhibited and the height of the steady-state plasma glucose concentration is considered as a direct measure of the ability of identical amounts of exogenous insulin to promote disposition of the infused glucose load [10, 11, 22, 25]. However, adrenaline and propranolol exert direct effects on carbohydrate and lipid metabolism, and may interfere with the ability of insulin to stimulate glucose disposal and to influence lipolysis.

Somatostatin inhibits both insulin and glucagon release from the pancreas [9] without any direct effect on glucose uptake [3]. Given these properties, somatostatin can be regarded as an appropriate tool to study insulin sensitivity *in vivo* [12, 18]. Harano et al. [12] and Nagulesparan et al. [18] used somatostatin to assess the insulin resistance *in vivo* in diabetic patients and obese subjects with normal glucose tolerance. The present study was carried out to determine the degree of insulin resistance by means of a continuous infusion of somatostatin, glucose and insulin in non-obese subjects with impaired glucose tolerance.

Subjects and Methods

Seven non-obese male subjects with normal fasting plasma glucose concentration but with impaired glucose tolerance and four healthy age and weight matched male controls were studied (Table 1). Informed consent was obtained from all subjects after fully explaining the nature, purpose, and possible side effects involved in the study. The subjects did not suffer from any other disease as

Table 1. Clinical and metabolic characteristics of subjects classified as having normal or impaired glucose tolerance (mean ± SEM)

	No. of Subjects	Body weight (kg)	Relative body weight (%)	Age (years)	Fasting plasma glucose concentration (mmol/l)	Criteria of the glucose infusion test			
						Plasma glucose[a]		Plasma insulin (IRI)	
						Area-plasma glucose 60–120 min (mmol·l^{-1}·min^{-1})	Plasma glucose-150 min (mmol/l)	ΔIRI-area 0–5 min (nmol·l^{-1}·min^{-1})	ΔIRI-area 30–120 min (nmol·l^{-1}·min^{-1})
Healthy controls	4	72±4	100±5	37±1	3.8±0.3	552±47	3.1±0.2	0.6±0.2	23±3
Impaired glucose tolerance	7	73±2	104±3	41±4	4.1±0.5	1249±68 $p < 0.05$	13.4±1.6 $p < 0.01$	0.2±0.2	24±6

[a] Normal value: area plasma glucose 60–120 min < 850 mmol·l^{-1}·min^{-1}; plasma glucose 150 min < 6.7 mmol/l

determined by physical examination and routine laboratory studies. None was under treatment with drugs. All studies were performed after a diet (50% carbohydrate, 30% fat and 20% protein) including 250 g of carbohydrate for the 3 days before the study with the subject at complete bed rest following an overnight fast. Table 1 shows some of the clinical characteristics as well as estimates of glucose tolerance and insulin response in the two groups. The relative body weight was estimated according to the tables of Möhr and Johnson [17]. Glucose tolerance and insulin response patterns were characterized by means of a glucose infusion test consisting of an initial injection of 0.33 g glucose/kg body weight followed by 12 mg/kg body weight/min for 2 h.

The criteria for the evaluation of glucose tolerance were derived on the basis of discriminant analysis from the results obtained in 115 healthy normal weight subjects without a family history of diabetes [16]. Glucose tolerance was estimated by determining the area of plasma glucose below the glucose curve at 60–120 min (normal value < 850 mmol/l^{-1} min^{-1}) and the plasma glucose concentration at 150 min (normal value < 6.7 mmol/l). The acute and late phases of insulin (IRI) response 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.

Three days after a glucose infusion test, a modification of the procedure of Shen et al. [25] was used for studying insulin resistance. After the same dietary preparation and following a 12-h overnight fast, an indwelling teflon cannula was inserted into an antecubital vein. All subjects received an IV injection of 125 μg cyclic somatostatin (Serono, Freiburg, FRG) within 5 min followed by a combined constant infusion of 250 μg/h somatostatin, 12 mg kg^{-1} min^{-1} glucose and 1 mU kg^{-1} min^{-1} Actrapid insulin (Novo Industrias, Copenhagen, Denmark) for 150 min. Blood samples were drawn at −30, −5, 0, 5, 15, 30, 60, 90, 105, 120, 135, 150 and 180 min for the determination of plasma glucose (Beckman analyzer), non-esterified fatty acid (NEFA) [7], glycerol [8], growth hormone [15] and plasma IRI concentrations [26].

The coefficient of variation of the insulin radioimmunoassay was 6.1%. Pancreatic glucagon was measured by radioimmunoassay using specific antiserum R 4 obtained from a rabbit by immunization with cross-linked glucagon [27]. This antiserum R 4 did not cross-react with porcine gut-glucagon-like immunoreactivity in a physiological range. The coefficient of variation in the concentration range of 17–25 pmol/l of glucagon for any plasma sample was found to be 15%. C-peptide was measured after the removal of pro-insulin and following on alcoholic extraction. In order to bind the proinsulin, which cross-reacts with the C-peptide antiserum, 0.5 ml of plasma was incubated with guinea-pig anti-insulin serum for 4 h. After alcoholic precipitation C-peptide immunoreactivity remained in the supernatant and the aqueous phase was frozen out following the addition of ether. Samples were lyophilized and dissolved with 0.1 ml glycine buffer (0.05 mol/l, pH 8.6) containing 3 g/l human albumin and assayed for C-peptide immunoreactivity by the back titration method using alcohol as a separating agent. The recovery of C-peptide from plasma was found to be 77.5 ± 1.7% (mean ± SEM; $n = 20$).

Results are presented as mean ± SEM. Student's t-test for paired or unpaired data defined the significance of difference between the means within a group or between groups.
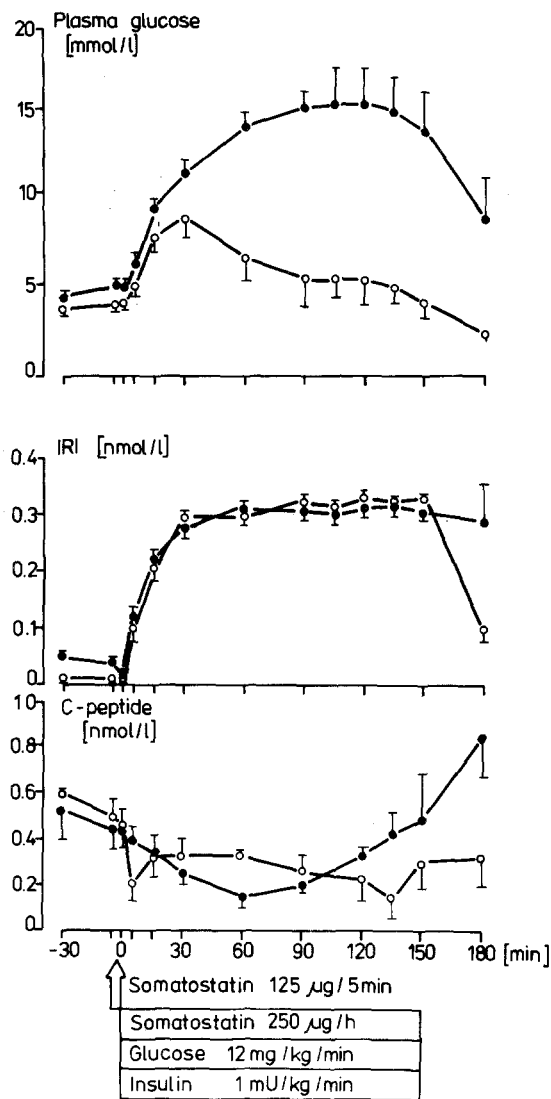
Results

Mean plasma glucose and insulin concentrations of controls and subjects with impaired glucose tolerance during the glucose infusion test are presented in Fig. 1. The fasting plasma glucose values were comparable in both groups, whereas the glucose response was more than twice in subjects with impaired glucose tolerance compared with normal subjects (Table 1). The insulin response in subjects with impaired glucose tolerance was characterized by a reduced initial insulin secretion phase, whereas the plasma insulin concentration after termination of the glucose infusion was significantly higher in comparison with controls (Fig. 1 and Table 1).

Mean basal plasma C-peptide levels before the somatostatin infusion were not significantly different between controls and patients with impaired glucose tolerance (Fig. 2). Endogenous insulin secretion was inhibited during the combined infusion of somatostatin, glucose and insulin in both groups, as indicated by suppression of C-peptide levels (Fig. 2). Mean basal plasma C-peptide levels decreased from 0.46 ± 0.11 to a minimum of 0.10 ± 0.07 nmol/l in controls
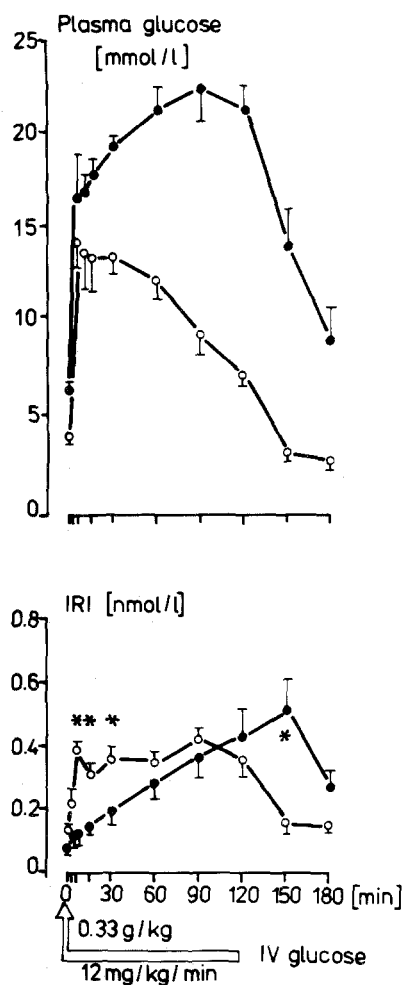
Fig. 1. Plasma glucose and plasma insulin responses (mean ± SEM) during a glucose infusion test in healthy controls (O—O, $n = 4$) and subjects with impaired glucose tolerance (●—●, $n = 7$). *$p < 0.05$

Fig. 2. Plasma glucose, plasma insulin, and C-peptide levels (mean ± SEM) of healthy controls (O—O) and subjects with impaired glucose tolerance (●—●) during a combined infusion of somatostatin, glucose and insulin of 150 min

(NS) and from 0.40 ± 0.08 to 0.16 ± 0.06 nmol/l ($p < 0.05$) in subjects with impaired glucose tolerance. Pancreatic glucagon concentrations were also suppressed during the infusion of somatostatin, glucose and insulin in the two groups (Fig. 3). Mean basal pancreatic glucagon concentration decreased from 22 ± 6 to a minimum of 11 ± 3 pmol/l in controls ($p < 0.05$) and from 37 ± 5 to 7 ± 1 pmol/l in subjects with impaired glucose tolerance ($p < 0.01$; Fig. 3). Pancreatic glucagon levels remained parallel during the infusion and were not significantly different in the two groups. A slight increase of pancreatic glucagon was noted after termination of the infusion in both groups (Fig. 3). Plasma growth hormone

levels were also suppressed in controls and subjects with impaired glucose tolerance to a minimum of 0.03 ± 0.01 and 0.01 ± 0.01 nmol/l respectively (Fig. 3).

During the combined infusion of somatostatin, glucose and insulin, plasma insulin levels increased in a similar manner in controls and subjects with impaired glucose tolerance (Fig. 2). Steady-state plasma insulin concentration was reached after 90 min in the two groups. The mean steady-state plasma insulin concentration during the time interval 90–135 min was closely similar in controls and subjects with impaired glucose tolerance (0.34 ± 0.03 versus 0.33 ± 0.04 nmol/l) In contrast, Fig. 2 demon-

strates clear differences of the groups regarding plasma glucose response to an infusion of somatostatin, glucose and insulin. Steady-state plasma glucose levels were reached after 90 min and remained constant until 135 min. The mean steady-state plasma glucose levels were significantly higher in subjects

Fig. 3. Pancreatic glucagon (IRG) and growth hormone responses (HGH) (mean ± SEM) in healthy controls (○——○) and subjects with impaired glucose tolerance (●——●) during a combined infusion of somatostatin, glucose and insulin of 150 min. *$p < 0.05$, **$p < 0.01$ versus values before the combined infusion

with impaired glucose tolerance compared with controls (14.6 ± 1.8 versus 5.1 ± 1.2 mmol/l; $p < 0.01$). There was a direct correlation between the steady-state plasma glucose and estimates of glucose tolerance for the two groups combined (Fig. 4). This relationship was not found when the two groups were analyzed separately. There was no relationship between steady-state plasma glucose levels and estimates of insulin secretion (fasting IRI-level, ΔIRI-area 0–5 min, ΔIRI-area 30–120 min), fasting pancreatic glucagon, or fasting growth hormone levels.

Mean plasma NEFA and glycerol concentrations decreased in response to the infusion of somatostatin, glucose and insulin in both groups (Fig. 5). Plasma NEFA levels were significantly higher in subjects with impaired glucose tolerance compared with the control subjects at 15, 30 and 60 min, whereas no differences were apparent from 90 to 135 min. Both the absolute and the relative decrease in plasma NEFA and glycerol concentration were comparable in both groups of subjects. The steady-state plasma glucose was not correlated with either the absolute or the relative decrease in NEFA or glycerol concentrations.
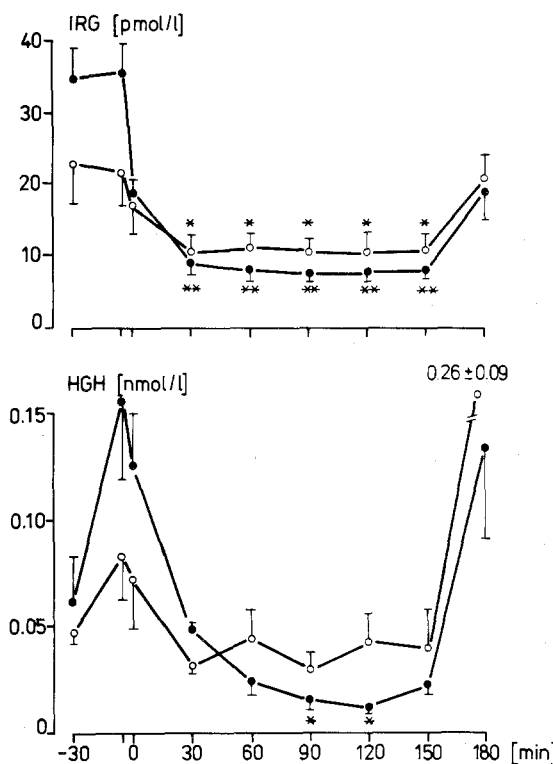
Discussion

Our results confirm previous studies that patients with mild decreases of carbohydrate tolerance respond to an IV glucose load with significant hyperinsulinaemia after termination of glucose infusion [24]. Apart from disturbances of insulin secretion, insulin resistance is well documented by several studies [10, 11, 20, 22]. Insulin resistance was previously determined by a method involving a continuous IV infusion of adrenaline, propranolol, glucose and insulin. Under these experimental conditions
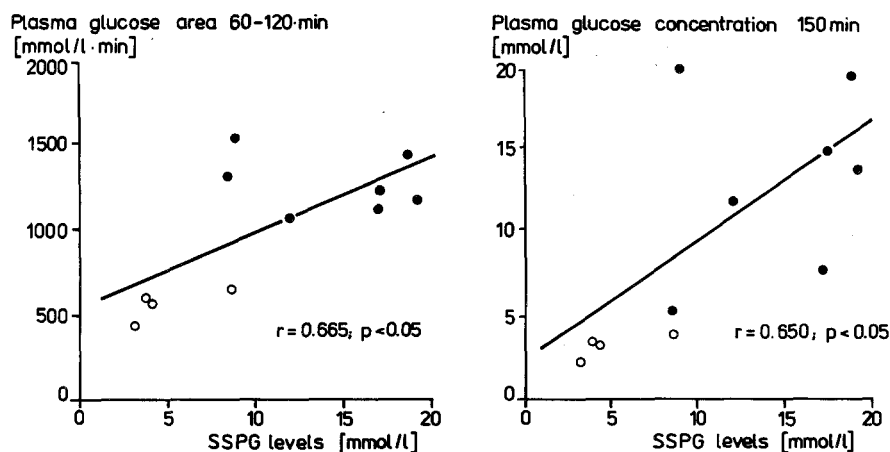

Fig. 4. The relationship between estimates of glucose tolerance (plasma glucose area 60–120 min and plasma glucose concentration at 150 min) and steady-state plasma glucose levels (SSPG) in all subjects studied (○ controls, ● subjects with impaired glucose tolerance)
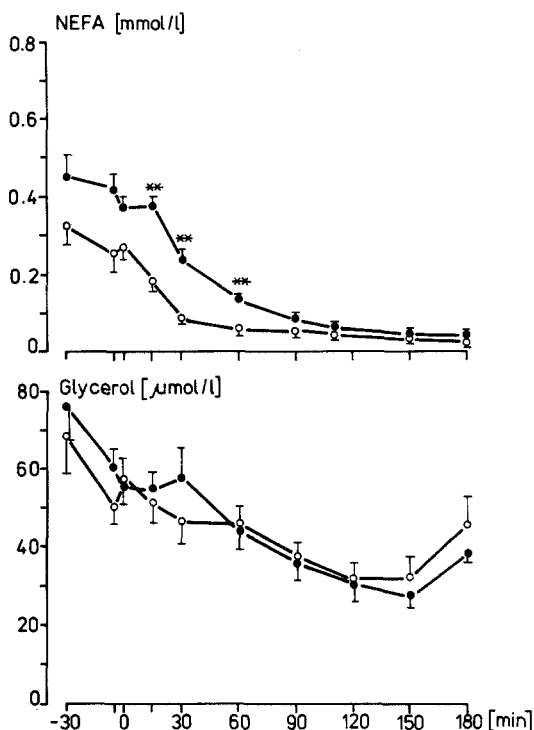
Fig. 5. Plasma NEFA and glycerol responses (mean ± SEM) in healthy controls (O——O) and subjects with impaired glucose tolerance (●——●) during a combined infusion of somatostatin, glucose and insulin. **$p < 0.01$

endogenous insulin secretion was inhibited and steady-state plasma glucose and exogenous insulin levels were reached after 90 min [25]. Bernstein et al. [2] have pointed out, however, that adrenaline and propranolol could interfere with the ability of insulin to stimulate glucose disposal and/or inhibit hepatic glucose production. Adrenaline increases glucose production, decreases glucose clearance [23], and impairs tissue sensitivity to insulin in man [5]. Propranolol had no effect on insulin mediated glucose metabolism [5]. Thus different pharmacological effects in patients with diabetes could account for the fact that these patients have higher steady-state plasma glucose concentrations. Another technique to measure insulin resistance is the euglycaemic and hyperglycaemic clamp technique [6]. The procedure is complicated and, if hyperglycaemia is produced, endogenous insulin release is stimulated. For this reason somatostatin has recently been used to suppress endogenous insulin release, and thereby it becomes possible to investigate the efficiency of exogenous insulin on glucose metabolism in vivo [12, 18]. Somatostatin also has the advantage of having no direct effect on basal or insulin stimulated glucose uptake of skeletal muscle, or on hepatic glucose production [3, 9].

The results of the present study indicate that the mean steady-state plasma glucose was significantly higher in subjects with impaired glucose tolerance than in controls demonstrating the existence of insulin resistance in the former group. In contrast to this observation the decrease in plasma NEFA and glycerol was comparable in the two groups suggesting no reduced insulin sensitivity of adipose tissue lipolysis in subjects with impaired glucose tolerance. This is in agreement with findings of Howard et al. [13] who have demonstrated marked sensitivity to the anti-lipolytic effect of insulin in obese Type 2 (insulin independent) diabetics considered to be resistant to its glucose lowering action. However, the effect of varying doses of insulin was not studied in the present study so that dose-response curves could not be obtained. For this reason insensitivity to insulin can be missed if the studies were carried out on the maximal part of the insulin dose-response curve.

Theoretically, higher steady-state plasma glucose levels in subjects with impaired glucose tolerance could be due to the presence of circulating insulin antagonists or to direct tissue resistance to the action of insulin. Growth hormone and glucagon are well recognised insulin antagonists but they were decreased to a comparable level in both groups of subjects studied. In addition, elevated NEFA concentrations in subjects with impaired glucose tolerance might contribute to insulin resistance [1] and glucose intolerance [21]. However, we found plasma NEFA concentrations to be suppressed to the same level both in controls and subjects with impaired glucose tolerance.

Olefsky and Reaven [19, 20] have demonstrated a reduced number of insulin receptors in "chemical" and non-ketotic diabetics and the decreased insulin binding in these subjects was inversely correlated with the degree of insulin resistance. Moreover, abnormalities in intracellular glucose metabolism might also contribute to insulin resistance observed in patients with mild diabetes [4].

In conclusion, our results demonstrate that insulin resistance exists in non-obese patients with impaired glucose tolerance and add further support to the view that the degree of glucose intolerance in these patients is proportional to their degree of insulin resistance [10]. Furthermore, the validity of these conclusions has been confirmed by the use of a technique [12, 18] which avoids the effects of adrenaline and propranolol on glucose metabolism.

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