

Impaired glucose tolerance (IGT) is associated with reduced insulin-induced suppression of glucagon concentrations

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Abstract

Aims/hypothesis. We aimed to examine whether impaired glucose tolerance is associated with reduced suppression of glucagon concentrations.

Methods. Eighty-four non-diabetic women of Caucasian origin and 61 years of age, of whom 48 had normal glucose tolerance (NGT) and 36 had IGT, underwent a 75 g OGTT and a hyperinsulinaemic, euglycaemic clamp with measurement of glucagon, insulin and glucose concentrations.

Results. At 2 h after 75 g oral glucose, glucagon concentrations were reduced by 7.1 ± 1.1 ng/l in NGT vs 8.0 ± 1.4 ng/l in IGT, (NS). However, the 2 h reductions in glucagon per mmol/l increase in 2 h glucose or per pmol/l increase in 2 h insulin were both impaired in IGT ($p = 0.002$ and $p = 0.043$, respectively) because the 2 h increases in glucose and insulin were higher in IGT than in NGT. Furthermore, suppression of glucagon concentrations during a euglycaemic

clamp at hyperinsulinaemic concentrations (NGT: 607 ± 19 pmol/l, IGT: 561 ± 21 pmol/l) was lower in IGT (13.6 ± 1.6 ng/l) than in NGT (23.1 ± 1.2 ng/l; $p < 0.001$). The suppression of glucagon concentrations during the hyperinsulinaemic, euglycaemic clamp correlated with insulin sensitivity ($r = 0.24$, $p = 0.027$) and with the 2 h glucose value during the OGTT ($r = -0.52$, $p < 0.001$).

Conclusion/interpretation. Impaired glucose tolerance is associated with reduced insulin-induced suppression of glucagon secretion, which could be caused by A-cell insulin resistance. Inappropriately high glucagon secretion could therefore contribute to the metabolic perturbations in IGT. [Diabetologia (2001) 44: 1998–2003]

Keywords Glucagon secretion, suppression, impaired glucose tolerance, IGT, Type II diabetes, insulin clamp, OGTT, humans.

Type II (non-insulin-dependent) diabetes mellitus is associated with increased hepatic glucose production which is linked to fasting hyperglycaemia [1–3]. This could be explained by glucagon, because along with impairment in insulin action and insulin secretion diabetic patients also have reduced suppression of glucagon secretion [4–7]. Reduced suppression of glucagon has, furthermore, been shown to contribute to postprandial hyperglycaemia in Type II diabetic pa-

tients [8]. Similarly, lack of suppression of glucagon during glucose administration increases the hyperglycaemia in non-diabetic subjects [9] as well as in patients with Type I (insulin-dependent) diabetes mellitus [10]. We have recently shown that subjects with impaired glucose tolerance (IGT) show increased glucagon secretion in response to an arginine challenge at high glucose [11] and that increased glucagon concentrations predict worsening of glucose tolerance in non-diabetic subjects [12]. Furthermore, reduced suppression of glucagon during OGTT testing has been observed in subjects with IGT [13]. Inappropriately high glucagon concentrations and reduced suppression of glucagon could therefore be involved in the metabolic perturbations in diabetic patients

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and in subjects with increased risk for developing diabetes, in conjunction with impaired insulin secretion. The inappropriately high glucagon concentrations could be explained by the reduced ability of insulin to inhibit glucagon secretion as a sign of insulin resistance in the A cells [14]. We explored this possibility by examining whether subjects with IGT or NGT show different suppression of glucagon concentrations during an OGTT and during a hyperinsulinaemic, euglycaemic clamp.

Subjects and methods

Study design. Oral glucose tolerance tests and euglycaemic hyperinsulinaemic clamps [15] were carried out in 84 non-diabetic female subjects of Caucasian origin, all 61 years of age. Both studies were done in the morning after an overnight fast with at least one week between the visits. The ethics committee of Lund University approved the study and informed written consent was obtained from all participants before entry into the study.

Subjects. 84 participants were recruited from a larger cohort of 841 postmenopausal women living in the city of Malmö, Sweden who participate in a health screening which identified that 27% of the subjects had IGT [16]. The subjects were randomly selected from their 2 h glucose concentration at an oral glucose tolerance; 48 had NGT and 36 had IGT. All 84 subjects were healthy and none were taking any medication known to affect carbohydrate metabolism (Table 1).

Oral glucose tolerance test. An intravenous catheter was inserted into an antecubital vein. After a baseline sample, an oral glucose tolerance test was carried out as a standard WHO 75 g glucose load [18]. Blood samples were taken before and at 30, 60, 90 and 120 min after the oral glucose ingestion. The subjects spent the two hours in a semi-recumbent position.

Hyperinsulinaemic, euglycaemic clamp. Insulin sensitivity was measured with the euglycaemic, hyperinsulinaemic clamp as described previously [15]. Intravenous catheters were inserted into antecubital veins in both arms. One arm was used for infusion of glucose and insulin; the contralateral arm was used for intermittent sampling. After obtaining two baseline samples, a primed-constant infusion of insulin (Actrapid 100 U/ml, Novo Nordisk, Bagsvaerd, Denmark) with a constant infusion rate of 0.28 nmol/m² body surface area per min was started. After 4 min, a variable rate 20% glucose infusion was added and its infusion rate was adjusted manually throughout the clamp procedure to maintain the blood glucose concentration at 5.0 mmol/l. Blood glucose was measured at the participants' bedside every 5 min. Mean blood glucose during the second hour of the clamp test was 5.1 ± 0.1 (means ± SEM) mmol/l. Samples for analysis of insulin and glucagon were taken at 0, 60 and 120 minutes.

Analyses. Blood glucose concentration was measured from the patients at their bedside by the glucose dehydrogenase technique with an Accutrend® (Boehringer Mannheim Scandinavia AB, Bromma, Sweden). Blood samples for analysis of glucagon, insulin and glucose were immediately centrifuged at 5°C and serum or plasma frozen at -20°C until analysis in

Table 1. Characteristics of the 84 women examined divided into the two groups of normal glucose tolerance (NGT) and impaired glucose tolerance (IGT)

Variable	NGT (n = 48)	p	IGT (n = 36)
Age	61 year 10 ± 5 mo	NS	61 year 8 ± 5 mo
Body mass index (kg/m ²)	25.4 ± 3.5	NS	25.8 ± 3.3
Waist circumference (cm)	81.5 ± 9.1	NS	83.4 ± 8.3
Fasting blood glucose (mmol/l)	4.7 ± 0.6	0.005	5.1 ± 0.8
2 h blood glucose (mmol/l)	6.5 ± 0.8	< 0.001	9.0 ± 0.1
ΔAUC _{glucose} (mmol/l x 120 min)	174 ± 88	< 0.001	333 ± 126
Fasting serum insulin (pmol/l)	78 ± 25	0.045	91 ± 34
30 min serum insulin (pmol/l)	496 ± 46	< 0.001	352 ± 43
30 min insulin response (pmol/l)	418 ± 32	< 0.001	261 ± 36
ΔAUC _{insulin} (nmol/l x 120 min)	42.9 ± 27.9	NS	41.9 ± 21.2
Fasting plasma glucagon (ng/l)	52.1 ± 3.7	NS	49.2 ± 2.7
ΔAUC _{glucagon} (ng/l x 120 min)	-547 ± 127	NS	-553 ± 129
Fasting plasma cholesterol (mmol/l)	5.9 ± 0.9	NS	6.1 ± 1.1
Fasting plasma triglycerides (mmol/l)	1.2 ± 0.6	0.021	1.5 ± 0.6
Insulin sensitivity (nmol glucose · kg body weight ⁻¹ · minute ⁻¹ / pmol insulin · l ⁻¹)	75.6 ± 28	0.046	62.3 ± 24

ΔAUC indicates the area under the curve from baseline value. Means ± SD are shown. p indicates probability of random difference between the groups

duplicate. Plasma glucagon was measured with double-antibody radioimmunoassay in duplicate using guinea pig antihuman glucagon antibodies specific for pancreatic glucagon, ¹²⁵I-glucagon as tracer and glucagon standard (Linco Research, St Charles, Mo., USA). Serum insulin concentrations were analysed with double-antibody RIA technique using guinea pig anti-human insulin antibodies, human insulin standard and mono-¹²⁵I-Tyr-human insulin (Linco). Plasma glucose concentrations were analysed using the glucose oxidase method. All concentrations were taken as means of the duplicate samples.

Calculations. Data are given as means ± SEM, unless otherwise noted. Suppression of glucagon during the OGTT was calculated as the difference between glucagon concentrations at time 0 and 120 min. This difference was also divided by the change in glucose or insulin concentrations during the 2 h to evaluate the reduction in glucagon concentrations per increased mmol/l glucose or pmol/l insulin. The suprabasal or subbasal areas under the curve (AUC) during the 2 h OGTT were also calculated for glucose, insulin and glucagon using the trapezoid rule. Suppression of glucagon during the hyperinsulinaemic, euglycaemic clamp was calculated as the difference in glucagon concentrations at time 0 vs the mean at 60 and 120 min. Insulin sensitivity (nmol glucose · kg body weight⁻¹ · min⁻¹ / pmol insulin · l⁻¹) was calculated as glucose infusion rate divided by the mean insulin at 60 and 120 min [15].

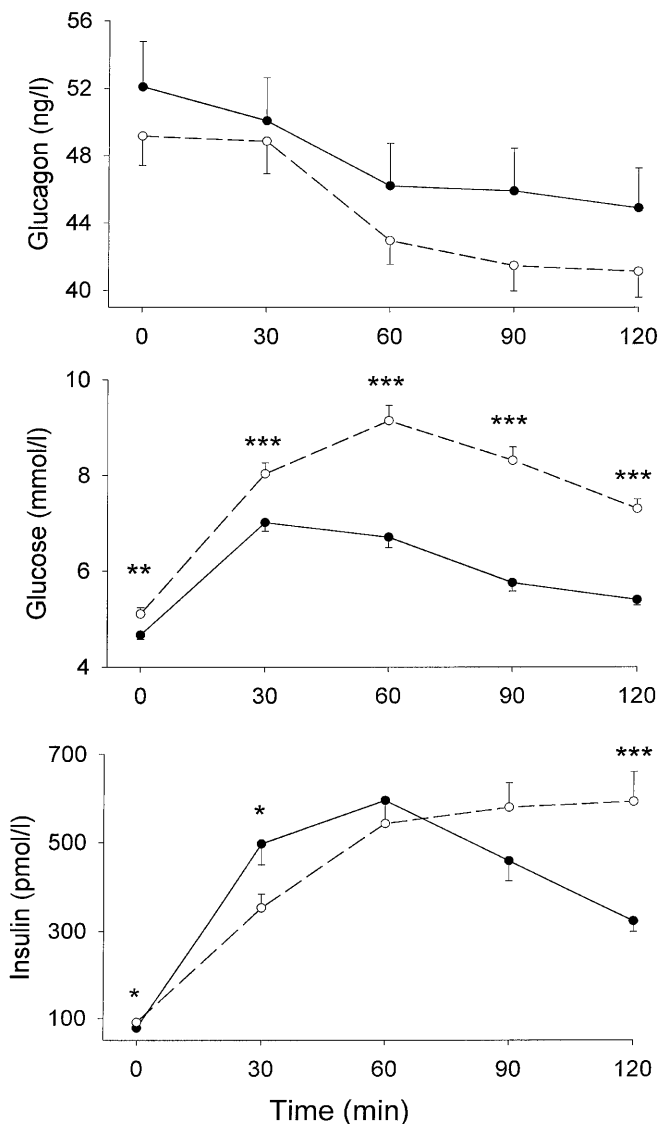


Fig. 1. Plasma concentrations of glucagon and glucose and serum insulin during a 75 g OGTT in healthy women, aged 61 years, with normal glucose tolerance (NGT, $n = 48$, ●) or impaired glucose tolerance (IGT, $n = 36$, ○). Means \pm SEM are shown. Asterisks indicate the probability of random difference between the two groups; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Statistics. Statistical analyses were done with the SPSS for Windows system (SPSS Inc., Chicago, IL, USA). Mann-Whitney U test was used for testing differences between the groups. Spearman regression coefficients were obtained to estimate correlation. Linear stepwise forward multiple regression was used to assess the independent effect of several variables. A probability of random difference of p less than 0.05 was considered to be statistically significant.

Results

Glucagon and insulin responses to oral glucose. Baseline glucagon did not differ significantly between IGT and NGT (Table 1). After the 75 g oral glucose in-

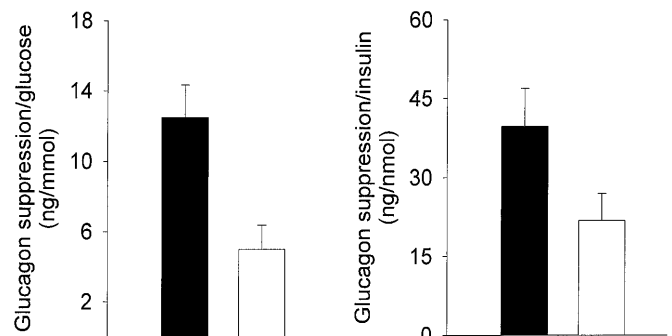


Fig. 2. Suppression of glucagon concentrations during a 75 g OGTT (i.e., glucagon concentrations at time 0 min minus those at time 120 min) divided by the 2 h increase in plasma glucose (left panel, $p = 0.002$) or the 2 h increase in serum insulin (right panel, $p = 0.043$) in healthy women, aged 61 years, with normal glucose tolerance (NGT, $n = 48$, ■) or impaired glucose tolerance (IGT, $n = 36$, □). Means \pm SEM are shown. p indicates the probability of random difference between the two groups.

take, circulating glucagon was reduced in both groups (Fig. 1). The reduction in glucagon during the 2 h study period was 8.0 ± 1.4 ng/l or 14.7 ± 2.9 % in IGT vs 7.1 ± 1.1 ng/l or 12.4 ± 1.9 % in NGT (NS). The AUC of the subbasal glucagon during the OGTT did not differ between the groups (Table 1). Both baseline and 2 h concentrations of glucose and insulin were higher in IGT than in NGT, whereas the 30 min insulin response was lower in IGT than in NGT (Table 1, Fig. 1). Also the 2 h increases in glucose and insulin during the OGTT were higher in IGT (2.2 ± 0.2 mmol/l and 500 ± 63 pmol/l) than in NGT (0.8 ± 0.1 mmol/l and 243 ± 22 pmol/l; both $p < 0.001$). Therefore, in IGT, the reduction in glucagon concentrations was reduced in relation to the increases in glucose and insulin as judged by dividing the 2 h suppression of glucagon with the 2 h increase in glucose ($p = 0.002$) and in insulin ($p = 0.043$; Fig. 2). In contrast, when dividing the suppression of glucagon concentrations by the 30 min insulin concentration no difference between the groups was observed.

Glucagon response during hyperinsulinaemic, euglycaemic clamp. During the steady state second hour of the hyperinsulinaemic, euglycaemic clamp, insulin concentrations were 561 ± 21 pmol/l in IGT vs 607 ± 19 pmol/l in NGT. Baseline glucagon did not differ between the groups but during the hyperinsulinaemic, euglycaemic clamp study, glucagon concentrations were higher in IGT (37.3 ± 1.8 ng/l) than in NGT (28.7 ± 2.5 ng/l; $p = 0.007$, Fig. 3). The suppression of glucagon during the clamp was therefore reduced in subjects with IGT (13.6 ± 1.6 ng/l) compared to NGT (23.1 ± 1.2 ng/l; $p < 0.001$, Fig. 3). Also the insulin sensitivity, as measured by the hyperinsulinaemic, euglycaemic clamp, was lower in IGT than in

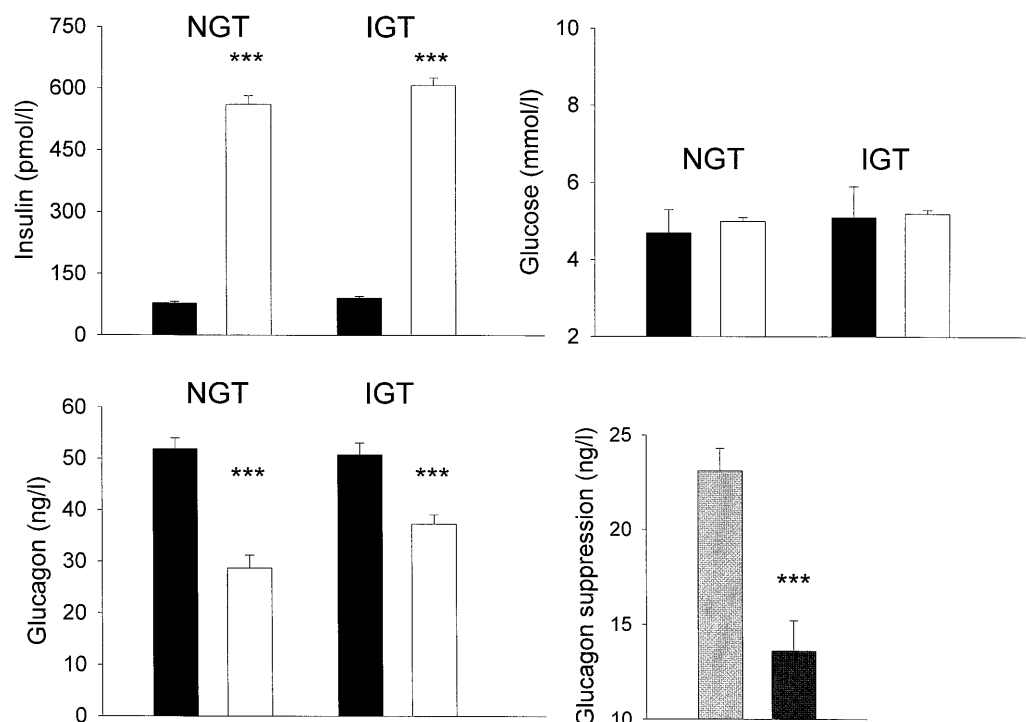


Fig. 3. Fasting serum insulin, plasma glucose and plasma glucagon before the euglycaemic, hyperinsulinaemic clamp (■) vs the mean of the 60 and 120 min values during the clamp (□) in healthy women, aged 61 years, with normal glucose tolerance (NGT, $n = 48$) or impaired glucose tolerance (IGT, $n = 36$). Lower right (NGT, $n = 48$, ■, IGT, $n = 36$, ■) shows the difference between the glucagon concentrations before and during the clamp, i. e., the suppression of glucagon concentrations. Means \pm SEM are shown. Asterisks indicate the probability of random difference during the clamp; *** $p < 0.001$

Table 2. Stepwise forward multiple regression model to determine whether suppression of glucagon concentrations during hyperinsulinaemic, euglycaemic, clamp and insulin sensitivity was the best predictor of the 2 h glucose value during an oral glucose tolerance test in 84 non-diabetic women, aged 61

Step	Variable	B	SE (B)	r	p
1	Suppression of glucagon	0.21	0.03	-0.50	< 0.001
2	Insulin sensitivity	0.036	0.004	-0.64	< 0.001

NGT ($p = 0.046$, Table 1). The suppression of glucagon correlated significantly with the insulin sensitivity ($r = 0.24$, $p = 0.027$). Both the suppression of glucagon during the clamp ($r = -0.52$, $p < 0.001$) and insulin sensitivity ($r = -0.50$; $p < 0.001$) correlated negatively to the 2 h glucose concentration in the OGTT. A multivariate stepwise analysis with the 2 h glucose concentration as the independent variable and the suppression of glucagon during the clamp and the insulin sensitivity as independent variables showed that both these variables significantly and independently predicted the 2 h glucose value (Table 2, $r = -0.64$, $p < 0.001$).

Discussion

In patients with Type II diabetes, glucagon concentrations are usually increased [4–7] and suppression of glucagon concentrations reduces glycaemia after food intake [8]. This would support that agents inhibiting glucagon secretion or counteracting glucagon action might be beneficial for patients with diabetes [18]. This has been reinforced by animal studies showing that immunoneutralization of glucagon by a specific antiserum lowers circulating glucose in fed rats [19], in rats subjected to neonatal administration of streptozotocin [20] and in alloxan-diabetic rabbits [21].

We have previously shown that subjects with IGT have increased glucagon secretory response to iv arginine at high glucose and that this augmentation predicts future deterioration of glucose tolerance [11,12], suggesting augmented A-cell responsiveness in IGT. We examined the suppressibility of glucagon in IGT and found that the reduction of glucagon concentrations during OGTT was not significantly different in subjects with IGT compared with subjects with NGT. This would suggest that suppression of glucagon concentrations is not altered in IGT. However, as both 2 h glucose and 2 h insulin concentrations were higher in IGT than in NGT, a larger suppression of glucagon concentrations would be anticipated in IGT. Since this was not observed, we conclude that the suppression of glucagon concentrations is reduced in IGT. When the suppression of glucagon concentrations was divided by the increased glucose and insulin concentrations, significantly lower suppression was evident in IGT. This confirms the previous

observation that the insulin to glucagon ratio after oral glucose was substantially higher in subjects with IGT than in subjects with NGT [13]. Hence, lack of appropriate suppression of glucagon, as in Type II diabetes [8] is also evident in subjects at risk of developing diabetes.

The subjects with IGT had significantly higher fasting insulin concentrations than the subjects with NGT, which suggests that they had reduced insulin sensitivity, which was supported by the results of the hyperinsulinaemic, euglycaemic clamp. Also the initial 30 min rise in insulin was lower in the subjects with IGT, despite a higher glucose concentration at this time point. This confirms a previous study that defective early insulin response to glucose is a characteristic of subjects with IGT [13]. Following this early phase after oral glucose, plasma insulin concentrations were higher in the subjects with IGT. The 2 h plasma insulin concentrations correlated with the 2 h glucose value ($r = 0.64$, $p < 0.001$) which supports that it is the achieved higher glucose concentrations which cause the 2 h hyperinsulinemia. This supports the hypothesis that a primary defect in IGT is a defective early insulin response which results in inappropriately high glucose concentrations which in turn augments insulin secretion during the later time points of the test [13, 22, 23]. Also the defective relative suppression of glucagon concentrations might contribute to the high 2 h glucose concentration by augmenting the release of glucose into the circulation [13]. This is supported by the studies in non-diabetic subjects showing that lack of suppression of glucagon results in hyperglycaemia when insulin secretion is impaired [10]. Hence, the impaired glucose tolerance seems to be caused by a combination of defective early insulin response in combination with defective glucagon suppression. Whether the slight reduction in insulin sensitivity, which supports previous reports in IGT [24,25], also contributes to the perturbations or whether it is caused by the hyperglycaemia and hyperinsulinemia can not be established from this cross sectional study.

The mechanism of the defective suppression of glucagon secretion in IGT is not known. An important factor to inhibit glucagon secretion is insulin through activation of insulin receptors on the A cells [26–28]. Since the subjects with IGT had whole body insulin resistance, the impaired suppression of glucagon observed in these subjects might be explained by insulin resistance at the level of the A cell. We examined this possibility by measuring glucagon during the hyperinsulinaemic, euglycaemic clamp, i.e., a condition in which circulating insulin was raised to a similar concentration in the two groups. We found that the reduction in glucagon concentrations during the hyperinsulinaemic clamp was impaired in IGT. The results therefore suggest that a failure to suppress glucagon secretion in IGT is dependent on a rel-

ative inability of insulin to inhibit the A cells. This is further supported by the correlation between insulin sensitivity and suppression of glucagon during the hyperinsulinaemic, euglycaemic clamp. Inability of insulin to suppress glucagon in IGT might also explain the increased glucagon response to arginine in IGT, as previously reported [11], because arginine elicits a strong insulin secretory response which will tend to counteract the direct action of arginine to stimulate glucagon secretion.

Of interest, the suppression of glucagon concentrations during the hyperinsulinaemic, euglycaemic clamp correlated negatively to the 2 h glucose concentration in the OGTT and was, in association with the reduced insulin sensitivity, an independent predictor of the 2 h glucose concentration in a multivariate analysis. This further suggests that defective glucagon suppression contributes to the metabolic perturbations in IGT.

Our results show that the inability of insulin to suppress glucagon concentrations is evident at an early stage during the development of Type II diabetes and it could be caused by A-cell insulin resistance. This supports a strategy of developing agents which inhibit glucagon secretion or counteract glucagon action for the early treatment of diabetes.

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